UNIVERSIDADE DE SÃO PAULO
INSTITUTO DE QUÍMICA DE SÃO CARLOS

Desenvolvimento de novos materiais e dispositivos para preparo miniaturizado de amostras e seu acoplamento *on-line* com cromatografia líquida de alta eficiência

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DEDICATÓRIA

Dedico essa tese aos meus pais Idalina e José Luis (in memoriam) que nunca mediram esforços e sacrifícios para que eu sempre trilhasse o caminho dos estudos. Dedico também à minha esposa Jessica. Seu amor, carinho, zelo e companheirismo foram essenciais durante todos esses anos. Pelo constante apoio e incentivo. Com vocês eu sou forte, obrigado!

LISTA DE FIGURAS

RESUMO

Esta tese de doutorado é orientada a resultados relacionados ao desenvolvimento da etapa de preparo de amostras, os quais são apresentados em forma de quatro artigos, um artigo por capítulo. As isoflavonas formam uma importante subclasse dos fitoestrógenos. Compostos presentes em todo o reino vegetal, mais facilmente em leguminosas como soja, feijão, grão de bico e sementes de girassol, estão diariamente presentes em nossa alimentação. A ação antioxidante e propriedades que previnem inflamações, aterosclerose, trombose, osteoporose, infecções virais, tumores e doenças cardiovasculares, fazem com que as isoflavonas sejam associadas a inúmeros benefícios à saúde humana, fatores que despertaram o interesse da comunidade científica, ocasionando um expressivo aumento no número de publicações relacionadas a estes compostos nas últimas duas décadas. Ainda que micro técnicas de preparo de amostra como a SPME e a MEPS já estejam bem desenvolvidas, o uso de técnicas clássicas de preparo de amostra ainda prevalece e é o mais utilizado. O uso de micro técnicas ainda é escasso limitando-se, principalmente, à trabalhos de desenvolvimento analítico. Foi sintetizada um fase extratora que consiste em aminopropil silica recoberta com nanofolhas de grafeno e funcionalizadas com β-ciclodextrina e aplicada em micro extração por sorvente empacotado (MEPS) com a finalidade de desenvolver uma metodologia simples, fácil, rápida e de baixo custo para análises de isoflavonas em alimentos líquidos vendidos comercialmente. Nesta etapa do trabalho foi constatada a seletividade da fase extratora proposta por meio de experimentos que avaliaram a influência do efeito de matriz. Uma nova abordagem para o acoplamento *on-line* entre micro extração em fase sólida (SPME) e cromatografia líquida foi desenvolvida. A fase extratora desenvolvida foi aplicada à técnica de extração chamada *needle-sleeve* SPME e um método analítico foi desenvolvido, automatizado e acoplado a cromatografia líquida de alta eficiência. Além da hifenização do SPME *on-line*, esta abordagem de extração permitiu tratar alguns problemas conhecidos como o entupimento do sistema e/ou da coluna analítica, onde em uma abordagem tipo *column switching*, como *in-tube* SPME, costuma ocorrer. O efeito dos principais parâmetros relacionados à extração e o desempenho do método *online* SPME-LC automatizado desenvolvido, foram estudados e o método se mostrou uma nova estratégia analítica sensível, confiável e direta para a determinação de compostos orgânicos em amostras complexas.

Palavras-Chave: Ciclodextrina; Grafeno; Isoflavonas; MEPS; Needle-sleeve SPME.

ABSTRACT

This thesis is oriented towards results related to the development of the sample preparation stage, which are presented in the form of four articles, one article per chapter. Isoflavones are an important subclass of phytoestrogens. Compounds present throughout the plant kingdom, more easily in legumes such as soybeans, beans, chickpeas and sunflower seeds, are daily present in our food. The antioxidant action and properties that prevent inflammation, atherosclerosis, thrombosis, osteoporosis, viral infections, tumors and cardiovascular diseases, cause isoflavones to be associated with many benefits to human health, factors that aroused the interest of the scientific community, indicating a significant increase in the number of publications related to these compounds in the last two decades. Although micro sample preparation techniques such as SPME and MEPS are already well developed, the use of classic sample preparation techniques is still the most used. The use of micro techniques is still scarce, mainly limited to analytical development work. An extraction phase was synthesized, consisting of aminopropyl silica coated with graphene nanosheets and functionalized with β-cyclodextrin and applied in micro extraction by packed sorbent (MEPS) in order to develop a simple, easy, fast and low cost method for analysis of isoflavones in liquid foods sold commercially. In this stage of the work, the selectivity of the proposed extraction phase was verified through experiments that evaluated the influence of the matrix effect. A new approach for on-line coupling between solid phase micro extraction (SPME) and liquid chromatography was developed. The developed extraction phase was applied to the extraction technique called needle-sleeve SPME and an analytical method was developed, automated and coupled to high-performance liquid chromatography. In addition to the hyphenation of the SPME online, this extraction approach allowed to treat some known problems such as system and/or analytical column clogging, where in a column switching approach, such as in-tube SPME, it usually occurs. The effect of the main parameters related to extraction and the performance of the developed automated SPME-LC online method were studied and the method proved to be a new sensitive, reliable and direct analytical strategy for the determination of organic compounds in complex samples.

Keywords: Cyclodextrin; Graphene; Isoflavones; MEPS; Needle-sleeve SPME.

SUMÁRIO

CAPÍTULO 1

INTRODUÇÃO

1 INTRODUÇÃO

1.1 Panorama geral

Atualmente a análise química de amostras complexas geralmente envolve algumas etapas em comum, dentre as quais destacam-se: (i) o preparo da amostra, (2) uma separação cromatográfica e (3) detecção e quantificação dos analitos de interesse. Análises químicas de resíduos, contaminantes, ou analitos em concentrações muito baixas, dependem de uma etapa de preparo de amostra capaz de fornecer uma pré-concentração dos analitos de interesse e, em matrizes complexas, a necessidade de um *clean-up* que remova os interferentes sem perda dos analitos alvo. Portanto, o preparo de amostra visa promover a extração, remoção dos interferentes, e a concentração dos analitos de interesse. É uma etapa muito onerosa, podendo chegar a consumir cerca de 80% do tempo de uma análise, passível de introduzir erros - tal como perda de analitos e contaminação da amostra – e, por isso, deve-se ter um cuidado especial na escolha da técnica, para que ela seja adequada aos objetivos da análise e sejam obtidos resultados confiáveis e exatos (1).

O preparo de amostra bem realizado pode promover a remoção de interferentes que coeluem com os analitos de interesse, e que promovem efeito de matriz, até remover impurezas que diminuem a vida útil de todo o sistema cromatográfico, incluindo as colunas, o sistema de injeção e os detectores. Essa etapa da análise, se bem sucedida, diminui a frequência de necessidade de limpeza e manutenção de todo o sistema analítico (2).

Quando bem desenvolvido e executado, um bom método de preparo de amostra resulta em uma menor exigência posterior do método cromatográfico (3); por isso a sua escolha e otimização não é uma questão simples a ser resolvida. Entretanto busca-se, como ideal, que esta etapa seja a mais simples possível, afim de não estender demais o tempo de análise e não introduzir erros devido ao grande número de etapas. O método deve ser seletivo de modo a eliminar interferentes e promover maior detectabilidade, além de ser rápido, consumir quantidades mínimas de solventes e reagentes, e possuir baixo custo (1).

Sabendo a importância que a etapa de preparo da amostra exerce em toda a análise química é de se esperar que ela sempre esteja presente na rotina de um laboratório, principalmente envolvendo separações cromatográficas. Sabe-se, também, que a evolução e surgimento de novas técnicas é constante. Esta evolução tornará a etapa de preparo da amostra mais rápida e precisa, além da automatização completa do sistema (3). Contudo, tornar-se dispensável e o desaparecimento desta etapa do método analítico, é pouco esperado e até mesmo improvável.

O avanço tecnológico da instrumentação analítica utilizada em cromatografia geralmente não elimina a necessidade de uma boa etapa de preparo de amostra e, ainda, exige avanços que os acompanhem e tornem compatíveis para obtenção de resultados precisos e confiáveis. A evolução instrumental da cromatografia líquida pode ser descrita pelo avanço tecnológico das colunas analíticas. Por volta de 1960 o desafio consistia em otimizar o empacotamento das colunas analíticas de modo a obter fases estacionárias térmica e mecanicamente estáveis, produção de partículas esféricas de fases estacionárias e a redução do diâmetro das partículas. Já em 1970 foram utilizadas para empacotas as colunas partículas totalmente porosas, um dos principais tipos de partículas utilizadas em HPLC atualmente, com diâmetro médio das partículas em torno de 10 µm, o que permitiu um empacotamento mais denso. Em 1980 era popular o uso de partículas de 5 µm de diâmetro; em 1990 de 3 a 3,5 µm de diâmetro; em 2000 partículas de diâmetro médio de 2,5 µm. Em 2004 foi introduzido o conceito de partículas de diâmetro inferior a 2 µm (sub-2) pela empresa Waters Corporation®. Atualmente, são disponíveis comercialmente colunas com partículas de até 1,3 µm de diâmetro médio. Na última década, tornou-se comum o uso de colunas contendo partículas superficialmente porosas (“*core shell”)*, com diâmetro inferior a 3 µm (sub-3). Essas colunas, as quais contém um núcleo sólido revestido com uma camada de partículas porosas, foram desenvolvidas para apresentarem eficiência similar ou superior às sub-2 µm sem requererem o uso de elevadas pressões, surgindo como uma alternativa que combina alta eficiência e o uso de aparelhos cromatográficos convencionais, sem a necessidade de equipamentos de custo mais elevado como o UHPLC (4).

O desenvolvimento de colunas analíticas mais eficientes, contendo partículas menores, levou à necessidade de aparelhos cromatográficos capazes de operar em

pressões elevadas, ocasionando o desenvolvimento da técnica cromatográfica como um todo.

De forma análoga, a etapa de preparo de amostra evoluiu para atender aos novos requisitos que surgiam. Os avanços promovidos pela diminuição do tamanho de partículas, do diâmetro interno das colunas e até mesmo o desenvolvimento de colunas não empacotadas (denominadas tubulares abertas), levaram a necessidade de menores volumes de injeção na coluna cromatográfica, exigindo maior capacidade de concentração da etapa de preparo de amostra (5).

Diversas técnicas foram desenvolvidas para o preparo de amostras; com sua evolução podemos atualmente separá-las em: técnicas clássicas, técnicas semi-clássicas, micro técnicas e técnicas automatizadas.

Como técnicas clássicas podemos destacar a extração sólido-líquido (SLE) e a extração líquido-líquido (LLE), técnicas muito versáteis ainda indicadas por órgãos reguladores, porém que utilizam de grandes volumes de amostra e solventes, geram muitos resíduos, expõem o analista a grandes volumes de solventes orgânicos, e são demoradas e tediosas, fatores que podem levar a erros grosseiros (2).

Um marco para as chamadas técnicas semi-clássicas, foi o desenvolvimento da extração em fase sólida (SPE), uma técnica amplamente conhecida, desenvolvida em 1976 com o intuito de suprir as desvantagens apresentada pela LLE. A técnica deveria possibilitar um menor consumo de solventes e, consequentemente, menor geração de resíduos, alta recuperação dos analitos, possibilidade de aumentar a concentração dos analitos seletivamente. A disponibilidade de uma grande variedade de produtos comerciais e facilidade de automatização da técnica se sobrepõem às desvantagens da técnica, incluindo o custo elevado dos dispositivos comerciais e dos cartuchos descartáveis, eventual dificuldade em selecionar a fase extratora adequada para a análise, os cartuchos serem utilizados uma única vez e o tempo total da análise relativamente elevado. Ainda assim, a técnia é muito aplicada até hoje em diversas áreas tais como na indústria alimentícia, farmacêutica, meio ambiente, em bioquímica e química orgânica (1,6).

Arthur e Pawliszyn em 1990 desenvolveram a micro extração em fase sólida (SPME), considerada a primeira técnica miniaturizada de preparo de amostras que

foi bem sucedida, dando início as chamadas micro técnicas de preparo de amostras. Diferentemente das técnicas clássicas e semi-clássicas baseadas na extração exaustiva, as micro técnicas são baseadas no equilíbrio de partição do analito entre a amostra e a fase extratora (3,7,8), permitindo o desenvolvimento de métodos rápidos, precisos, com baixo consumo de solventes e pequenos volumes de amostras.

A extração dos analitos em SPME ocorre por absorção e adsorção, dependendo da fibra utilizada. As fibras absorventes extraem pelo processo de partição, que é baseado na distribuição do analito entre duas fases imiscíveis. A quantidade de analito que uma fibra de SPME pode extrair está relacionada à espessura do filme da fibra, ou seja, na quantidade de material sorvente e em condição de equilíbrio a quantidade de analito extraída depende do coeficiente de partição fibra/amostra, do volume da fibra, volume de amostra e a concentração inicial do analito na amostra (8).

Desenvolvida em 2004 por Abdel-Rehim, a técnica micro extração em sorbente empacotado – MEPS, consiste em uma pequena quantidade de material sólido empacotado dentro de um pequeno cilindro fixado em uma seringa, entre filtros de polietileno de encaixe, ou como um cartucho entre o corpo da seringa e a agulha. Esta técnica baseia-se na partição do analito entre a amostra e o material sorvente que foi empacotado na seringa (6,9). Pensada como uma opção à SPME, o MEPS-se parece como uma miniaturização da SPE; entretanto, ao contrário da SPE, o cartucho MEPS pode ser usado mais de uma vez, chegando a mais de 400 vezes quando se trabalha com amostra de água (10), dependendo diretamente da amostra e do material sólido empacotado.

Esta abordagem para o preparo de amostra tem se popularizado por ser fácil, rápida e de baixo custo, além de ser possível uma automatização total do procedimento. A MEPS permite que se trabalhe com pequenas quantidades de amostra e reduz muito o uso de solventes orgânicos. Em comparação com a SPME, primeira técnica miniaturizada de preparo de amostras bem sucedida, a MEPS se mostra mais robusta, podendo ser utilizada em amostras complexas, enquanto a SPME é mais sensível à natureza da amostra (6,10). Entretanto, esta técnica também possui desvantagens, tal como a formação de bolhas que podem atrapalhar o procedimento, e pontos que requerem atenção, tal como a velocidade de deslocamento do em-

bolo, que deve ser realizada com velocidade adequada. Movimentos muito rápidos podem atrapalhar a recuperação dos analitos comprometendo a repetibilidade dos resultados (6).

No meio acadêmico há uma crescente busca por processos analíticos que apresentem maior desempenho, confiabilidade, melhoria da velocidade, redução do custo, melhora da sensibilidade e seletividade. Vantagens e desvantagens de métodos analíticos que são discutidas desde o século passado (11). Além do desenvolvimento de novas técnicas de preparo de amostra e a miniaturização, uma vertente na busca por melhores resultados é o desenvolvimento de novos materiais sorventes.

Geralmente, a escolha da fase extratora para o desenvolvimento da etapa de preparo de amostra segue as mesmas regras aplicadas à cromatografia, onde se analisa primeiramente as características físico-químicas do analito, propriedades do sorvente e a composição da matriz. Isso se deve ao fato dos mecanismos de separação nas técnicas baseadas em extração em fase sólida (adsorção, partição, exclusão e troca iônica) serem os mesmos na etapa de preparo de amostra e na separação cromatográfica. A maioria dos sorventes, ou fases sólidas, utilizados baseiam-se em grupos orgânicos C8, C18, cicloexil, fenil, cianopropil, aminopropil, HLB (estes quimicamente ligados à sílica), e polímeros como poliestireno-divinilbenzeno (PS-DVB) e polidimetilsiloxano (PDMS)) (12). Conhecer os grupos funcionais presentes nos analitos de interesse e na fase extratora, buscando semelhanças que não são encontradas nos interferentes presentes na matriz, é um bom começo para garantir a seletividade do material sorvente (1).

Originalmente, em SPME empregava-se um filme de PDMS como sorvente. O desenvolvimento da extração por sorção em barras de agitação - SBSE, técnica baseada na SPME, mostrou que o aumento da espessura do filme de PMDS resulta em um aumento do rendimento da extração. Porém o único material comercialmente disponível continuou ainda por anos sendo o PDMS, o que limitava o uso dessas técnicas a analises de compostos de baixa a moderada polaridade. O desenvolvimento da MEPS, utilizando um cartucho (chamado de BIN) empacotado, assemelhou esta micro técnica à conhecida SPE, possibilitando o uso de um grande número de sorventes. Dentre as várias micro técnicas desenvolvidas, as baseadas em mate-

riais sortivos são bastante utilizadas, resultando em um amplo interesse no desenvolvimento de novos materiais sorventes (13).

Muitos novos materiais foram reportados na literatura ultimamente, tais como polímeros molecularmente impressos (MIPs), os quais oferecem ganho de seletividade; nanomateriais à base de carbono que se popularizaram devido a sua grande área superficial e capacitade de sorção, boa estabilidade física, química e térmica e baixo custo; nanopartículas magnéticas; líquidos iónicos e outros. Cada material apresenta características únicas, vantagens e desvantagens (12). A combinação de micro técnicas de preparo de mostra e materiais sorventes mais seletivos, possibilita uma diminuição do uso de solventes tóxicos e o reuso dos materiais que, consequentemente, resultam em menor geração de resíduos, contribuindo também com o conceito de química verde.

O desenvolvimento de novas técnicas de preparo de amostra, novos materiais sorventes, instrumentação analítica cada vez mais robusta, tempos de análises cada vez menores, e maior velocidade na aquisição e tratamento dos dados, não parece ter sentido quando pensamos que o preparo de amostra pode custar um tempo maior que todo o resto do procedimento analítico somado. É contra produtivo que um cromatógrafo seja capaz de injetar automaticamente as amostras e que cada corrida cromatográfica possa ser realizada em 10 minutos, obtendo-se 6 análises por hora, enquanto a etapa de preparo de amostra tenha uma limitação de uma amostra por hora, ou pior. Estes são apenas alguns dos problemas que podem ser solucionados com técnicas *on-line* de preparo de amostra.

A automatição dos métodos analíticos já é uma realidade, porém ainda com um amplo campo a ser desenvolvido. A *in-tube* SPME, por exemplo, é uma técnica *on-line* de preparo de amostra já bem conhecida; entre 1999 e 2014 o número de trabalhos publicados relacionados a esta técnica teve considerável aumento (14). Independentemente da técnica utilizada, a abordagem automatizada da etapa de preparo de amostra desperta o interesse científico e vantagens tem sido apresentadas como o fácil acoplamento com técnicas cromatográficas, redução do tempo de análise, redução do volume de amostra, redução do consumo de solventes, menor geração de resíduos e consumo de energia, além da melhora da precisão, exatidão e sensibilidade (14,15,16)

1.2 Panorama específico

1.2.1 Grafeno e óxido de grafeno

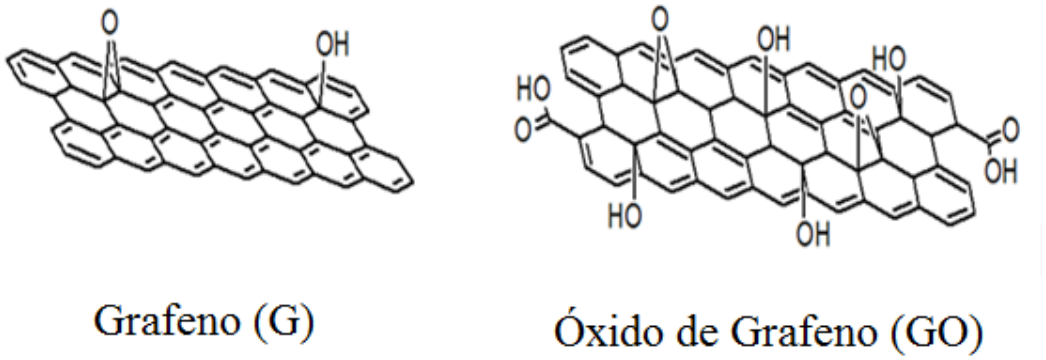
Em 2004 Novoselov e colaboradores reportaram a obtenção de uma estrutura de átomos de carbono arranjados em monocamadas, através de esfoliação mecânica, denominada folha de grafeno (17). Desde então, há um grande interesse em suas aplicações - hoje já exploradas em diversos campos tais como na eletrônica, produção de energia limpa, biomedicina e como materiais sorbentes em preparo de amostras (18,19).

O grafeno (G) é uma forma alotrópica do carbono que possui hibridização sp^2, orbitais σ no plano de sua superfície e elétrons π delocalizados, conferindo ao material resistência mecânica e térmica, propriedades elétricas e ópticas. Somadas à grande área superficial, o grafeno e óxido de grafeno (GO) são considerados adsorventes superiores com elevada capacidade de sorção e grande afinidade por compostos aromáticos (20). O grafeno e o óxido de grafeno diferem quanto a polaridade, pois o GO apresenta mais grupos hidroxilas (–OH) e carboxilas (–COOH). Estes grupos funcionais propiciam maior facilidade para unir o GO à outros materiais, como, por exemplo, a aminopropil-sílica. A Figura 1 ilustra a estrutura desses dois compostos.

A aplicação do G e do GO em preparo de amostras tem crescido muito nos últimos anos. A maioria dos trabalhos relata aplicações em matrizes ambientais e alimentos, com grande potencial para a aplicação destes materiais em análises de matrizes biológicas (19).

A primeira aplicação de grafeno em preparo de amostra, consistiu na extração de compostos clorofenólicos em água com o uso da técnica de extração em fase sólida (SPE) por Liu et al. (21,22). Outros trabalhos reportam a aplicação de materiais à base de grafeno em determinação do tripeptídeo glutaniona (GSH) em plasma

Figura 1 – Representação da estrutura do grafeno e do óxido de grafeno.



FONTE: LIU, Q.; SHI, J.; JIANG, G. Application of graphene in analytical sample preparation. **TrAC - Trends in Analytical Chemistry**, v. 37, p. 1–11, 2012.

e também na determinação de cocaína e adenosina em plasma (23,24). Estudos comparativos mostram que o grafeno é superior em absorção, dessorção e recuperação que outros sorventes mais utilizados, tal como sílica–C18 (22).

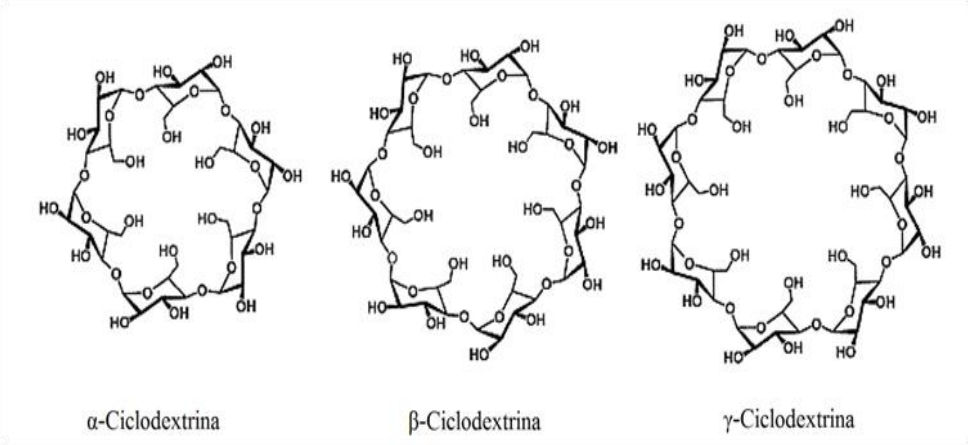
Em técnicas modernas de preparo de amostra, é reportado o uso de materiais à base de grafeno em análises de parabenos em água, empregando MEPS como técnica de preparo de amostra (25); análise de tetraciclinas em leite bovino empregando MEPS como técnica de preparo de amostra (26); análises de aldeídos em fragrâncias empregando SPME como técnica de preparo de amostra (27); e análise de triazinas em água empregando in-tube SPME como técnica de preparo de amostra (15). Estes exemplos demonstram o grande potencial e versatilidade deste material. Outras técnicas com extração em fase sólida dispersiva – dSPE, MSPD, SBSE e on-line SPE também são reportadas na literatura com o emprego de grafeno (28).

1.2.2 Ciclodextrinas

Ciclodextrinas (CDs) são oligossacarídeos naturais formados pela digestão do amido por bactérias. As CDs mais comuns são: α–CD, β–CD e γ–CD, com 6, 7 e 8

anéis de glicopiranose, respectivamente (Figura 2). Possuem um formato de cone, com uma cavidade oca hidrofóbica e uma superfície externa hidrofílica devido aos

Figura 2 – Representação da estrutura química das α, β e γ ciclodextrinas



FONTE: LEUDJO TAKA, A.; PILLAY, K.; YANGKOU MBIANDA, X. Nanosponge cyclodextrin polyurethanes and their modification with nanomaterials for the removal of pollutants from waste water: A review. **Carbohydrate Polymers**, v. 159, p. 94–107, 2017.

grupos –OH existentes na sua parede externa (29,30,31).

As CDs são utilizadas na indústria alimentar como estabilizadores de sabores, odores, cores, vitaminas e emulsões. São também muito utilizadas na área de fármacos para inibir sabores e odores desagradáveis e para aumentar a solubilidade em água destes compostos, através da formação de complexos de inclusão onde o hospedeiro é a CD e o hóspede é o fármaco. Esta propriedade faz com que o fármaco possua maior solubilidade, permeação e biodisponibilidade. Por exemplo, a complexação com γ–CD aumenta a eficiência e a potência da digoxina e doxorrubicina, que são fármacos pouco solúveis em água (29). Enfim, as aplicações são inúmeras e vão desde a indústria farmacêutica até a indústria têxtil e também em proteção ambiental (30).

Em química analítica, as CDs podem ser utilizadas em técnicas de preparo de amostras tais como SPE, SPME e SBSE. Por exemplo, β–CD foi utilizada eficientemente na extração de oito hormônios esteroides em água, em determinação de hormônios vegetais derivados de naftaleno em tomate, garantindo um ganho de seletividade frente ao método padrão, e em determinação de flavonóides provenientes de frutas, garantindo seletividade de compostos quirais (31,32,33,34).

1.2.3 Grafeno e ciclodextrinas

A estrutura específica das ciclodextrinas favorece a formação de complexos de inclusão por meio de ligações de hidrogênio, interações hidrofóbicas e Van der Waals (34,35). Seus grupos hidroxila podem ser substituídos a fim de melhorar a solubilidade e capacidade de inclusão e, até mesmo, para sua imobilização em um suporte sólido (36). Somado à correspondência de tamanho, possuem uma grande capacidade de reconhecimento de compostos fenólicos, tornando comum o emprego de materiais à base de grafeno funcionalizados com ciclodextrinas como detectores eletroquímicos (36) , detector eletrocatalítico (37) e material eletrocatalítico (38).

Em 2012 foi apresentado pela primeira vez um nanocomposto magnético de óxido de grafeno funcionalizado com β−CD. Primeiramente o GO foi preparado por oxidação ácida do grafite pelo método de Hummers, depois o Fe_3O_4 foi funcionalizado com GO por precipitação química, formando o GO/ Fe_3O_4. O próximo passo foi funcionalizar o híbrido GO/ Fe_3O_4 com β−CD, formando o nano composto GO/Fe_3O_4/ β−CD, o qual foi utilizado como uma nova fase estacionária em uma coluna cromatográfica para uma separação eficiente de enantiomeros do triptofano (39). Outros estudos utilizam o acoplamento de ciclodextrinas ao GO para produção de sensores eletroquímicos para a detecção de pesticidas organofosforados, analise de crisoidina e detecção de triptofano (36,40,41).

Os compostos formados por GO-CDs podem ter as vantagens tanto das CDs quanto das nano folhas de GO (42), o que torna interessante o uso desta combinação em preparo de amostra. São reportados o emprego deste material em SPME (43), MSPE (44,45) e dSPE (46), resultando em alto fator de enriquecimento, maior eficiência de extração, capacidade de adsorção seletiva.

1.2.4 Fitoestrógenos

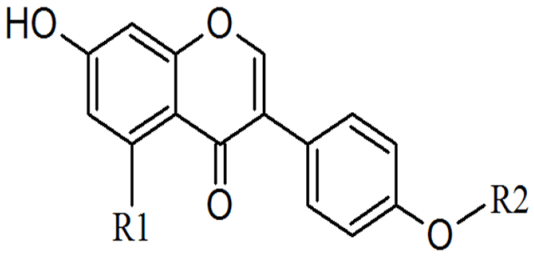
Fitoestrógenos são compostos não esteróides produzidos por plantas, os quais mimetizam a ação dos estrogênios endógenos (47). São divididos em três

classes principais: Isoflavonas, lignanos e coumestans, sendo isoflavonas e lignanos os principais. Os lignanos são encontrados na porção lenhosa de frutas e vegetais, cascas de sementes e farelo de grãos de cereais ricos em fibras, enquanto os coumestans são menos abundantes na dieta e, portanto, são menos estudados (48).

As isoflavonas (Figura 3) são os fitoestrógenos mais potentes e mais comuns. São metabólitos secundários de plantas, compostos polifenólicos estruturalmente semelhantes ao 17β-estradiol (47,49). As isoflavonas, genisteína (GEN) e daidzeína (DAI), são encontradas em várias plantas, sendo mais abundantes em leguminosas como soja e alimentos derivados de soja. Nos humanos a GEN e DAI são as isoflavonas biologicamente ativas mais importantes (50).

Aos fitoestrógenos são atribuídos vários benefícios à saúde, tal como diminuição do risco de câncer de mama e de próstata, prevenção de doenças cardiovasculares, osteoporose e alívio dos sintomas da menopausa (49,50,51). Em regiões onde se pratica uma dieta mediterrânea, a qual consiste em alimentos ricos em fitoestrógenos, a taxa de incidência das doenças citadas é bem menor do que nos países que praticam uma dieta rica em gorduras e proteínas (52). Entretanto, há trabalhos que relatam que os efeitos benéficos dos fitoestrógenos são evidenciados em doses elevadas, sendo que em doses baixas mimetizam o estrogênio podendo causar problemas tais como câncer de mama (48). Porém estudos associados à saúde humana ainda são inconclusivos (53). Neste contexto, faz se importante o estudo de métodos de identificação e quantificação de isoflavonas.

Figura 3 – Estrutura das isoflavonas.



	R1	R2
Daidzeína	H	H
Genisteína	OH	H
Formononetina	H	CH_3
Biochanina A	OH	CH_3

FONTE: Autoria própria

1.3 Objetivos

1.3.1 Objetivo geral

Esta tese teve como objetivo geral o desenvolvimento de métodos analíticos capaz de identificar e quantificar isoflavonas em matrizes complexas, empregando material à base de grafeno funcionalizado com β-ciclodextrina como fases extratoras em micro técnicas de preparo de amostra.

1.3.2 Objetivos específicos

- Síntese de um material sorvente baseado em grafeno, óxido de grafeno e ciclodextrina;
- Desenvolvimento de metodologia MEPS *off-line* para análise de isoflavonas em suco de soja, otimização da etapa de preparo de amostra, e qualificação do método desenvolvido.
- Desenvolvimento de metodologia SPME *on-line* para análise de isoflavonas em urina, otimização da etapa de preparo de amostra e qualificação do método desenvolvido.

1.4 A coletânea de artigos

Esta tese de doutorado é orientada a resultados relacionados ao desenvolvimento da etapa de preparo de amostras, os quais são apresentados em forma de quatro artigos, um artigo por capítulo. Alguns dos pontos positivos que podemos ressaltar sobre esta abordagem: o leitor pode ler qualquer capítulo desta tese de maneira independente, pois as informações contidas em cada capítulo são suficientes para a compreensão do mesmo e os trabalhos então publicados em revistas ci-

entíficas, muitas vezes de acesso restrito, agora totalmente acessíveis em forma de tese de doutorado. Entretanto, um possível ponto negativo desta abordagem é a repetição de conceitos, principalmente no início de cada capítulo, que corresponde a introdução e estado da arte de cada artigo.

Todos os trabalhos apresentados foram desenvolvidos sob a orientação e coautoria do Prof. Dr. Fernando Mauro Lanças. Dos quatro artigos três são de primeira autoria do doutorando, e um em colaboração. Esses trabalhos foram aceitos/publicados em periódicos nacionais e internacionais; parte de um deles foi apresentada no XVII Congresso Latino-Americano de Cromatografia e Técnicas Relacionadas – COLACRO 2019.

No **primeiro trabalho**[(1)] publicado, com a colaboração de Marcela Jordan-Sinistera, contido no **capítulo 2** (o capítulo 1 apresenta uma Introdução geral à tese), temos uma revisão da literatura sobre trabalhos voltados para a análise de isoflavonas em matrizes complexas. Neste contexto temos uma definição do que são as isoflavonas, uma importante subclasse dos fitoestrógenos. Compostos presentes em todo o reino vegetal, mais facilmente em leguminosas como soja, feijão, grão de bico e sementes de girassol, estão diariamente presentes em nossa alimentação. A ação antioxidante e propriedades que previnem inflamações, aterosclerose, trombose, osteoporose, infecções virais, tumores e doenças cardiovasculares, fazem com que as isoflavonas sejam associadas a inúmeros benefícios à saúde humana, fatores que despertaram o interesse da comunidade científica, ocasionando um expressivo aumento no número de publicações relacionadas a estes compostos nas últimas duas décadas.

[(1)] **Métodos analíticos para determinação de isoflavonas em matrizes complexas.**
*Luis Felipe da Silva; Marcela Jordan-Sinisterra; Fernando Mauro Lanças**
Scientia Chromatographica 2018; 10(4):219-228
http://dx.doi.org/10.4322/sc.2015.000

Este trabalho apresenta um levantamento bibliográfico entre os anos de 2010 a 2018 que nos permite perceber que diversas técnicas de preparo de amostra tais como extração líquido-líquido – LLE; extração sólido-líquido – SLE; extração em fase sólida – SPE; QuEChERS sigla proveniente das inicias das palavras palavras *quick* (rapido), *easy* (facil), *cheap* (barato), *effective* (eficaz), *rugged* (robusto) e *safe* (seguro); fase sólida magnética dispersa na matriz – MSPD, micro extração em fase sólida – SPME; e micro extração em sorbente empacotado – MEPS, são aplicadas em análises de isoflavonas em variadas matrizes como soja, produtos comerciais derivados de soja, leite bovino, urina, plasma, água e outros. Ainda que micro técnicas de preparo de amostra como a SPME e a MEPS já estejam bem desenvolvidas, o uso de técnicas clássicas de preparo de amostra ainda prevalece e é o mais utilizado. O uso de micro técnicas ainda é escasso limitando-se, principalmente, à trabalhos de desenvolvimento analítico.

O **segundo trabalho** publicado [2], o qual corresponde ao **capitulo 3** mostra que, uma vez conhecido o estado da arte para análises de isoflavonas em matrizes complexas e o uso de micro técnicas de preparo de amostra, buscou-se o desenvolvimento de uma fase extratora e um procedimento seletivo MEPS combinado com HPLC–MS/MS para análise de isoflavonas em amostras de suco à base de soja.

A primeira etapa deste trabalho concentrou-se na síntese da fase extratora e no desenvolvimento da etapa de preparo de amostra. Foram realizadas diversas sínteses na busca de um nanomaterial a base grafeno que apresentasse melhor taxa de recobrimento da aminopropil sílica pelas nano folhas de óxido de grafeno e melhor taxa de funcionalização com β-ciclodextrina, resultando na fase extratora Si@GO@βCD.

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[2] **Determination of isoflavones in soy-based juice using β-cyclodextrin coupled to graphene oxide supported on aminopropyl silica as a sorbent material for microextraction by packed sorbent (MEPS)**
Luis Felipe da Silva , Fernando Mauro Lanças
JSS Journal of Separation Science, published online on 5 October, 2020.
DOI: 10.1002/jssc.202000598

Foi desenvolvido, também para este trabalho, uma seringa *lab-made* como uma alternativa barata e de fácil acesso e utilização em relação as seringas comerciais utilizadas em MEPS.

O método de preparo de amostra foi desenvolvido e otimizado e experimentos para avaliar a performance da fase extratora foram realizados. A funcionalização da fase extratora com β-ciclodextrina teoricamente confere seletividade ao material proposto, o que foi constatado com os experimentos para avaliação do efeito de matriz. Os resultado obtidos foram apresentados no XVII Congresso Latino-Americano de Cromatografia e Técnicas Relacionadas – COLACRO 2019.

No seguimento do trabalho foi concluído o desenvolvimento e análise das figuras de mérito do método analítico proposto. Os resultados indicam que o método é reproduzível, preciso e de baixo custo. O uso de material sorvente à base de óxido de grafeno e ciclodextrina melhora a seletividade, resultando em um bom *clean-up* e ausência de efeito da matriz, sugerindo sua aplicabilidade em outras matrizes e também em outras classes de analitos, desde que compatíveis com o uso das ciclodextrinas.

O **capítulo 4** apresenta o **terceiro trabalho** desenvolvido e publicado. Com a colaboração de Deyber Arley Vargas Medina foi desenvolvida uma nova abordagem para o acoplamento *on-line* entre micro extração em fase sólida (SPME) e cromatografia líquida (LC). Um inovador dispositivo extrator que consiste numa agulha revestida com Si@GO@βCD foi desenvolvido e empregado na determinação *on-line* SPME-LC-UV de isoflavonas em urina. O uso de um robô baseado em motor de passos controlado por um sistema Aurdino, permitiu a automatização de todo o processo de preparo de amostra. Este robô é responsável pelo processo de extração, clean-up, eluição, injeção, *start* da corrida cromatográfica, lavagem da fase extratora e do *loop* de carregamento da amostra.

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(3) **Automated needle-sleeve based online hyphenation of solid-phase microextraction and liquid chromatography.**
Luis Felipe da Silva, Deyber Arley Vargas Medina, Fernando Mauro Lanças
Talanta. Available online 2 September 2020.
https://doi.org/10.1016/j.talanta.2020.121608

Além da hifenização do SPME *on-line*, esta abordagem de extração permitiu tratar alguns problemas conhecidos como o entupimento do sistema e/ou da coluna analítica, onde em uma abordagem tipo *column switching*, como *in-tube* SPME, costuma ocorrer. O efeito dos principais parâmetros relacionados à extração e o desempenho do método *online* SPME-LC automatizado desenvolvido, foram estudados e o método se mostrou uma nova estratégia analítica sensível, confiável e direta para a determinação de compostos orgânicos em amostras complexas.

O **quarto trabalho** apresentado, referente ao **capitulo 5**, é um trabalho em colaboração com outros alunos do grupo de pesquisa. O Grupo de Cromatografia do IQSC-USP vem desenvolvendo uma série de trabalhos objetivando a síntese, caracterização e aplicações de novos materiais à base de grafeno em ciências da separação, especialmente em micro técnicas de preparo de amostras e em cromatografia. No presente trabalho são apresentados conhecimentos e a experiência prática referentes à pesquisa de cada um dos integrantes que trabalharam no grupo, durante o período desta tese, com materiais à base de grafeno. O uso de grafeno em preparo de amostra é recente, e diversos materiais foram desenvolvidos e aplicados em inúmeras situações distintas, mostrando um enorme potencial para novos desenvolvimentos. Minha contribuição neste artigo consiste numa revisão e análise crítica de trabalhos publicados na literatura que apresentem materiais à base de grafeno funcionalizados com ciclodextrinas. Este trabalho permitiu uma descrição detalhada do material empregado nesta tese, estado da arte sobre sínteses, aplicações e resultados conhecidos. Esta contribuição enquadra o corpo de um trabalho de revisão completo sobre o estado da arte, até então, do uso de materiais à base de grafeno em preparo de amostra.

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(4) **The Current Role of Graphene-Based Nanomaterials in the Sample Preparation Arena.**
Edvaldo Vasconcelos Soares Maciel, Karen Mejía-Carmona, Marcela Jordan-Sinisterra, Luis Felipe da Silva, Deyber Arley Vargas Medina and Fernando Mauro Lanças
Frontiers in Chemistry, vol 8 (2020) 1-24,
doi: 10.3389/fchem.2020.00664

Referências bibliográficas

1 JARDIM, I. C. S. F. Extração em Fase Sólida : Fundamentos Teóricos e Novas Estratégias para Preparação de Fases Sólidas. **Scientia Chromatographica**, v. 2, n. 1, p. 13–25, 2010.

2 MOREIRA, B. J.; YOKOYA, J. M. C.; GAITANI, C. M. DE. Microextração líquido-líquido dispersiva (DLLME): fundamentos , inovações e aplicações biológicas. **Scientia Chromatographica**, v. 6, n. 3, p. 186–204, 2014.

3 FUMES, B. H.; ANDRADE, M. A.; FRANCO, M. S.; LANÇAS, F. M. On-line approaches for the determination of residues and contaminants in complex samples. **Journal of Separation Science**, v. 40, n. 1, p. 183–202, 2017.

4 BOCELLI, M. D.; BORSATTO, J. V. B.; SANTOS NETO, Á. J. DOS. Capillary UHPLC: theoretical and practical aspects. **Scientia Chromatographica**, v. 10, n. 1, 2018.

5 GOU, Y.; PAWLISZYN, J. In-Tube Solid-Phase Microextraction Coupled to Capillary LC for Carbamate Analysis in Water Samples. v. 72, n. 13, p. 2774–2779, 2000.

6 NOVÁKOVÁ, L.; VLČKOVÁ, H. A review of current trends and advances in modern bio-analytical methods: Chromatography and sample preparation. **Analytica Chimica Acta**, v. 656, n. 1–2, p. 8–35, 2009.

7 LORD, H.; PAWLISZYN, J. Evolution of solid-phase microextraction technology. **Journal of Chromatography A**, v. 885, p. 153–193, 2000.

8 PARREIRA, F. V.; CARDEAL, Z. DE L. Amostragem de Compostos Orgânicos Voláteis no Ar Utilizando a Técnica de Microextração em Fase Sólida. **Quimica Nova**, v. 28, n. 4, p. 646–654, 2005.

9 ABDEL-REHIM, M. New trend in sample preparation : on-line microextraction in packed syringe for liquid and gas chromatography applications I . Determination of local anaesthetics in human plasma samples using gas chromatography – mass spectrometry. **Journal of Chromatography B**, v. 801, n. 2, p. 317–321, 2004.

10 ABDEL-REHIM, M.; ANDERSSON, A.; BREITHOLTZ-EMANUELSSON, A.; SANDBERG-STÄLL, M.; BRUNFELTER, K.; PETTERSSON, K. J.; NORSTEN-HÖÖG, C. MEPS as a rapid sample preparation method to handle unstable compounds in a complex matrix: Determination of AZD3409 in plasma samples utilizing MEPS-LC-MS-MS. **Journal of Chromatographic Science**, v. 46, n. 6, p. 518–523, 2008.

11 AUGUSTO, F.; CARASEK, E.; SILVA, R. G. C.; RIVELLINO, S. R.; BATISTA, A. D.; MARTENDAL, E. New sorbents for extraction and microextraction techniques. **Journal of Chromatography A**, v. 1217, n. 16, p. 2533–2542, 2010.

12 WEN, Y.; CHEN, L.; LI, J.; LIU, D.; CHEN, L. Recent advances in solid-phase sorbents for sample preparation prior to chromatographic analysis. **TrAC - Trends in Analytical Chemistry**, v. 59, p. 26–41, 2014.

13 FUMES, B. H.; SILVA, M. R.; ANDRADE, F. N.; NAZARIO, C. E. D.; LANÇAS, F. M. Recent advances and future trends in new materials for sample preparation. **TrAC - Trends in Analytical Chemistry**, v. 71, p. 9–25, 2015.

14 FERNÁNDEZ-AMADO, M.; PRIETO-BLANCO, M. C.; LÓPEZ-MAHÍA, P.; MUNIATEGUI-LORENZO, S.; PRADA-RODRÍGUEZ, D. Strengths and weaknesses of in-tube solid-phase microextraction: A scoping review. **Analytica Chimica Acta**, v. 906, p. 41–57, 2016.

15 DE TOFFOLI, A. L.; FUMES, B. H.; LANÇAS, F. M. Packed in-tube solid phase microextraction with graphene oxide supported on aminopropyl silica: Determination of target triazines in water samples. **Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes**, v. 53, n. 7, p. 434–440, 2018.

16 ALTUN, Z.; ABDEL-REHIM, M.; BLOMBERG, L. G. New trends in sample preparation: On-line microextraction in packed syringe (MEPS) for LC and GC applications: Part III: Determination and validation of local anaesthetics in human plasma samples using a cation-exchange sorbent, and MEPS-LC-MS-MS. **Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences**, v. 813, n. 1–2, p. 129–135, 2004.

17 NOVOSELOV, K. S.; GEIM, A. K.; MOROZOV, S. V; JIANG, D.; ZHANG, Y.; DUBONOS, S. V.; GRIGORIEVA, I. V; FIRSOV, A. A. Electric Field Effect in Atomically Thin Carbon Films Supplementary. **Science**, v. 5, n. 1, p. 1–12, 2004.

18 LIU, Q.; SHI, J.; JIANG, G. Application of graphene in analytical sample preparation. **TrAC - Trends in Analytical Chemistry**, v. 37, p. 1–11, 2012.

19 NAZARIO, C. E. D.; FUMES, B. H.; DA SILVA, M. R.; LANÇAS, F. M. New materials for sample preparation techniques in bioanalysis. **Journal of Chromatography B**, v. 1043, p. 81–95, 2017.

20 ZHANG, X.; CHEN, S.; HAN, Q.; DING, M. Preparation and retention mechanism study of graphene and graphene oxide bonded silica microspheres as stationary phases for high performance liquid chromatography. **Journal of Chromatography A**, v. 1307, p. 135–143, 2013.

21 LIU, Q.; SHI, J.; ZENG, L.; WANG, T.; CAI, Y.; JIANG, G. Evaluation of graphene as an advantageous adsorbent for solid-phase extraction with chlorophenols as model analytes. **Journal of Chromatography A**, v. 1218, n. 2, p. 197–204, 2011.

22 HUANG, Z.; LEE, H. K. Materials-based approaches to minimizing solvent usage in analytical sample preparation. **TrAC - Trends in Analytical Chemistry**, v. 39, p. 228–244, 2012.

23 HUANG, K.-J.; JING, Q.-S.; WEI, C.-Y.; WU, Y.-Y. Spectrofluorimetric determination of glutathione in human plasma by solid-phase extraction using graphene as adsorbent. **Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy**, v. 79, n. 5, p. 1860–5, 2011.

24 GULBAKAN, B. et al. A dual platform for selective analyte enrichment and ionization in mass spectrometry using aptamer-conjugated graphene oxide. **Journal of the American Chemical Society**, v. 132, n. 49, p. 17408–17410, 2010.

25 FUMES, B. H.; LANÇAS, F. M. Use of graphene supported on aminopropyl silica for microextraction of parabens from water samples. **Journal of Chromatography A**, v. 1487, p. 64–71, 2017.

26 VASCONCELOS SOARES MACIEL, E.; HENRIQUE FUMES, B.; LÚCIA DE TOFFOLI, A.; MAURO LANÇAS, F. Graphene particles supported on silica as sorbent for residue analysis of tetracyclines in milk employing microextraction by packed sorbent. **Electrophoresis**, v. 39, n. 15, p. 2047–2055, 2018.

27 ZHANG, J.; YANG, L.; WU, M.; GUO, X.; ZENG, B.; ZHAO, F. Electrochemical preparation of poly(3-methylthiophene-carbazole)/graphene oxide composite coating for the highly effective solid-phase microextraction of some fragrance. **Talanta**, v. 171, n. February, p. 61–67, 2017.

28 DE TOFFOLI, A. L.; MACIEL, E. V. S.; FUMES, B. H.; LANÇAS, F. M. The role of graphene-based sorbents in modern sample preparation techniques. **Journal of Separation Science**, v. 41, n. 1, p. 288–302, 2018.

29 SAOKHAM, P.; LOFTSSON, T. Y-Cyclodextrin. **International Journal of Pharmaceutics**, v. 516, p. 278–292, 2017.

30 LEUDJO TAKA, A.; PILLAY, K.; YANGKOU MBIANDA, X. Nanosponge cyclodextrin polyurethanes and their modification with nanomaterials for the removal of pollutants from waste water: A review. **Carbohydrate Polymers**, v. 159, p. 94–107, 2017.

31 SONG, X. Y.; HA, W.; CHEN, J.; SHI, Y. P. Application of beta-cyclodextrin-modified, carbon nanotube-reinforced hollow fiber to solid-phase microextraction of plant hormones . **J.Chromatogr.A** , v. 1374, n. 1873- 3778 (Electronic), p. 23–30, 2014.

32 SAUVE, S. Using a novel sol – gel stir bar sorptive extraction method for the analysis of steroid hormones in water by laser diode thermal desorption / atmospheric chemical ionization tandem mass spectrometry. **Talanta**, v. 101, p. 337–345, 2012.

33 CAGLIERO, C.; BICCHI, C.; CORDERO, C.; RUBIOLO, P.; SGORBINI, B.; LIBERTO, E. Fast headspace-enantioselective GC-mass spectrometric-multivariate statistical method for routine authentication of flavoured fruit foods. **Food Chemistry**, v. 132, n. 2, p. 1071–1079, 2012.

34 ZHU, Q.-Y.; ZHANG, Q.-Y.; CAO, J.; CAO, W.; XU, J.-J.; PENG, L.-Q. Cyclodextrin-assisted liquid-solid extraction for determination of the composition of jujube fruit using ultrahigh performance liquid chromatography with electrochemical detection and quadrupole time-of-flight tandem mass spectrometry. **Food Chemistry**, v. 213, p. 485–493, 2016.

35 PINHO, E.; GROOTVELD, M.; HENRIQUES, M. Cyclodextrins as encapsulation agents for plant bioactive compounds. **Carbohydrate Polymers**, v. 101, p. 121–135, 2014.

36 WANG, C.; LI, T.; LIU, Z.; GUO, Y.; LI, C.; DONG, C.; SHUANG, S. An ultra-sensitive sensor based on β-cyclodextrin modified magnetic graphene oxide for detection of tryptophan. **Journal of Electroanalytical Chemistry**, v. 781, p. 363–370, 2016.

37 PHAM, T. S. H.; FU, L.; MAHON, P.; LAI, G.; YU, A. Fabrication of β-Cyclodextrin-Functionalized Reduced Graphene Oxide and Its Application for Electrocatalytic Detection of Carbendazim. **Electrocatalysis**, v. 7, n. 5, p. 411–419, 2016.

38 RAN, X.; YANG, L.; QU, Q.; LI, S.; CHEN, Y.; ZUO, L.; LI, L. Synthesis of well-dispersive 2.0 nm Pd–Pt bimetallic nanoclusters supported on β-cyclodextrin functionalized graphene with excellent electrocatalytic activity. **RSC Advances**, v. 7, n. 4, p. 1947–1955, 2017.

39 LIANG, R. P.; LIU, C. M.; MENG, X. Y.; WANG, J. W.; QIU, J. D. A novel open-tubular capillary electrochromatography using β-cyclodextrin functionalized graphene oxide-magnetic nanocomposites as tunable stationary phase. **Journal of Chromatography A**, v. 1266, p. 95–102, 2012.

40 ZHAO, H.; JI, X.; WANG, B.; WANG, N.; LI, X.; NI, R. Biosensors and Bioelectronics An ultra-sensitive acetylcholinesterase biosensor based on reduced chitosan nanocomposites for organophosphorus pesticides detection. **Biosensors and Bioelectronic**, v. 65, p. 23–30, 2015.

41 WANG, X.; LI, X.; LUO, C.; SUN, M.; LI, L.; DUAN, H. Ultrasensitive molecularly imprinted electrochemical sensor based on magnetism graphene oxide/β-

cyclodextrin/Au nanoparticles composites for chrysoidine analysis. **Electrochimica Acta**, v. 130, p. 519–525, 2014.

42 XU, J.; WANG, Q.; XUAN, C.; XIA, Q.; LIN, X.; FU, Y. Chiral Recognition of Tryptophan Enantiomers Based on β-Cyclodextrin-platinum Nanoparticles/Graphene Nanohybrids Modified Electrode. **Electroanalysis**, v. 28, n. 4, p. 868–873, 2016.

43 DENG, J.; ZHANG, P.; JIN, T.; ZHOU, H.; CHENG, J. Graphene oxide/β-cyclodextrin composite as fiber coating for high efficiency headspace solid phase microextraction of organophosphate ester flame retardants in environmental water. **RSC Advances**, v. 7, n. 86, p. 54475–54484, 2017.

44 LIU, G.; LI, L.; XU, D.; HUANG, X.; XU, X.; ZHENG, S.; ZHANG, Y.; LIN, H. Metal–organic framework preparation using magnetic graphene oxide–β-cyclodextrin for neonicotinoid pesticide adsorption and removal. **Carbohydrate Polymers**, v. 175, p. 584–591, 2017.

45 CAO, S.; CHEN, J.; LAI, G.; XI, C.; LI, X.; ZHANG, L.; WANG, G.; CHEN, Z. A high efficient adsorbent for plant growth regulators based on ionic liquid and β-cyclodextrin functionalized magnetic graphene oxide. **Talanta**, v. 194, n. October 2018, p. 14–25, 2019.

46 TAN, P.; HU, Y. Improved synthesis of graphene/β-cyclodextrin composite for highly efficient dye adsorption and removal. **Journal of Molecular Liquids**, v. 242, p. 181–189, 2017.

47 BUSTAMANTE-RANGEL, M.; DELGADO-ZAMARREÑO, M. M.; PÉREZ-MARTÍN, L.; CARABIAS-MARTÍNEZ, R. QuEChERS method for the extraction of isoflavones from soy-based foods before determination by capillary electrophoresis-electrospray ionization-mass spectrometry. **Microchemical Journal**, v. 108, p. 203–209, 2013.

48 YU, J.; WU, Q.; QIAO, S.; YU, Z.; JIN, N.; YU, B. Simultaneous determination of phytoestrogens and key metabolites in breast cancer patients' urine by liquid chromatography-tandem mass spectrometry. **Journal of Pharmaceutical and Biomedical Analysis**, v. 50, n. 5, p. 939–946, 2009.

49 DAEMS, F.; ROMNEE, J. M.; RASSE, C.; FROIDMONT, É.; HEUSKIN, S.; LOGNAY, G. Quantification of Four Isoflavones in Forages with UPLC®-MS/MS, Using the Box–Behnken Experimental Design to Optimize Sample Preparation. **Chromatographia**, v. 79, n. 11–12, p. 711–725, 2016.

50 BUSTAMANTE-RANGEL, M.; DELGADO-ZAMARREÑO, M.; CARABIAS-MARTÍNEZ, R.; DOMÍNGUEZ-ÁLVAREZ, J. Analysis of isoflavones in soy drink by capillary zone electrophoresis coupled with electrospray ionization mass spectrometry. **Analytica Chimica Acta**, v. 709, p. 113–119, 2012.

51 SOUKUP, S. T.; AL-MAHARIK, N.; BOTTING, N.; KULLING, S. E. Quantification of soy isoflavones and their conjugative metabolites in plasma and urine: An automated and validated UHPLC-MS/MS method for use in large-scale studies. **Analytical and Bioanalytical Chemistry**, v. 406, n. 24, p. 6007–6020, 2014.

52 LÓPEZ-BIEDMA, A.; SÁNCHEZ-QUESADA, C.; DELGADO-RODRÍGUEZ, M.; GAFORIO, J. J. The biological activities of natural lignans from olives and virgin olive oils: A review. **Journal of Functional Foods**, v. 26, p. 36–47, 2016.

53 VEJDOVSZKY, K.; SCHMIDT, V.; WARTH, B.; MARKO, D. Combinatory estrogenic effects between the isoflavone genistein and the mycotoxins zearalenone and alternariol in vitro. **Molecular Nutrition and Food Research**, v. 61, n. 3, p. 1–12, 2017.

CAPÍTULO 2

UMA REVISÃO BIBLIOGRÁFICA

[(1)] **Métodos analíticos para determinação de isoflavonas em matrizes complexas.**
*Luis Felipe da Silva; Marcela Jordan-Sinisterra; Fernando Mauro Lanças**
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Métodos analíticos para determinação de isoflavonas em matrizes complexas

Analytical methods for determination of isoflavones in complex matrices

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Resumo

As isoflavonas formam uma importante subclasse dos fitoestrógenos. Encontradas em todo o reino vegetal, estão diariamente presentes em nossa alimentação e são associadas a diversos benefícios à saúde humana, como prevenção de doenças cardiovasculares e de câncer de mama. Esses fatores despertaram o interesse da comunidade científica, ocasionando um expressivo aumento no número de publicações relacionadas a estes compostos nas últimas duas décadas. Este trabalho traz uma visão geral das técnicas de preparo de amostra; técnicas cromatográficas e eletroforéticas de separação; e técnicas de detecção acopladas, assim como suas aplicações em análises de isoflavonas nas mais diversas matrizes, desde 2010 até 2018. São relatados trabalhos de análises de isoflavonas em soja, produtos comerciais derivados de soja, leite bovino, urina, plasma, água e outros. Dentre as técnicas de preparo de amostra destacam-se os trabalhos com LLE, SLE, SPE, QuEChERS, MSPD SPME e MEPS. O uso de técnicas clássicas de preparo de amostra ainda é o mais comum; mesmo com micro técnicas já bem desenvolvidas como a SPME e a MEPS, o uso destas ainda é escasso limitando-se, principalmente, à trabalhos de desenvolvimento analítico. Dentre as técnicas de separação, as cromatográfica, como a HPLC, UHPLC e GC são destaque; as técnicas eletroforéticas também encontram aplicações na área. Dentre os detectores mais empregados estão os espectrômetros massas e os espectrofotômetros na região UV-Vis, respectivamente.

Palavras chaves: isoflavonas, matrizes complexas, métodos analíticos, preparo de amostra, técnicas de separação.

Abstract

Isoflavones are an important subclass of phytoestrogens. They are throughout the plant kingdom, often present in our diet and are associated with several benefits to human health, such as prevention of cardiovascular diseases and breast cancer. These factors have aroused the interest of the scientific community, causing a significant increase in the number of publications related to these compounds in the last two decades. In this work we present an overview on sample preparation techniques, chromatographic separation techniques, and chromatography coupled to different detection techniques applied to the analysis of isoflavones in several matrices, from 2010 to the present days. Studies have reported on the analysis of isoflavones in soybean, soy derivatives, bovine milk, urine, plasma, water and others. Among the sample preparation techniques, there are reports involving LLE, SLE, SPE, QuEChERS, MSPD SPME and MEPS. Classical sample preparation techniques are often used. There are well-developed micro-techniques such as SPME and MEPS, however their use in sample preparation aiming to determine isoflavones is very limited, practically restricted to analytical development studies. Among the chromatographic techniques HPLC, UHPLC and GC are the most popular ones, being followed by the electrophoretic techniques. The most common detectors in this analytical niche are mass spectrometers and UV-Vis spectrophotometers, respectively.

Keywords: isoflavones, complex matrices, analytical methods, sample preparation, separation techniques.

1. Introdução

Fitoestrógenos são metabólitos secundários de plantas não esteroides, biologicamente ativos, os quais possuem estrutura química semelhante à do estradiol, ou seja, propriedades semelhantes a hormônios sexuais femininos endógenos, o que possibilita que se liguem a receptores de estrogênio exercendo efeitos estrogênicos ou antiestrogênicos (1,2). Vários benefícios à saúde tem sido atribuídos aos fitoestrógenos e em regiões do mundo onde se pratica dietas conhecidas como dieta mediterrânea a incidência de doenças como câncer e doenças cardiovasculares é menor do que em regiões onde a dieta é rica em gorduras e proteínas (3). Entretanto, há trabalhos que relatam que os efeitos benéficos à saúde, proporcionados pelos fitoestrógenos, somente ficam evidentes em exposição à doses elevadas, sendo que em doses baixas estes compostos acabam realizando função contrária ao esperado e gerando malefícios à saúde, tal como o câncer de mama (4,5). Contudo, os estudos associados à saúde humana parecem ainda controversos e inconclusivos (6).

Dentre os fitoestrógenos, os flavonóides (flavonas, flavonóis, flavanonas, antocianinas, antocianidinas, auronas, chalconas e isoflavonas entre outros) formam uma importante classe de substâncias naturais encontradas em frutas, legumes, grãos, casca, raízes, caules e flores em todo o reino vegetal. Possuem ação antioxidante e propriedades que previnem inflamações, aterosclerose, trombose, osteoporose, infecções virais, tumores e doenças cardiovasculares (7,8). A estrutura química básica dos flavonóides inclui um polifenol formado por um anel aromático e um anel heterocíclico condensados, mais um anel aromático ligado ao anel heterocíclico, onde o grau de oxidação do anel heterocíclico difere as subclasses (9). Por exemplo, para as isoflavonas o anel aromático se liga ao anel heterocíclico na posição 3, tal como mostrado na Figura 1.

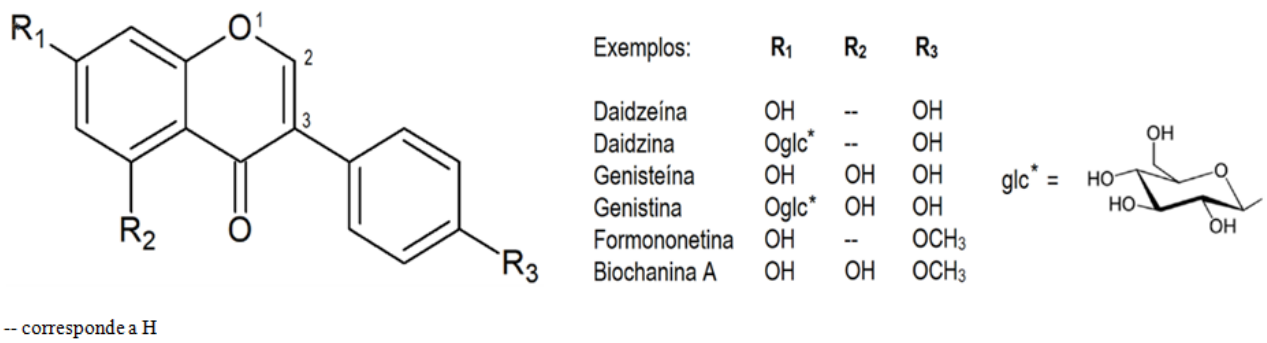

Figura 1. Estrutura básica para as isoflavonas, formada por um anel aromático e um anel heterocíclico condensados mais um anel aromático ligado ao anel heterocíclico na posição 3.

As isoflavonas formam a mais importante subclasse dos fitoestrógenos. São encontradas mais facilmente em leguminosas como soja, feijão, grão de bico e sementes de girassol, atuando na proteção contra fungos, herbívoros, radiação ultravioleta e na regulação hormonal (10). Encontradas em uma grande variedade de plantas e grãos, as isoflavonas estão frequentemente presentes em nossa dieta, possibilitando potenciais benefícios para a saúde humana, tal como diminuição do risco de câncer (11,12), prevenção de obesidade e diabetes (2), doenças cardiovasculares (13), osteoporose (14) e diminuição dos sintomas da menopausa (15).

Nos vegetais, as isoflavonas estão presentes na forma inativa como glicosídeos (ex: daidzina e genistina)

e quando ingeridos são hidrolisados no intestino e convertidos em agliconas (ex: daidzeína e genisteína), agora bioativas, e que são absorvida mais facilmente pelo organismo no intestino, conjugadas à glicuronídeos no fígado, e reabsorvidas ou excretadas na urina. As isoflavonas daidzeína, genisteína e equol são as principais isoflavonas detectadas no sangue e na urina de humanos e animais (2,16).

Os benefícios à saúde associados às isoflavonas aumentou o interesse da sociedade científica para estes compostos. Nas duas últimas décadas é notável o aumento no número de publicações e de citações relacionadas às isoflavonas. Uma rápida busca de publicações nas bases de dados, em língua inglesa, empregando o termo isoflavonas, mostra um aumento expressivo do número de publicações e citações de 1999 até 2018 (Figura 2).

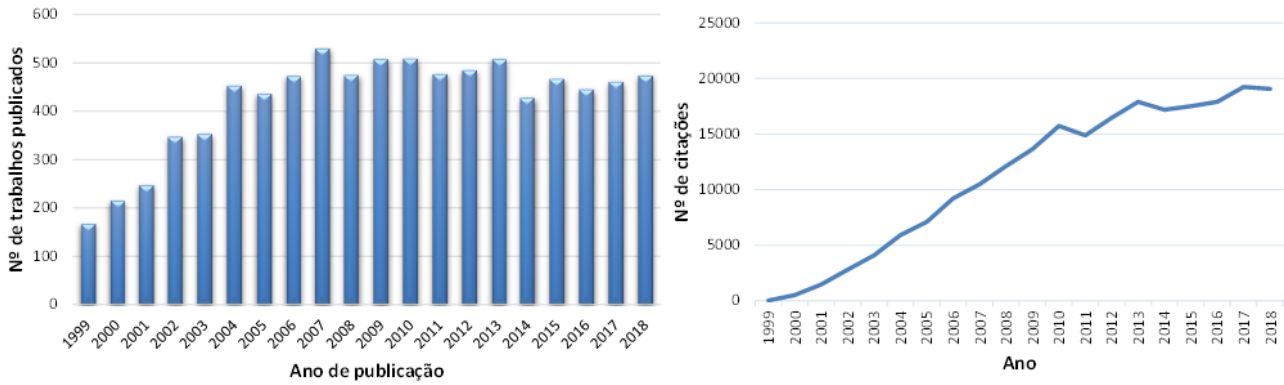

Figura 2. Distribuição do número de artigos publicados e citações sobre isoflavonas em função do tempo. Fonte dos dados: Web of Science.

O interesse pelas isoflavonas se espalha por diversas áreas, como farmacologia, nutrição, química, genética e outras. A figura 3 mostra a porcentagem da distribuição dos trabalhos publicados sobre isoflavonas nos últimos 20 anos nas áreas com maior número de trabalhos. O aumento geral do interesse pelas isoflavonas pode ser associado ao desenvolvimento de novas técnicas analíticas, tanto de separação e detecção quanto de preparo de amostras.

Este trabalho tem como objetivo proporcionar uma visão geral da literatura desde 2010 até os dias atuais envolvendo as principais técnicas analíticas empregadas em análises de isoflavonas nas mais diversas matrizes, com ênfase nas técnicas cromatográficas.

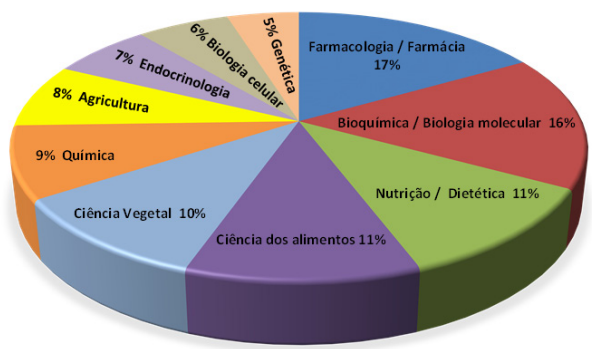

Figura 3. Distribuição dos artigos sobre isoflavonas nas diferentes áreas de aplicação. Fonte dos dados: Web of Science.

2. Preparo De Amostras Para Análise De Isoflavonas

O preparo de amostra é uma etapa importante para qualquer método analítico, pois está diretamente ligado à confiabilidade e precisão do mesmo. É comum nas técnicas mais usuais nos depararmos com etapas de secagem, homogeneização, extração, eliminação de interferentes (*clean-up*), pré-concentração, re-suspensão, etc. Levar em consideração a natureza da matriz e as propriedades químicas e físico-químicas dos analitos é um requisito básico para se obter sucesso na escolha da técnica de preparo de amostra e das condições mais adequadas para cada caso. Para análises de isoflavonas, técnicas mais convencionais como extração líquido-líquido e sólido-líquido são, de longe, as mais aplicadas. No entanto, com o desenvolvimento de novas técnicas, a busca por métodos cada vez mais rápidos, mais eficientes, confiáveis, precisos, que consumam menos tempo e solventes, específicos e seletivos, resultou em uma ampla gama de técnicas de preparo de amostra aplicáveis à extração de isoflavonas em matrizes complexas.

Podemos dividir as matrizes contendo isoflavonas em duas categorias principais, sendo elas as de origem vegetal como soja e seus produtos, e matrizes biológicas como urina, sangue e plasma. Dentro da categoria de matrizes vegetais ainda podemos ter amostras sólidas, como grãos e folhas, e amostras líquidas de produtos derivados, como leite e sucos à base de soja. As técnicas de preparo de amostra também podem ser divididas em três grupo: clássicas, intermediárias e micro técnicas. A seguir serão abordados aspectos técnicos sobre a etapa de preparo de amostra e sua aplicação em análises de isoflavonas.

2.1. Técnicas clássicas

Para amostras sólidas, como grãos, folhas e raízes a técnica de preparo de amostra mais usual é a extração com solventes orgânicos como metanol, etanol, acetonitrila, acetona e diclorometano, puros ou em soluções aquosas (17–24). É um processo relativamente simples, onde a amostra sólida é picada ou moída, para aumentar a superfície de contato e, também, facilitar a interação entre os analitos e o solvente de extração. É deixada por um determinado tempo em repouso ou agitação à uma certa temperatura (variações do método que influenciam no processo de extração) e, em alguns casos, se faz o uso de ultrassom. Variáveis como temperatura, tempo de extração, polaridade e volume do solvente e natureza da amostra afetam a eficiência do processo. Entretanto este procedimento de extração usualmente envolve muitas etapas, tais como centrifugação, diversas filtrações, secagem e resuspensão, resultando em um processo bastante demorado que pode custar dias de trabalho para o preparo de uma única amostra (18,25,26).

A extração sólido-líquido (SLE), e suas variações, permite obter-se bom rendimento (27) e, quando combinada com separação cromatográfica empregando-se HPLC com detecção UV-vis, o método apresenta precisão, linearidade, seletividade, limite de detecção (LD) por volta de 0.3 mg L^{-1} e limite de quantificação (LQ) por volta de 1 mg L^{-1} (28). Porém, o uso de grandes volumes de solvente e a lentidão do processo são desvantagens do método.

Diversas modificações podem ser feitas na extração sólido-líquido para aumentar a eficiência da extração, tal como o uso de micro-ondas. Lee e Choung (26) relataram o uso de micro-ondas para assistir a hidrólise de isoflavonas, favorecendo a análise de isoflavonas totais em alimentos, com aumento de eficiência da extração, diminuição no uso de solventes e no tempo de extração.

A extração líquido-líquido (LLE) é uma técnica clássica e versátil de preparo de amostras, sendo indicada por muitos órgãos reguladores. Porém, a LLE convencional utiliza grandes quantidades de solventes, expondo o analista e gerando muitos resíduos potencialmente tóxicos. Por envolver várias etapas, também é considerada uma operação demorada e tediosa, podendo ocasionar contaminação e perda de analitos durante o processo (29).

Esta técnica de preparo de amostra consiste basicamente em preparar uma mistura com a amostra e

um solvente, agitar, separar as fases por decantação ou centrifugação, coletar a fase de interesse e analisar. Estas etapas ainda podem ser modificadas ou complementadas com uso de ultrassom, adição de sais, uso de misturas diversificadas e etapas de filtração para melhor rendimento e diminuição de tempo, por exemplo (20,27,30–33).

Há trabalhos que se utilizam desta técnica para análises de isoflavonas em amostras comerciais de leite de soja (33), iogurtes e leite bovino (34), em urina (35) e plasma (36). A Tabela 1 mostra alguns trabalhos que utilizam LLE como etapa de preparo de amostra em análises de isoflavonas que relatam métodos com limite de detecção (LD) e limite de quantificação (LQ) na faixa de ng mL^{-1} e pg mL^{-1}, com boa recuperação, alta sensibilidade, especificidade e precisão.

Tabela 1. Trabalhos publicados utilizando LLE como técnica de preparo de amostra para análise de isoflavonas

Autores	Ano	Matriz	Técnica de análise	LD (ng mL^{-1})	LQ (ng mL^{-1})	Recuperação (%)
KUNISUE et al. (35)	2010	Urina	HPLC - MS/MS (Triplo quadrupolo)	0.15 - 0.84	----	96.7 – 115
MA et al. (36)	2012	Plasma de rato	UPLC - Q-TOF-MS	0.5	1	média de 77.21 ± 1.63
PARK; JUNG. (33)	2017	Leite de soja	HPLC - MS/MS (Triplo quadrupolo)	0.001 - 0.03	0.004 - 0.099	89.4 – 103.2
KAŠPAROVSKÁ et al. (34)	2017	Leite e derivados	HPLC–MS (TOF)	0.3 - 0.7	0.9–2.2	103 – 109
YAO et al. (37)	2018	Plasma	HPLC - MS/MS (Triplo quadrupolo)	----	0.1–0.4	85.7 – 100.2

----- Os autores não relataram nenhum resultado

2.2. Técnicas semi-clássicas

A extração em fase sólida, mais conhecida como SPE - sigla proveniente do inglês *solid-phase extration* - foi desenvolvida em 1976 com o intuito de suprir as desvantagens apresentadas pela extração líquido-líquido. Ainda hoje é a técnica de preparo de amostra mais popular, sendo aplicada em diversas áreas tais como na indústria alimentícia, farmacêutica, meio ambiente, em bioquímica e química orgânica (38,39).

São relatados vários trabalhos utilizando SPE como técnica de preparo de amostras para a análise de isoflavonas em amostras de plasma humano (40–42), urina de humanos (43,44), plasma de ratos (45) e em água (46). A Tabela 2 mostra alguns dados dos métodos empregados para análise de isoflavonas que utilizam SPE para o preparo de amostra. Os métodos apresentam alta sensibilidade, seletividade, precisão e boa recuperação. O uso de fases extratoras baseadas em octadecilsilano (C18) são as mais comuns (44), porém a fase extratora Oasis HLB é também muito empregada. Este copolímero hidrofílico-lipofílico balanceado de fase reversa, desenvolvido para extrair compostos ácidos, básicos e neutros, se mostra

muito eficiente no *clean-up* de matrizes complexas e na recuperação dos analitos. Para análises de isoflavonas, a fase extratora Oasis HLB permite a eliminação dos interferentes mais comuns, como açúcares e lipídios (47).

Tabela 2. Trabalhos publicados utilizando SPE como técnica de preparo de amostra para análise de isoflavonas.

Autores	Ano	Matriz	Técnica de análise	LD (ng mL^{-1})	LQ (ng mL^{-1})	Recuperação (%)	Fase extratora
HOSODA; FURUTA; ISHII. (40)	2010	Plasma humano	HPLC–UV (DAD)	7.9–9.4	21.1–23.4	76.6% - 109.4	Oasis HLB
SARACINO; RAGGI. (41)	2010	Plasma humano	HPLC–ESA (ESA Coulochem III)	0.008 – 0.15	0.25 – 0.5	>90.0	Oasis HLB
BARANOWSKA; MAGIERA; BARANOWSKI. (43)	2011	Urina de humano	HPLC–UV	10.7 – 78.6	32.2 – 235.9	70.14 - 99.85	Oasis HLB
LI et al.(45)	2013	Plasma de rato	HPLC–MS	0.17	0.51	89.36±3.49 – 98.90±5.21	Zorbax SB-C18
RODRIGUEZ-MORATO et al. (42)	2015	Plasma humano	HPLC – MS/MS (Triplo quadrupolo)	0.6 – 1	5	79 – 86.2	Oasis MAX
REDRUELLO et al. (44)	2015	Urina de humano	UHPLC–PDA–FLR	2.93 – 15.17nM*	2 – 12 nM*	70 –114	C18
PROCHÁZKOVÁ et al. (46)	2017	Água	UHPLC-MS/MS	-----	$3\ 10^{-6}$ – $3\ 10^{-3}$	< 50	Oasis HLB

*Concentração em nanomolar

----- Os autores não relataram nenhum resultado

Em matrizes biológicas como sangue e urina, as isoflavonas são encontradas na forma glicosilada, as quais são hidrolisadas por micro-organismos formando agliconas as quais, por sua vez, são absorvidas e convertidas em seus conjugados glucuronídeo ou sulfato como metabólitos de fase II. Por serem compostos ácidos altamente polares há baixa recuperação dos metabólitos das isoflavonas pelas fases convencionais empregadas em SPE, sendo um revés nos casos de análise simultânea de isoflavonas e seus metabólitos. Este fato contribui para a popularidade da fase Oasis HLB em análise de isoflavonas (40).

Um enfoque utilizado no preparo de amostra comum na indústria, para análise de isoflavonas em alimentos, é denominada QuEChERS. A sigla é proveniente das iniciais das palavras *quick* (rápido), *easy* (fácil), *cheap* (barato), *effective* (eficaz), *rugged* (robusto) e *safe* (seguro).

Originalmente, o procedimento consistia em uma extração com acetonitrila seguida de partição com sulfato de magnésio, isoladamente ou em combinação com outros sais, geralmente NaCl, seguido da técnica d-SPE (*dispersive solid-phase extraction*) com o objetivo de eliminar interferentes. Esta técnica foi desenvolvida para análises de resíduos de pesticidas em frutas e vegetais e, posteriormente, passou por várias modificações. Atualmente a técnica é aplicada em análises de pesticidas, fármacos, micotoxinas, PAHs (48) e também isoflavonas.

Delgado-Zamarreño e colaboradores relataram o desenvolvimento de um método analítico para determinação de isoflavonas em legumes utilizando a técnica QuEChERS para o preparo da amostra na determinação de oito isoflavonas. O método apresentou LD na faixa de 0.7 a 1.5 ng mL^{-1}, recuperação de 72 a 119% e RSD menor que 25% para a precisão inter-dia. Os autores concluíram que o método é capaz de identificar isoflavonas livres e conjugadas, é preciso, seletivo e não consome muito tempo (49).

Merlanti e colaboradores relatam o desenvolvimento de um método analítico para determinação de isoflavonas em peixes (músculo de truta) expostos a dieta baseada em grãos de soja e derivados de soja, utilizando QuEChERS como técnica de preparo de amostra e LC – MS como técnica de separação e detecção. O método apresentou LD na faixa de 0.003 a 0.098 µg kg^{-1} e LQ de 0.2 µg kg^{-1} , recuperação de 78.77 a 109.93% e RSD de 1.10 a 12.67% para a precisão intra-dia e 2.80 a 14.46% inter-dia, satisfazendo os requisitos adotados como referência no trabalho. O método pode ser classificado como simples, rápido, eficiente, reprodutível e preciso, tal como sugere o nome da técnica de preparo de amostra, além de fornecer um menor LQ do que os procedimentos propostos na literatura atualmente (50).

Benedetti e colaboradores compararam a técnica QuEChERS com SPE na análise de isoflavonas em hambúrgueres de soja; obtiveram maior recuperação, menor RSD e menor efeito de matriz utilizando QuEChERS, concluindo que esta técnica é mais adequada que SPE para este tipo de amostra (47).

A técnica MSPD "*matrix solid-phase dispersion*" pode ser utilizada no preparo de amostras biológicas sólidas, semi-sólidas ou líquidas altamente viscosas, permitindo que seja realizada a extração e *clean-up* em uma única etapa para analitos de diversas classes, em diferentes matrizes naturais (51,52).

O procedimento MSPD é muito parecido com a SPE. A matriz é previamente tratada, misturada e homogeneizada com a fase estacionária e depois empacotada em um cartucho de SPE como uma coluna. Então um solvente adequado realiza a eluição dos analitos. Outras colunas podem ser adicionadas ao procedimento para auxiliar na separação dos analitos ou no *clean-up* da amostra (52 ,53). As fases estacionárias mais comuns são C18, sílica gel, forisil (silicato de magnésio) e alumina.

Em análise de isoflavonas, MSPD foi utilizada por Visnevschi Necrasov e colaboradores no desenvolvimento de um método analítico para análise de isoflavonas em vegetais, tendo MSPD como técnica de preparo de amostra e análise por HPLC-DAD. O método apresentou LD na faixa de 11 a 171 ng mL^{-1} e LQ de 37 a 569 ng mL^{-1}, recuperação de 82 a 104% e RSD de 4 a 9%. O método possibilita a extração e *clean-up* da amostra em uma única etapa, requer pequenas quantidades de amostra, baixo consumo de solvente e tempo de análise, sendo uma boa escolha para análises de rotina (54).

Xu e colaboradores relatam uma modificação na MSPD para análise de isoflavonas, onde foi utilizado titânia (óxido de titânio) devido a sua propriedade de troca iônica, propondo que a interação entre titânia e açúcar, que é um interferente, libera H^+ e é favorecida em pH alto (55).

2.3. Micro técnicas

O desenvolvimento de novas técnicas de preparo de amostra nos leva a percepção de que se busca métodos cada vez mais rápidos, confiáveis, precisos e que consumam menos tempo e solventes, porém não é uma tarefa simples em se tratando de matrizes complexas que envolvem uma gama muito ampla de componentes.

Em 1990 Arthur e Pawilszyn propuseram a micro extração em fase sólida, conhecida como SPME do inglês "*solid-phase microextraction*", que se tornou muito popular. É uma técnica baseada no equilíbrio de partição do analito entre a matriz e a fase estacionária, de fácil operação, rápida e pequeno ou nenhum consumo de solvente. Entretanto, deve-se sempre avaliar a natureza da

matriz para se obter um bom desempenho da SPME no preparo de amostras (56,57).

A SPME é aplicada em análises de isoflavonas em bebidas à base de soja, onde foram utilizadas fibras comerciais com fase estacionária contendo polidimetilsiloxano (PDMS) recomendado para compostos apolares, polidimetilsiloxano – divinilbenzeno (PDMS-DVB) que é recomendado para uso geral e poliacrilato (PA) recomendado para extrair analitos muito polares de amostras polares. Todas se mostraram capazes de extrair as isoflavonas da matriz, porém a fase PDMS-DVB apresentou maior eficiência em comparação com as outras em estudo (58).

A escolha do tipo do material que reveste a fibra é importante para a SPME, porém a espessura do revestimento também deve ser avaliada, pois é um fator que altera o tempo de equilíbrio e a sensibilidade do método. Revestimentos de maior espessura necessitam de um tempo maior mas, por outro lado, aumentam a recuperação (57,58).

Outros fatores, além da fase estacionária empregada, podem influenciar na eficiência da extração de isoflavonas, como a temperatura, que pode diminuir o tempo necessário para que seja atingido o equilíbrio de partição do analito entre a fase estacionária e a matriz. Entretanto, o pH possui papel relevante, uma vez que em meio ácido há um aumento na extração das isoflavonas devido à baixa solubilidade destas no meio. A força iônica do meio pode ser alterada pelo feito "*salting-out*" que, como o pH, altera a solubilidade das isoflavonas na matriz (58).

Uma aplicação interessante da SPME para análise de isoflavonas é relatada por Calvello e colaboradores (59), onde é investigado o potencial anti-inflamatório de leite bovino e leite de soja e das isoflavonas equol, daidzeína e genisteína, utilizando um modelo in vitro de células epiteliais intestinais. O autor relata que a aplicação da SPME como técnica de preparo de amostra produziu resultados comparáveis a trabalhos anteriores.

A técnica de micro extração em sorbente empacotado, conhecida como MEPS sigla proveniente do inglês "*microextraction in packet sorbent*" também é aplicada em análises de isoflavonas em matrizes complexas. Esta técnica foi desenvolvida por Abdel-Rehim no ano de 2004 como uma proposta de miniaturização da SPE (60). Como na sua predecessora, o uso da MEPS para preparo de amostras em análises de isoflavonas tem alguns parâmetros a serem estudados como a fase estacionária empregada, etapa de *clean-up* e melhor solvente de eluição. É uma técnica baseada no equilíbrio de partição do analito entre a matriz e a fase estacionária tal como a SPME, e possui uma ampla gama de fases estacionárias como a SPE, podendo até ser produzidas fases estacionárias "*lab-made*" diferenciadas. Gonçalves e colaboradores (61) relatam o uso de MEPS como técnica de preparo de amostra para análises de polifenóis, dentre as quais duas isoflavonas, em vinho. Foram avaliadas fases estacionárias compostas por C2, C8, C18, sílica e um mix de 80% de C8 e 20% de SCX, onde a fase de C8 apresentou melhores resultados. O processo de extração foi classificado como simples, eficiente, rápido, requer pouco volume de solventes e aplicável para pequenos volumes de amostra.

2.4. Técnicas automatizadas

Na atualidade, trabalhos completamente automatizados para a análise de isoflavonas ainda são escassos. Entretanto, vem se desenvolvendo estratégias com o objetivo de num futuro próximo conseguir realizar estudos "on-line". Foi relatado, por exemplo, o desenvolvimento de um dispositivo em formato de microchip de separação de microfluidos acoplado a MS, o qual incrementa notoriamente as bioanálises por eletroforese capilar e por LC. Chang e colaboradores utilizaram o microchip com LC-MS para determinar isoflavonas em soja, fazendo a extração assistida por ultrassom. O extrato foi filtrado e carregado num cartucho HLB de SPE, identificando isoflavonas tais como geisteina na semente de soja, pela primeira vez (62). Comparando com o método de análise convencional HPLC-MS, o

procedimento proposto foi mais rápido e a quantidade de analitos detectados no primeiro teste foi maior. O método foi aplicado em 6 diferentes amostras reais, observando-se que três isoflavonas representam quase 80% do conteúdo total de flavonoides, sendo a quantidade maior na soja colhida na primavera do que a colhida no verão.

Na literatura, ainda não foram encontrados muitos trabalhos automatizados de quantificação de isoflavonas em alimentos usando eletroforese capilar acoplada a espectrometria de massas (CE-MS), mas estão sendo desenvolvidos métodos automatizados para estas análises, como o relatado por Bustamante-Rangel e colaboradores (63).

3. Separação das isoflavonas por técnicas cromatográficas e eletroforéticas

As isoflavonas foram classicamente separadas por cromatografia de camada delgada usando como principal sorvente poliamida (64). Nos últimos anos, a cromatografia liquida em coluna vem se destacando com uso, por exemplo, de sorventes como C8 e C18 em fase reversa, sendo a separação determinada pela solubilidade dos analitos em água, pois na fase reversa a separação ocorre segundo as interações hidrofóbicas de cada uma delas (64). Além disso, outros fatores também são destacáveis para obter-se uma boa separação cromatográfica, como a afinidade com a fase estacionária, a composição da fase móvel, o gradiente de eluição, a temperatura da coluna, entre outros (65).

Trabalhos vem sendo relatados há bastante tempo em matrizes alimentares (66), ressaltando-se alimentos como soja (67) na qual realiza-se um processamento que inclui evaporação, cocção, torrefação e fermentação microbiana para a quebra da ligação glicosídea permitindo a formação das agliconas que, junto com os glicosídeos, vão ser separados facilmente em uma coluna de fase reversa (68). Neste caso empregar uma eluição isocrática não é suficiente; o uso de um gradiente é mais aconselhável, começando geralmente com uma porcentagem entre 8 e 15% v/v de solvente orgânico (acetonitrila, metanol entre outros), sendo as agliconas eluidas nos conteúdos mais altos de solvente orgânico. Fiechter e colaboradores, obtiveram uma separação em 3 minutos, sendo o tempo total entre as injeções de 8 minutos, usando um gradiente com ácido fórmico 0,3% e metanol (69).

Outra das atuais modificações que tem melhorado a separação das isoflavonas é a aplicação de colunas de diâmetro interno menor, o que faz que o tamanho de partícula da fase estacionária também seja menor, usualmente inferior a 2 µm, diminuindo os tempos de analises e incrementando a efetividade da separação. Isto vem sendo relatado em pesquisas em alimentos (70,71) ao comparar-se essas colunas com as clássicas que empregam como fases estacionarias C18. Dentre essas colunas miniaturizadas, vem se destacando as colunas monolíticas, as quais são relativamente simples de obter, mostrando bons resultados na separação das isoflavonas (72).

Outro conjunto de técnicas de separação, o qual vem aumentando o número de publicações desde a década passada para analisar matrizes naturais (73), é conhecido como técnicas de eletromigração capilar, sendo sua principal vantagem a facilidade de acoplamento dos detectores eletroquímicos (DE) sensíveis, pois se trabalha com sistemas de eletrólitos muito simples (geralmente tampão de borato a concentrações milimolar) (74). A desvantagem desta técnica é a limitação que apresenta devido à presença de modificadores orgânicos nas fases móveis.

O termo "técnicas de eletromigração capilar" faz referência a um grupo de técnicas de separação que utilizam uma corrente elétrica para obter variações em seus princípios de separação, e inclui (i) eletroforese capilar de zona (CZE); (ii) eletroforese capilar em gel (CGE); (iii) cromatografia eletro cinética micelar (MECK); (iv) eletrocromatografia capilar (CEC); (v) focalização isoelétrica capilar (CIF); e (vi) isotacoforese capilar (CIP).

Os primeiros relatos de separação de isoflavonas por CZE ocorreram em 1994, sendo esta técnica a mais utilizada para estudo destes analitos (75). Bons resultados foram obtidos no estudo de soja, lupino e ervilhas verdes quando comparados com o uso de HPLC utilizando como detector diodos UV-Vis-diode (DAD). Por outro lado, a HPLC obtém melhor seletividade.

Estudos recentes tem descrito combinações de CZE com detectores eletroquímicos para análise de diversas formas glicosiladas de glicitina, daidzina e genistina, e agliconas como formononetina, biocanina A, glicitina, daidzeína e genisteína em amostras comerciais (76).

A MECK também tem sido utilizada para separar compostos polifenólicos pouco solúveis em água. A ononina, daidzina, genistina, biochanina A, formononetina, puerarina, genisteína e daidzeína foram separadas da pueraria (77) usando MEKC em combinação com um detector de UV. Foram comparadas várias fases estacionarias, incluindo surfactante aniônico, dodecilsulfato de sódio (SDS); surfactante catiônico, brometo de hexadeciltrimetilamonio; surfactante neutro polioxietileno sorbitato monolaurate (Tween 20); um tensoativo do tipo líquido iónico, tetrafluoroborato de 1-dodecil-3-metilimidazolio ($C_{12}MIMBF_4$); aditivo (modificador), tetrafluoroborato de 1-butil-3-metilimidazolio ($BMImBF_4$); e micelas mistas de SDS + Tween 20 e $C_{12}MIMBF_4$ + Tween 20. Se demostrou que SDS com $BMImBF_4$ e SDS + Tween 20, tivessem maior eficiência de separação nos oito compostos estudados em tempos inferiores a 10 minutos. A diferença entre CZE e MECK, é que esta última permite a separação efetiva das agliconas hidrófobas que são pouco solúveis nos tampões da CZE (78).

A Tabela 3 mostra alguns trabalhos recentes que usam diversas técnicas de separação como as descritas no texto, para analises de isoflavonas, incluindo os limites de detecção (LD) obtidos com boa recuperação, alta sensibilidade, especificidade e precisão.

4. Identificação e quantificação das isoflavonas via espectrometria de massas

Nas últimas décadas, a espectrometria de massas tem demostrado ser uma via muito efetiva na identificação de compostos em diversas matrizes, principalmente amostras complexas. Por sua alta sensibilidade, especificidade e acoplamento simples com a cromatografia, a espectrometria de massas tem sido escolhida como uma das principais técnicas de identificação pelos analistas, usando para a análise de isoflavonas principalmente os métodos descritos a seguir.

4.1. Cromatografia de gases – espectrometria de massas (GC-MS)

Os compostos voláteis geralmente são analisados por esta técnica que mistura as destacáveis propriedades da separação por GC com a sensibilidade e seletividade da MS, a qual depende da fonte de íons e do modo de obter esses íons. Devido à excessiva fragmentação que se pode apresentar durante a análise, especialmente empregando a ionização com elétrons, os íons moleculares podem estar ausentes no espectro obtido. Neste caso o uso de baixa voltagem de ionização, ou ionização química, é recomendado para verificar-se o íon molecular. Na análise de amostras complexas é muito comum o uso de monitoramento de íons selecionados (SIM) para obter um espectro mais específico (88). A desvantagem é que para bioflavonoides precisa-se de derivatização dos compostos não voláteis, sendo ainda pior para os metabolitos biológicos ou compostos termicamente lábeis dos flavonoides (89).

4.2. Bombardeamento com átomos rápidos (FAB) e espectrometria de massa de íons secundários líquidos (LSIMS)

Para evitar a derivatização dos compostos, pode-se empregar um destes dois métodos. No FAB, o impacto de uma partícula energética inicia a evaporação da amostra e os processos de ionização dos analitos,

Tabela 3. Aplicações de diversas técnicas de separação para a análise de isoflavonas em diferentes matrizes.

Autores	Ano	Matriz	Técnica de análise	LD	LQ	Recuperação %
FIECHTER et al. (79)	2013	Soja	UV-UPLC	0.5 – 0.9 mg g^{-1}	1.8 – 3.1 mg g^{-1}	90±2 - 99± 3
BUSTAMANTE et al. (80)	2013	Alimentos à base de soja	QuEChERS CE-ESI-MS	0.21 – 2.0 µg L^{-1}	0.69 – 6.6 µg L^{-1}	92- 102
MARTÍ et al. (81)	2017	Tomate	MECK HPLC	0.8 – 3.8 mg kg^{-1}	2.6 – 12.6 mg kg^{-1}	77 - 106
SONG et al. (82)	2013	Flores *Trollius* comerciais	HPLC	0.01 – 0.08 µg mL^{-1}	0.03 - 0.29 µg mL^{-1}	95.8 - 105.1
GANZERA et al. (83)	2015	Soja	SFC	0.03 – 0.21 µg mL^{-1}	0.11 - 0.65 µg mL^{-1}	97.6 - 102.4
VANDERMOLEN et al. (84)	2013	Suco De Toranja	UPLC	0.78 – 69 µM	2.6 - 230 µM	-----
BARFI et al. (85)	2013	Sucos de frutas cítricas	SM-USA-MSPD HPLC	23.3 – 46.8 ng mL^{-1}	74.8 - 141.5 ng mL^{-1}	84.6–101.5
BADJAH et al. (86)	2014	Diversos méis iemenitas	HPLC	0.018 – 0.14 µg mL^{-1}	0.06 - 0.46 µg mL^{-1}	< 2.5
SHIM et al. (87)	2015	Soja, feijão vermelho, feijão preto e pasta de soja	UHPLC/PDA	0.03 – 0.33 mg kg^{-1}	0.10 - 0.33 mg kg^{-1}	85.6 ± 5.7 - 113.5 ± 5.7

----- Os autores não relataram nenhum resultado

evitando ter que volatilizar termicamente a amostra. No LSIMS, a matriz tem que ser liquida (usa-se glicerol, tioglicerol, entre outros que apresentam alta temperatura de ebulição), e a evaporação e ionização dos analitos é causado por um feixe primário de íons Césio. Estes dois métodos de ionização produzem íons fragmento em menor quantidade, sendo adequados para analisar compostos iônicos e termolábeis (90).

4.3. Ionização por electrospray (ESI)-MS e ionização química à pressão atmosférica (APCI)-MS

Outra técnica empregadas para evitar-se a realização de processos de derivatização prévios é a chamada ionização por electrospray (ESI)-MS, na qual se geram partículas altamente carregadas que produzem a expulsão dos íons durante o processo de evaporação. Um campo elétrico é obtido no nebulizador pela aplicação de alta voltagem e pela proximidade do contra eletrodo, sendo os íons atraídos pelo campo elétrico para as partículas geradas, segundo a polaridade escolhida, sendo as interfaces ESI frequentemente empregadas com espectrômetros de massas tendo como analisador de massas um quadrupolo (91).

Outra forma de ionização empregada no acoplamento LC-MS é a chamada ionização química à pressão atmosférica (APCI), a qual envolve reações

íon-molécula para que os íon se encontrem na fase gasosa. Estas duas tecnologias (ESI e APCI) estão sendo utilizadas para estudar uma variedade de flavonoides como as isoflavonas, uma vez que a ESI apresenta uma maior sensibilidade que as técnicas de FAB e LSIMS; além disso a ESI tem uma melhor relação sinal-ruído, porque se obtém um quantidade reduzida de íons ficando na faixa inferior aos 300 Da, região importante para aqueles analitos (92).

Parets e colaboradores relataram recentemente a obtenção de uma melhora na eficiência da ionização usando a técnica de fotoionização (93).

Uma das desvantagens das técnicas ESI e APCI é que não são compatíveis com o uso de modificadores comuns na fase móvel no HPLC como ácido trifluoroacético ou fosfato de sódio, pois interferem no processo de ionização. Assim, é necessário trabalhar-se com modificadores tipo ácido fórmico ou acetato de amônio no caso dos tampões de fosfato. Embora ambas técnicas possam ser empregadas no modo de íons positivos ou negativos, as isoflavonas geralmente são analisadas no modo negativo (94).

4.4. Eletroforese capilar - espectrometria de massa (CE-MS)

A eletroforeses capilar de zona (CZE) foi descrita pela primeira vez em 1981 (95), e é considerada relativamente nova em relação à GC e HPLC. A CE baseia-se na diferença das mobilidades eletroforéticas dos analitos em solução, carregados num campo elétrico em capilares de pequenos diâmetros – comumente entre 50 e 100 µm - que permitem uma rápida separação e alta resolução. O volume empregado, da ordem de nanolitros, permite obter excelentes limites de detecção empregando-se um espectrômetro de massas como detector. Esta técnica vem sendo usada em diferentes modos, como descrito anteriormente (76,77). Como as isoflavonas são ácidos fracos, usa-se tampões alcalinos para garantir que o resíduo fenólico é carregado por separação eletroforética, pois a estrutura e composição do tampão influi no comportamento eletroforético das amostra a analisar (96).

Ao comparar o fluxo de trabalho do CE (geralmente inferior a 1µL min^{-1}) com o fluxo do HPLC convencional (1 mL min^{-1}), observa-se que é mais adequado trabalhar com CE quando usa-se um MS como detector, sendo possível introduzir o efluente do CE no MS através da interfase ESI, sem divisão do fluxo. Assim, é possível manter-se a eficiência e a boa resolução na separação. A primeira interfase CE-MS foi relatada no ano 1987 por Smith e colaboradores (97), sendo desenvolvidas muitas outras interfaces ao longo do tempo (98). Zhao e colaboradores (99) apresentaram o avanço da CE e CEC nas análises fitoquímicas durante 2012 e 2013, assim como para amostras biomédicas, farmacêuticas, ambientais e de alimentos, contendo analitos como isoflavonas.

Ainda hoje a conexão entre CE e MS é considerada difícil; as altas concentrações de isoflavonas nas amostras discutidas faz com que as análises apresentem sensibilidade adequada, aumentando assim a importância do desenvolvimento das técnicas de CE-MS para estudos em matrizes fisiológicas como tecidos, sangue e urina.

4.5. Ionização e desorção assistida pela matriz, acoplada a espectrometria de massas em tempo-de-voo (MALDI-TOF-MS)

A ionização e desorção por laser assistida pela matriz, MALDI, foi estudada pela primeira vez por Karas e colaboradores (100), com o objetivo de converter amostras sólidas em amostras gasosas para serem detectadas por MS. A técnica MALDI-TOF-MS apresenta como vantagem, ao ser comparada com as outras apresentadas, a alta velocidade de analise a boa sensibilidade; a boa tolerância com contaminantes; e a produção, principalmente, de íons com carga única, o que não acontece com ESI-MS (101). Por isso, é possível determinar, simultaneamente, compostos presentes em amostras complexas com alto e baixo peso molecular.

Embora a MALDI-TOF-MS seja usada principalmente como ferramenta para análises de biomoléculas, recentemente vem sendo empregada em analises das isoflavonas (102) em matrizes alimentícias.

4.6. Técnicas combinadas e espectrometria de massas empregando transformada de Fourier e ressonância ciclotrônica de íons (FT-ICR-MS)

Existe uma grande variedade de técnicas baseadas na espectrometria de massas, as quais podem ser empregadas no estudo das isoflavonas, como vem sendo relatado nesta revisão. Na atualidade vem-se estudando a combinação destas técnicas com o objetivo de aproveitar as vantagens que cada uma delas pode oferecer, como é o caso da LC-ESI-MS, na qual o analisador quadrupolo pode ser trocado por um analisador de íons - "*ion trap*") ou um de analisador do tipo tempo-de-voo (ToF). O *Íon Trap* tem a vantagem de fazer a fragmentação sequencial primeiro do íon molecular principal e depois dos íons secundários, o qual é muito útil na análise de flavonóides isômeros como, por exemplo, glucósidos de genisteína, uma isoflavona, a qual apresenta a mesma massa molecular e um comportamento cromatográfico muito similar à apigenina, uma flavona (103).

Na proteômica, frequentemente usa-se um instrumento hibrido TOF-TOF, onde íons presentes no primeiro analisador TOF são selecionados temporalmente numa região. Os íons então vão colidir com um gás (por exemplo argônio) para serem de novo acelerados para o segundo analisador TOF. A técnica é considerada rápida e permite analisar muitas amostras em pouco tempo (104). Porém, os baixos valores m/z dos bioflavonoides e seus metabolitos são um impedimento para obter uma ótima separação como já foi relatado, mas continua-se tentando obter avanços no uso de instrumentos híbridos para análises de isoflavonas.

Em FT-ICR-MS, os íons são inseridos em uma cela cilíndrica localizada no centro do campo magnético de um ímã supercondutor, sendo a resolução função do campo magnético. Os íons ficam ao redor do centro do campo magnético em orbitas muito próximos. A radiação do cíclotron incrementa a energia dos íons, levando eles a criarem orbitas maiores. Quando a radiação é removida, os íons excitados voltam a seus estados iniciais, criando assim um sinal em queda livre (FID), no tempo, similar a um experimento RMN. Este sinal é rapidamente processado pela transformada de Fourier, na frequência obtendo-se, assim, um espectro de massas.

Esta técnica tem demostrado ser muito promissora na análise de peptídeos em análises proteômicas, mas ainda não é muito explorada na análise de isoflavonas, embora já existam publicações onde é usado (105), pois sua capacidade para determinar as massas com precisão, garante a identificação correta dos metabólitos presentes nas matrizes em estudo.

A Tabela 4 resume diferentes técnicas de identificação e quantificação de isoflavonas, via espectrometria de massas, em diferentes tipos de matrizes.

5. Conclusões

Isoflavonas formam uma subclasse muito importante de fitoestrógenos e estão associadas à prevenção de diversas doenças. Metabólitos secundários de plantas, são encontradas em todo o reino vegetal e constantemente presentes em nossa dieta, seja por consumo direto de vegetais ou de produtos industrializados, o que causa uma constante vigilância sobre a bioatividade e propriedades nutricionais destes compostos.

Na última década, o crescente interesse pelas isoflavonas resultou em um aumento no número de pesquisas científicas relacionadas ao tema. Desde 2010, os trabalhos relatam análises de isoflavonas nas mais variadas matrizes, como soja, produtos industrializados a base de soja, leite bovino, urina e plasma humano, utilizando diversas técnicas de separação e detecção, como HPLC – UV , UHPLC – MS/MS, CE – UV e GC – MS/MS.

Mesmo com o avanço das técnicas de preparo de amostra, onde atualmente temos micro técnicas bem desenvolvidas e até mesmo métodos *on-line* de preparo de amostra, o uso de técnicas clássicas como as extrações com solvente ainda são muito comuns, principalmente nos trabalhos que visam apenas aplicação de um método já desenvolvido. Técnicas como a SPME e MEPS, as quais são mais recentes, são empregadas em análises de

Tabela 4. Técnicas de identificação e quantificação das isoflavonas em diferentes tipos de matrizes empregando a espectrometria de massas.

Autores	Ano	Matriz	Técnica de análise	LD	LQ	Recuperação (%)
SCHMIDT et al. (88)	2013	Urina	GC-MS/MS	0.1 - 0.6 μg L^{-1}	0.3 – 2.0 μg L^{-1}	40 – 111
NAKATA et al. (90)	2018	Plantas à base de soja	IT-TOF-MS	-------	-------	-------
YAN et al. (91)	2014	Plasma de rato	LC–ESI-MS/MS	-------	1.7 - 17.6 ng mL^{-1}	72.9 ± 9.8 – 117.4 ± 7.6
LEI et al. (92)	2015	Produto de uma planta natural	UHPLC-MS	-------	-------	-------
SCHMIDT et al. (94)	2019	Resveratrol	ESI–HCD (MS)	-------	-------	-------
PEREZ-MARTIN et al. (96)		Legumes	CE-MS LC-MS/MS	0.20 - 1.8 μg L^{-1}	-------	80 - 120
SAKAMOTO et al. (102)	2015	Soja	MALDI-TOF-MS	-------	-------	99.9 e 108.8
GIMÉNEZ-CASSINA et al. (105)	2014	Própolis vermelha	ESI(-)-FT-ICR–MS	-------	-------	-------

----- Os autores não relataram nenhum resultado

isoflavonas em trabalhos que visam o desenvolvimento de métodos analíticos e são mais facilmente encontrados em revistas científicas da área de cromatografia.

O uso de técnicas eletroforéticas, como a CZE e MEKC, é muito comum na separação de isoflavonas. Nos últimos anos a diminuição do diâmetro interno das colunas analíticas, destacando o uso de colunas capilares monolíticas, tem resultado em melhora na efetividade das separações e diminuição do tempo de análises. Dentre os detectores destacam-se o uso de espectrômetros de massas assim como técnicas combinadas de cromatografia líquida, gasosa e eletroforese à espectrometria de massas.

Espera-se, em um futuro próximo, o aumento do uso de técnicas analíticas totalmente automatizadas para a análise "on-line" de isoflavonas, incluindo o preparo das amostras, separação e a detecção por espectrometria de massas, em uma única etapa.

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Referências

[1] Wu Q, Wang M, Simon JE. Analytical methods to determine phytoestrogenic compounds. J Chromatogr B Anal Technol Biomed Life Sci. 2004 Dec;812(1–2 SPEC. ISS.):325–55. Available from: https://doi.org/10.1016/j.jchromb.2004.08.008

[2] Bhathena SJ, Velasquez MT. Beneficial role of dietary phytoestrogens in obesity and diabetes. Am J Clin Nutr. 2002 Dec;76(6):1191–201. Available from: https://doi.org/10.1093/ajcn/76.6.1191

[3] López-Biedma A, Sánchez-Quesada C, Delgado-Rodríguez M, Gaforio JJ. The biological activities of natural lignans from olives and virgin olive oils: A review. J Funct Foods. 2016 Oct;26:36–47. Available from: https://doi.org/10.1016/j.jff.2016.07.005

[4] Yu J, Wu Q, Qiao S, Yu Z, Jin N, Yu B. Simultaneous determination of phytoestrogens and key metabolites in breast cancer patients' urine by liquid chromatography-tandem mass spectrometry. J Pharm Biomed Anal. 2009 Dec;50(5):939–46. Available from: https://doi.org/10.1016/j.jpba.2009.06.032

[5] Matsumura A, Ghosh A, Pope GS, Darbre PD. Comparative study of oestrogenic properties of eight phytoestrogens in MCF7 human breast cancer cells. J Steroid Biochem Mol Biol. 2005 Apr;94(5):431–43. Available from: https://doi.org/10.1016/j.jsbmb.2004.12.041

[6] Vejdovszky K, Schmidt V, Warth B, Marko D. Combinatory estrogenic effects between the isoflavone genistein and the mycotoxins zearalenone and alternariol in vitro. Mol Nutr Food Res. 2017 Oct;61(3):1–12. Available from: https://doi.org/10.1002/mnfr.201600526

[7] Nijveldt RJ, Nood Ev, van Hoorn DEC, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: a review of probable mechanisms of action. Am J Clin Nutr. 2001 Oct;74:418–25. Available from: https://doi.org/10.1093/ajcn/74.4.418

[8] Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996 Nov;20(7):933–56. Available from: https://doi.org/10.1016/0891-5849(95)02227-9

[9] Pietta PG. Flavonoids as antioxidants. J Nat Prod. 2000 May;63(7):1035–42. Available from: https://pubs.acs.org/doi/abs/10.1021/np9904509

[10] Ren MQ, Kuhn G, Wegner J, Chen J. Isoflavones, substances with multi-biological and clinical properties. Eur J Nutr. 2001 Aug;40(4):135–46. Available from: https://doi.org/10.1007/PL00007388

[11] Wu AH, Ziegler RG, Horn-Ross PL, Nomura AM, West DW, Kolonel LN, Rosenthal JF, Hoover RN, Pike MC. Tofu and risk of breast cancer in Asian-Americans. Cancer Epidemiol Biomarkers Prev [Internet]. 1996 Nov;5(11):901–6. Available from: http://cebp.aacrjournals.org/content/5/11/901.short

[12] Adlercreutz H. Phytoestrogens and breast cancer. J Steroid Biochem Mol Biol. 2002 Dec;83(1–5):113–8. Available from: https://doi.org/10.1016/S0960-0760(02)00273-X

[13] Anthony MS, Clarkson TB, Williams JK. Effects of soy isoflavones on atherosclerosis: Potential mechanisms. Am J Clin Nutr. 1998 Dec;68(6 SUPPL.):1390–3. Available from: https://doi.org/10.1093/ajcn/68.6.1390S

[14] Zhi CD, Lowik C. Dose-dependent effects of phytoestrogens on bone. Trends Endocrinol Metab. 2005 Jul;16(5):207–13. Available from: https://doi.org/10.1016/j.tem.2005.05.001

[15] Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. Obstet Gynecol. 1996 May;87(5):897–904. Available from: https://www.sciencedirect.com/science/article/pii/S0029784496804613

[16] Tsunoda N, Pomeroy S, Nestel P. Absorption in Humans of Isoflavones from Soy and Red Clover Is Similar. J Nutr. 2018 Aug;132(8):2199–201. Available from: https://doi.org/10.1093/jn/132.8.2199

[17] Manchón N, D'Arrigo M, García-Lafuente A, Guillamón E, Villares A, Ramos A, Martínez, JA, Rostagno, MA. Fast analysis of isoflavones by high-performance liquid chromatography using a column packed with fused-core particles. Talanta. 2010 Oct;82(5):1986–94. Available from: https://doi.org/10.1016/j.talanta.2010.08.050

[18] Lee JH, Choung MG. Determination of curcuminoid colouring principles in commercial foods by HPLC. Food Chem. 2011 Feb;124(3):1217–22. Available from: https://doi.org/10.1016/j.foodchem.2010.07.049

[19] Yanaka K, Takebayashi J, Matsumoto T, Ishimi Y. Determination of 15 isoflavone isomers in soy foods and supplements by high-performance liquid chromatography. J Agric Food Chem. 2012 Mar;60(16):4012–6. Available from: https://pubs.acs.org/doi/abs/10.1021/jf205154x

[20] Jankowiak L, Kantzas N, Boom R, Van Der Goot AJ. Isoflavone extraction from okara using water as extractant. Food Chem. 2014 Oct;160:371–8. Available from: https://doi.org/10.1016/j.foodchem.2014.03.082

[21] Desfontaine V, Guillarme D, Francotte E, Nováková L. Supercritical fluid chromatography in pharmaceutical analysis. J Pharm Biomed Anal [Internet]. 2015 Sep;113:56–71. Available from: https://doi.org/10.1016/j.jpba.2015.03.007

[22] Yuk HJ, Song YH, Curtis-Long MJ, Kim DW, Woo SG, Lee YB, Uddin Z, Kim CY, Park KH. Ethylene Induced a High Accumulation of Dietary Isoflavones and Expression of Isoflavonoid Biosynthetic Genes in Soybean (Glycine max) Leaves. J Agric Food Chem. 2016 Sep;64(39):7315–24. Available from: https://pubs.acs.org/doi/abs/10.1021/acs.jafc.6b02543

[23] Yilmaz A, Rudolph HL, Hurst JJ, Wood TD. High-Throughput Metabolic Profiling of Soybean Leaves by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Anal Chem. 2016 Dec;88(2):1188–94. Available from: https://pubs.acs.org/doi/abs/10.1021/acs.analchem.5b03340

[24] Zhang M, Sun J, Chen P. Development of a Comprehensive Flavonoid Analysis Computational Tool for Ultrahigh-Performance Liquid Chromatography-Diode Array Detection-High-Resolution Accurate Mass-Mass Spectrometry Data. Anal Chem. 2017 Jun;89(14):7388–97. Available from: https://pubs.acs.org/doi/abs/10.1021/acs.analchem.7b00771

[25] Góes-Favoni SP, Carrão-Panizzi MC, Beleia A. Changes of isoflavone in soybean cotyledons soaked in different volumes of water. Food Chem. 2010 Apr;119(4):1605–12. Available from: https://doi.org/10.1016/j.foodchem.2009.09.051

[26] Lee JH, Choung MG. Determination of optimal acid hydrolysis time of soybean isoflavones using drying oven and microwave assisted methods. Food Chem. 2011 Nov;129(2):577–82. Available from: https://doi.org/10.1016/j.foodchem.2011.04.069

[27] Terigar BG, Balasubramanian S, Boldor D, Xu Z, Lima M, Sabliov CM. Continuous microwave-assisted isoflavone extraction system: Design and performance evaluation. Bioresour Technol. 2010 Apr;101(7):2466–71. Available from: https://doi.org/10.1016/j.biortech.2009.11.039

[28] Zafra-Gómez A, Garballo A, García-Ayuso LE, Morales JC. Improved sample treatment and chromatographic method for the determination of isoflavones in supplemented foods. Food Chem. 2010 Dec;123(3):872–7. Available from: https://doi.org/10.1016/j.foodchem.2010.05.009

[29] Moreira BJ, Yokoya JMC, Gaitani CM De. Microextração líquido-líquido dispersiva (DLLME): fundamentos , inovações e aplicações biológicas. Sci Chromatogr. 2014 Dec;6(3):186–204. Available from: http://dx.doi.org/10.4322/sc.2015.005

[30] Nara K, Nihei KI, Ogasawara Y, Koga H, Kato Y. Novel isoflavone diglycoside in groundnut (Apios americana Medik). Food Chem. 2011 Feb;124(3):703–10. Available from: https://doi.org/10.1016/j.foodchem.2010.05.107

[31] Zhang Wd, Yang Wj, Wang Xj, Gu Y, Wang R. Simultaneous determination of tectorigenin, irigenin and irisflorentin in rat plasma and urine by UHPLC-MS/MS: Application to pharmacokinetics. J Chromatogr B Anal Technol Biomed Life Sci. 2011 Dec;879(31):3735–41. Available from: https://doi.org/10.1016/j.jchromb.2011.10.022

[32] Matsumoto D, Kotani A, Hakamata H, Takahashi K, Kusu F. Column switching high-performance liquid chromatography with two channels electrochemical detection for high-sensitive determination of isoflavones. J Chromatogr A. 2010 Apr;1217(17):2986–9. Available from: https://doi.org/10.1016/j.chroma.2010.02.050

[33] Park HJ, Jung MY. One step salting-out assisted liquid-liquid extraction followed by UHPLC-ESI-MS/MS for the analysis of isoflavones in soy milk. Food Chem. 2017 Aug;229:797–804. Available from: https://doi.org/10.1016/j.foodchem.2017.02.145

[34] Kašparovská J, Dadáková K, Lochman J, Hadrová S, Křížová L, Kašparovský T. Changes in equol and major soybean isoflavone contents during processing and storage of yogurts made from control or isoflavone-enriched bovine milk determined using LC–MS (TOF) analysis. Food Chem. 2017 May;222:67–73. Available from: https://doi.org/10.1016/j.foodchem.2016.12.010

[35] Kunisue T, Tanabe S, Isobe T, Aldous KM, Kannan K. Profiles of phytoestrogens in human urine from several Asian countries. J Agric Food Chem. 2010 Aug;58(17):9838–46. Available from: https://pubs.acs.org/doi/abs/10.1021/jf102253j

[36] Ma Y, Zhang L, Zhao X, Shen Q. Analysis of daidzein in nanoparticles after oral co-administration with sodium caprate to rats by ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry. J Chromatogr B. 2012 Oct;907:21–6. Available from: https://doi.org/10.1016/j.jchromb.2012.08.026

[37] Fiechter G, Raba B, Jungmayr A, Mayer HK. Characterization of isoflavone composition in soy-based nutritional supplements via ultra performance liquid chromatography. Anal Chim Acta. 2010 Jul;672(1–2):72–8. Available from: https://doi.org/10.1016/j.aca.2010.04.004

[38] Jardim ICSF. Extração em Fase Sólida : Fundamentos Teóricos e Novas Estratégias para Preparação de Fases Sólidas. Sci Chromatogr. 2010;2(1):13–25. Available from: http://www.iicweb.org/scientiachromatographica.com/files/v2n1a2.pdf

[39] Nováková L, Vlčková H. A review of current trends and advances in modern bio-analytical methods : Chromatography and sample preparation. Anal Chim Acta. 2009 Dec;656:8–35. Available from: https://doi.org/10.1016/j.aca.2009.10.004

[40] Hosoda K, Furuta T, Ishii K. Simultaneous determination of glucuronic acid and sulfuric acid conjugated metabolites of daidzein and genistein in human plasma by high-performance liquid chromatography. J Chromatogr B Anal Technol Biomed Life Sci. 2010 Mar;878(7–8):628–36. Available from: https://doi.org/10.1016/j.jchromb.2010.01.028

[41] Saracino MA, Raggi MA. Analysis of soy isoflavone plasma levels using HPLC with coulometric detection in postmenopausal women. J Pharm Biomed Anal. 2010 Nov;53(3):682–7. Available from: https://doi.org/10.1016/j.jpba.2010.06.001

[42] Rodríguez-Morató J, Farré M, Pérez-Maná C, Papaseit E, Martínez-Riera R, de la Torre R, Pizarro, N. Pharmacokinetic Comparison of Soy Isoflavone Extracts in Human Plasma. J Agric Food Chem. 2015 Jul;63(31):6946–53. Available from: https://pubs.acs.org/doi/abs/10.1021/acs.jafc.5b02891

[43] Baranowska I, Magiera S, Baranowski J. UHPLC method for the simultaneous determination of β-blockers, isoflavones, and flavonoids in human urine. J Chromatogr Sci. 2011 Mar;49(10):764–73. Available from: https://doi.org/10.1016/j.jchromb.2011.01.026

[44] Redruello B, Guadamuro L, Cuesta I, Álvarez-Buylla JR, Mayo B, Delgado S. A novel UHPLC method for the rapid and simultaneous determination of daidzein, genistein and equol in human urine. J Chromatogr B Anal Technol Biomed Life Sci. 2015 Nov;1005:1–8. Available from: https://doi.org/10.1016/j.jchromb.2015.09.029

[45] Li T, Wang Y, Wang Y, Liang R, Zhang D, Zhang H, Chen L, Yang W. Development of an SPE-HPLC-MS method for simultaneous determination and pharmacokinetic study of bioactive constituents of Yu Ping Feng San in rat plasma after oral administration. J Ethnopharmacol. 2013 Feb;145(3):784–92. Available from: https://doi.org/10.1016/j.jep.2012.12.010

[46] Procházková T, Sychrová E, Javůrková B, Večerková J, Kohoutek J, Lepšová-Skácelová O, Bláha L, Hilscherová K. Phytoestrogens and sterols in waters with cyanobacterial blooms - Analytical methods and estrogenic potencies. Chemosphere. 2017 Mar;170:104–12. Available from: https://doi.org/10.1016/j.chemosphere.2016.12.006

[47] Benedetti B, Di Carro MD, Magi E. Phytoestrogens in soy-based meat substitutes: Comparison of different extraction methods for the subsequent analysis by liquid chromatography-tandem mass spectrometry. J Mass Spectrom. 2018 Jul;53(9):862–70. Available from: https://doi.org/10.1002/jms.4268

[48] González-Curbelo MÁ, Socas-Rodríguez B, Herrera-Herrera AV, González-Sálamo J, Hernández-Borges J, Rodríguez-Delgado MÁ. Trends in Analytical Chemistry Evolution and applications of the QuEChERS method. Trends Anal Chem [Internet]. 2015 Sep;71:169–85. Available from: https://doi.org/10.1016/j.trac.2015.04.012

[49] Delgado-Zamarreño MM, Pérez-Martín L, Bustamante-Rangel M, Carabias-Martínez R. A modified QuEChERS method as sample treatment before the determination of isoflavones in foods by ultra-performance liquid chromatography-triple quadrupole mass spectrometry. Talanta. 2012 Oct;100:320–8. Available from: https://doi.org/10.1016/j.talanta.2012.07.070

[50] Merlanti R, Lucatello L, Inacio JL, Pastore RM, Laverda S, Capolongo F. Isoflavones quantification in rainbow trout muscle by QuEChERS tecnique and liquid chromatography coupled with mass spectrometry. J Food Compos Anal [Internet]. 2018 Jul;70:114–24. Available from: https://doi.org/10.1016/j.jfca.2018.04.009

[51] Capriotti AL, Cavaliere C, Giansanti P, Gubbiotti R, Samperi R, Laganà A. Recent developments in matrix solid-phase dispersion extraction. J Chromatogr A. 2010 Apr;1217:2521–32. Available from: https://doi.org/10.1016/j.chroma.2010.01.030

[52] Barker SA. Matrix solid phase dispersion (MSPD). J Biochem Biophys Methods. 2007 Mar;70:151–62. Available from: https://doi.org/10.1016/j.jbbm.2006.06.005

[53] Peng L, Li Q, Chang Y, An M, Yang R, Tan Z, Hao J, Cao J, Xu JJ, Hu SS. Determination of natural phenols in olive fruits by chitosan assisted matrix solid-phase dispersion microextraction and ultrahigh performance liquid chromatography with quadrupole time-of-flight tandem mass spectrometry. J Chromatogr A [Internet]. 2016 Jul;1456:68–76. Available from: http://dx.doi.org/10.1016/j.chroma.2016.06.011

[54] Visnevschi-Necrasov T, Barreira JCM, Cunha SC, Pereira G, Oliveira MBPP. Advances in Isoflavone Profile Characterisation using Matrix Solid-phase Dispersion Coupled to HPLC / DAD in Medicago Species. Phytochem Anal. 2015 Aug;26:40–6. Available from: https://doi.org/10.1002/pca.2534

[55] Xu, L.; Shi, H.; Liang, T.; Feng, J.; Jin, Y.; Ke, Y.; Liang X. Selective separation of flavonoid glycosides in Dalbergia odorifera by matrix solid-phase dispersion using titania. J Sep Sci. 2011 Apr;34(11):1347–54. Available from: https://doi.org/10.1002/jssc.201100024

[56] Balasubramanian S, Panigrahi S. Solid-Phase Microextraction (SPME) Techniques for Quality Characterization of Food Products: A Review. Food Bioprocess Technol. 2011 Jan;4(1):1–26. Available from: https://doi.org/10.1007/s11947-009-0299-3

[57] Aulakh JS, Malik AK, Kaur V, Schmitt-Kopplin P. A review on solid phase micro extraction - High performance liquid chromatography (SPME-HPLC) analysis of pesticides. Crit Rev Anal Chem. 2005 Oct;35(1):71–85. Available from: https://doi.org/10.1080/10408340590947952

[58] Aresta A, Di Grumo F, Zambonin C. Determination of Major Isoflavones in Soy Drinks by Solid-Phase Micro Extraction Coupled to Liquid Chromatography. Food Anal Methods. 2016 Jul;9(4):925–33. Available from: https://doi.org/10.1007/s12161-015-0260-1

[59] Calvello R, Aresta A, Trapani A, Zambonin C, Cianciulli A, Salvatore R, Clodoveo ML, Corbo F, Franchini C, Panaro MA. Bovine and soybean milk bioactive compounds : Effects on inflammatory response of human intestinal Caco-2 cells. Food Chem. 2016 Nov;210:276–85. Available from: https://doi.org/10.1016/j.foodchem.2016.04.067

[60] Abdel-Rehim M. New trend in sample preparation: on-line microextraction in packed syringe for liquid and gas chromatography applications I . Determination of local anaesthetics in human plasma samples using gas chromatography – mass spectrometry. J Chromatogr B. 2004 Mar;801(2):317–21. Available from: https://doi.org/10.1016/j.jchromb.2003.11.042

[61] Gonçalves J, Mendes B, Silva CL, Câmara JS. Development of a novel microextraction by packed sorbent-based approach followed by ultrahigh pressure liquid chromatography as a powerful technique for quantification phenolic constituents of biological interest in wines. J Chromatogr A. 2012 Mar;1229:13–23. Available from: https://doi.org/10.1016/j.chroma.2012.01.023

[62] Chang Y, Zhao C, Wu Z, Zhou J, Zhao S, Lu X, Xu, G. Chip-based nanoflow high performance liquid chromatography coupled to mass spectrometry for profiling of soybean flavonoids. Electrophoresis [Internet]. 2012 Jun;33(15):2399–406. Available from: http://doi.wiley.com/10.1002/elps.201100581

[63] Bustamante-Rangel M, Delgado-Zamarreño MM, Carabias-Martínez R, Domínguez-Álvarez J. Analysis of isoflavones in soy drink by capillary zone electrophoresis coupled with electrospray ionization mass spectrometry. Anal Chim Acta [Internet]. 2012 Jan;709:113–9. Available from: https://doi.org/10.1016/j.aca.2011.10.015

[64] Vacek J, Klejdus B, Lojková L, Kubán V. Current trends in isolation, separation, determination and identification of isoflavones: A review. J Sep Sci [Internet]. 2008 Jul;31(11):2054–67. Available from: http://doi.wiley.com/10.1002/jssc.200700569

[65] Engida AM, Kasim NS, Tsigie YA, Ismadji S, Huynh LH, Ju Y-H. Extraction, identification and quantitative HPLC analysis of flavonoids from sarang semut (Myrmecodia pendan). Ind Crops Prod [Internet]. 2013 Jan;41:392–6. Available from: https://doi.org/10.1016/j.indcrop.2012.04.043

[66] Merken HM, Beecher GR. Measurement of Food Flavonoids by High-Performance Liquid Chromatography: A Review. 2000 Feb; Available from: https://pubs.acs.org/doi/abs/10.1021/jf990872o

[67] Zaheer K, Akhtar MH. Critical Reviews in Food Science and Nutrition An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health. 2015 Nov; Available from: https://doi.org/10.1080/10408398.2014.989958

[68] Uifălean A, Farcaş A, Ilieş M, Hegheş SC, Ionescu C, Iuga CA. Assessment of isoflavone aglycones variability in soy food supplements using a validated hplc-uv method. Orig Res Clujul Med [Internet]. 2015 Jul;88(3):373–80. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4632898/pdf/cm-88-373.pdf

[69] Fiechter G, Opacak I, Raba B, Mayer HK. A new ultra-high pressure liquid chromatography method for the determination of total isoflavone aglycones after enzymatic hydrolysis: Application to analyze isoflavone levels in soybean cultivars. 2013 Mar; Available from: https://doi.org/10.1016/j.foodres.2011.03.038

[70] Klejdus B, Vacek J, Benešová L, Kopecký J, Lapčík O, Kubáň V. Rapid-resolution HPLC with spectrometric detection for the determination and identification of isoflavones in soy preparations and plant extracts. Anal Bioanal Chem [Internet]. 2007 Dec;389(7–8):2277–85. Available from: https://doi.org/10.1007/s00216-007-1606-3

[71] Repollés C, Herrero-Martínez JM, Ràfols C. Analysis of prominent flavonoid aglycones by high-performance liquid chromatography using a monolithic type column. J Chromatogr A [Internet]. 2006 Oct;1131(1–2):51–7. Available from: https://doi.org/10.1016/j.chroma.2006.07.012

[72] Jandera P. Advances in the development of organic polymer monolithic columns and their applications in food analysis—A review. J Chromatogr A [Internet]. 2013 Oct;1313:37–53. Available from: https://doi.org/10.1016/j.chroma.2013.08.010

[73] Herrero M, Ibáñez E, Cifuentes A. Analysis of natural antioxidants by capillary electromigration methods. J Sep Sci [Internet]. 2005 Jun;28(9–10):883–97. Available from: http://doi.wiley.com/10.1002/jssc.200400104

[74] Wieczorek P, Ligor M, Buszewski B. Applications of Electromigration Techniques: Applications of Electromigration Techniques in Food Analysis. In Springer, Berlin, Heidelberg; 2013 Apr; 105:299–333. Available from: https://doi.org/10.1007/978-3-642-35043-6_17

[75] Shihabi ZK, Kute T, Garcia LL, Hinsdale M. Analysis of isoflavones by capillary electrophoresis. J Chromatogr A [Internet]. 1994 Sep;680(1):181–5. Available from: https://doi.org/10.1016/0021-9673(94)80066-9

[76] Bustamante-Rangel M, Delgado-Zamarreño MM, Pérez-Martín L, Carabias-Martínez R. QuEChERS method for the extraction of isoflavones from soy-based foods before determination by capillary electrophoresis-electrospray ionization-mass spectrometry. Microchem J [Internet]. 2013 May;108:203–9. Available from: https://doi.org/10.1016/j.microc.2012.10.023

[77] Xiao W, Chen C, Zhang Q, Zhang Q-H, Hu Y-J, Xia Z-N, Yang, F-Q. Separation Study of Eight Isoflavones by MEKC with Different Surfactants. Chromatographia [Internet]. 2015 Nov;78(21–22):1385–93. Available from: https://doi.org/10.1007/s10337-015-2969-9

[78] Martí R, Valcárcel M, Herrero-Martínez JM, Cebolla-Cornejo J, Roselló S. Simultaneous determination of main phenolic acids and flavonoids in tomato by micellar electrokinetic capillary electrophoresis. Food Chem [Internet]. 2017 Apr;221:439–46. Available from: https://doi.org/10.1016/j.foodchem.2016.10.105

[79] Fiechter G, Opacak I, Raba B, Mayer HK. A new ultra-high pressure liquid chromatography method for the determination of total isoflavone aglycones after enzymatic hydrolysis: Application to analyze isoflavone levels in soybean cultivars. Food Research International. 2013 Mar; 50(2):586-592. Available from: https://doi.org/10.1016/j.foodres.2011.03.038

[80] Bustamante-Rangel M, Delgado-Zamarreño MM, Pérez-Martín L, Carabias-Martínez R. QuEChERS method for the extraction of isoflavones from soy-based foods before determination by capillary electrophoresis-electrospray ionization-mass spectrometry. Microchem J. 2013 May;108:203–9. Available from: https://doi.org/10.1016/j.microc.2012.10.023

[81] Martí R, Valcárcel M, Herrero-Martínez JM, Cebolla-Cornejo J, Roselló S. Simultaneous determination of main phenolic acids and flavonoids in tomato by micellar electrokinetic capillary electrophoresis. Food Chem. 2017 Apr;221:439–46. Available from: https://doi.org/10.1016/j.foodchem.2016.10.105

[82] Song Z, Hashi Y, Sun H, Liang Y, Lan Y, Wang H, Chen S. Simultaneous determination of 19 flavonoids in commercial trollflowers by using high-performance liquid chromatography and classification of samples by hierarchical clustering analysis. Fitoterapia. 2013 Dec;91:272–9. Available from: https://doi.org/10.1016/j.fitote.2013.09.006

[83] Ganzera M. Supercritical fluid chromatography for the separation of isoflavones. J Pharm Biomed Anal. 2015 Mar;107:364–9. Available from: https://doi.org/10.1016/j.jpba.2015.01.013

[84] VanderMolen KM, Cech NB, Paine MF, Oberlies NH. Rapid Quantitation of Furanocoumarins and Flavonoids in Grapefruit Juice using Ultra-Performance Liquid Chromatography. Phytochem Anal. 2013 Jun;24(6):654–60. Available from: https://doi.org/10.1002/pca.2449

[85] Barfi B, Asghari A, Rajabi M, Barfi A, Saeidi I. Simplified miniaturized ultrasound-assisted matrix solid phase dispersion extraction and high performance liquid chromatographic determination of seven flavonoids in citrus fruit juice and human fluid samples: Hesperetin and naringenin as biomarkers. J Chromatogr A. 2013 Oct;1311:30–40. Available from: https://doi.org/10.1016/j.chroma.2013.08.078

[86] Ahmed AYBH, Obbed MS, Wabaidur SM, AlOthman ZA, Al-Shaalan NH. High-Performance Liquid Chromatography Analysis of Phenolic Acid, Flavonoid, and Phenol Contents in Various Natural Yemeni Honeys Using Multi-walled Carbon Nanotubes as a Solid-Phase Extraction Adsorbent. J Agric Food Chem. 2014 Jun;62(24):5443–50. Available from: https://pubs.acs.org/doi/abs/10.1021/jf5011758

[87] Shim Y-S, Yoon W-J, Hwang J-B, Park H-J, Seo D, Ha J. Rapid method for the determination of 14 isoflavones in food using UHPLC coupled to photo diode array detection. Food Chem. 2015 Nov;187:391–7. Available from: https://doi.org/10.1016/j.foodchem.2015.04.077

[88] Schmidt L, Müller J, Göen T. Simultaneous monitoring of seven phenolic metabolites of endocrine disrupting compounds (EDC) in human urine using gas chromatography with tandem mass spectrometry. Anal Bioanal Chem [Internet]. 2013 Feb;405(6):2019–29. Available from: http://link.springer.com/10.1007/s00216-012-6618-y

[89] Roh C. Biotransformation of Isoflavone Using Enzymatic Reactions. Molecules [Internet]. 2013 Mar;18(3):3028–40. Available from: https://doi.org/10.3390/molecules18033028

[90] Nakata R, Yoshinaga N, Teraishi M, Okumoto Y, Huffaker A, Schmelz EA, Mori, N. A fragmentation study of isoflavones by IT-TOF-MS using biosynthesized isotopes. Biosci Biotechnol Biochem [Internet]. 2018 Aug;82(8):1309–15. Available from: https://www.tandfonline.com/doi/full/10.1080/09168451.2018.1465810

[91] Yan Y, Chai C-Z, Wang D-W, Wu J, Xiao H-H, Huo L-X, Zhu D-N, Yu B.Y. Simultaneous determination of puerarin, daidzin, daidzein, paeoniflorin, albiflorin, liquiritin and liquiritigenin in rat plasma and its application to a pharmacokinetic study of Ge-Gen Decoction by a liquid chromatography–electrospray ionization-tandem mass spectrometry. J Pharm Biomed Anal [Internet]. 2014 Jul;95:76–84. Available from: https://doi.org/10.1016/j.jpba.2014.02.013

[92] Lei Z, Jing L, Qiu F, Zhang H, Huhman D, Zhou Z, Sumner LW. Construction of an Ultrahigh Pressure Liquid Chromatography-Tandem Mass Spectral Library of Plant Natural Products and Comparative Spectral Analyses. Anal Chem [Internet]. 2015 Jun;87(14):7373–81. Available from: http://pubs.acs.org/doi/10.1021/acs.analchem.5b01559

[93] Parets L, Alechaga É, Núñez O, Saurina J, Hernández-Cassou S, Puignou L. Ultrahigh pressure liquid chromatography-atmospheric pressure photoionization-tandem mass spectrometry for the determination of polyphenolic profiles in the characterization and classification of cranberry-based pharmaceutical preparations and natural extracts. Anal Methods [Internet]. 2016 Jun;8(22):4363–78. Available from: http://xlink.rsc.org/?DOI=C6AY00929H

[94] Schmidt J. Negative ion electrospray high-resolution tandem mass spectrometry of polyphenols. J Mass Spectrom [Internet]. 2016 Jan;51(1):33–43. Available from: http://doi.wiley.com/10.1002/jms.3712

[95] Jorgenson JW, Lukacs KA. Zone electrophoresis in open-tubular glass capillaries: Preliminary data on performance. J High Resolut Chromatogr [Internet]. 1981 May;4(5):230–1. Available from: http://doi.wiley.com/10.1002/jhrc.1240040507

[96] Perez-Martin L, Bustamante-Rangel M, Delgado-Zamarreno MM. Determination of Isoflavones in Legumes by QuEChERS-Capillary Electrophoresis-Electrospray Ionization-Mass Spectrometry. Bentham Science Publishers. 2015 Apr;11(2):117-123 Available from: https://www.ingentaconnect.com/content/ben/cac/2015/00000011/00000002/art00008#

[97] Olivares JA, Nguyen NT, Yonker CR, Smith RD. On-line mass spectrometric detection for capillary zone electrophoresis. Anal Chem [Internet]. 1987 Apr;59(8):1230–2. Available from: http://pubs.acs.org/doi/abs/10.1021/ac00135a034

[98] Zhong X, Chen Z, Snovida S, Liu Y, Rogers JC, Li L. Capillary Electrophoresis-Electrospray Ionization-Mass Spectrometry for Quantitative Analysis of Glycans Labeled with Multiplex Carbonyl-Reactive Tandem Mass Tags. Anal Chem [Internet]. 2015 Jul;87(13):6527–34. Available from: http://pubs.acs.org/doi/10.1021/acs.analchem.5b01835

[99] Zhao J, Hu D-j, Lao K, Yang Z-m, Li S. Advance of CE and CEC in phytochemical analysis (2012-2013). Electrophoresis [Internet]. 2014 Jan;35(1):205–24. Available from: http://doi.wiley.com/10.1002/elps.201300321

[100] Karas M, Bachmann D, Bahr U, Hillenkamp F. Matrix-assisted ultraviolet laser desorption of non-volatile compounds. Int J Mass Spectrom Ion Process [Internet]. 1987 Sep;78:53–68. Available from: https://doi.org/10.1016/0168-1176(87)87041-6

[101] Theparee T, Das S, Thomson RB. Total Laboratory Automation and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Improve Turnaround Times in the Clinical Microbiology Laboratory: a Retrospective Analysis. J Clin Microbiol [Internet]. 2018 Jan;56(1):e01242-17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29118171

[102] Sakamoto S, Yusakul G, Pongkitwitoon B, Paudel MK, Tanaka H, Morimoto S. Simultaneous determination of soy isoflavone glycosides, daidzin and genistin by monoclonal antibody-based highly sensitive indirect competitive enzyme-linked immunosorbent assay. Food Chem [Internet]. 2015 Feb;169:127–33. Available from: https://doi.org/10.1016/j.foodchem.2014.08.004

[103] Cui Q, Peng X, Yao X-H, Wei Z-F, Luo M, Wang W, Zhao, C-J, Fu Y-J, Zu Y-G. Deep eutectic solvent-based microwave-assisted extraction of genistin, genistein and apigenin from pigeon pea roots. Sep Purif Technol [Internet]. 2015 Aug;150:63–72. Available from: https://doi.org/10.1016/j.seppur.2015.06.026

[104] Wang L, Wang ZQ, Hu DD, Cui J. Proteomic analysis of Trichinella spiralis muscle larval excretory-secretory proteins recognized by early infection sera. Biomed Res Int [Internet]. 2013 Jun;2013:139745. Available from: http://dx.doi.org/10.1155/2013/139745

[105] Giménez-Cassina B, Schmidt EM, Eberlin MN, Sawaya ACHF. Phytochemical markers of different types of red propolis. Food Chem [Internet]. 2014 Mar;146:174–80. Available from: https://doi.org/10.1016/j.foodchem.2013.09.063

CAPÍTULO 3

DESENVOLVIMENTO E APLICAÇÃO DE UMA FASE SÓLIDA EXTRATORA BASEADA EM GRAFENO E CICLODEXTRINA

[2] **β-cyclodextrin coupled to graphene oxide supported on aminopropyl silica as a sorbent material for determination of isoflavones**

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RESEARCH ARTICLE

β-Cyclodextrin coupled to graphene oxide supported on aminopropyl silica as a sorbent material for determination of isoflavones

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β-Cyclodextrin, coupled to graphene oxide supported on aminopropyl silica, was synthesized and characterized. This material was combined with microextraction by packed sorbent to act as the sample preparation step. The analytical method optimization was carried out by employing experimental design and had its figures of merit determined. The resulting linearity ranged from 1.0 to 200 μg/L for daidzein, from 2.0 to 200 μg/L for genistein, from 3.0 to 200 μg/L for formononetin, and from 2.0 to 200 μg/L for biochanin A with all R^2 values above 0.993 and limit of quantification ranging from 0.5 to 1.5 μg/L. The accuracy ranged from 93.3 to 123.3%, and intraday and interday precision reported by the relative standard deviations were <16%. This work aimed to synthesize and evaluate cyclodextrins coupled to graphene-based sorbents to be used as a high sorption capacity and selective sorbent for sample preparation of complex matrices using microextraction techniques. The synthesized material kept the high absorption characteristic of graphene-based materials while maintaining the cyclodextrins' selectivity to extract the target analyte. Four isoflavones were determined in soy-based juice samples from the local market, confirming the excellent performance of the proposed method.

KEYWORDS
cyclodextrins, graphene, microextraction by packed sorbent, sample preparation

1 | INTRODUCTION

The main goal of the sample preparation step in a chemical analysis workflow is to remove those matrix components that can interfere with the analysis. In solid-phase based techniques involving sorption processes, the correct choice of the sorbent material is crucial for proper analyte isolation. There are different materials and manufacturers available on the market, including, among many others, C8, C18, X-Strata, Oasis HLB, all widely applied in solid-phase based techniques [1]. However, the interest in new materials to be used in sample preparation has recently increased.

Although many screening techniques have been developed, most confirmatory methods use sorption-based sample preparation techniques. Aiming to obtain a higher specificity and selective enrichment, several new materials have been reported in the literature over the past

Article Related Abbreviations: APTES, (3-aminopropyl) trietoxisilane; BIO, biochanin A; CD, cyclodextrins; DAI, daidzein; DMF, dimethylformamide; EDC, N-ethyl-N'-(3-dimethyl aminopropyl) carbodiimide; FOR, formononetin; G, graphene; GEN, genistein; GO, graphene oxide; ME, matrix effect; MEPS, microextraction by packed sorbent; MRM, multiple reaction monitoring; NHS, N-hydroxysuccinimide; Si, amino-functionalized spherical silica gel; TGA, thermogravimetric analysis

10 years [2]. Since graphene (G) was discovered in 2004 by Novoselov et al. [3], its uses in several different areas have been investigated. Sample preparation is a specific area where the use of graphene-based materials, as sorbent, has shown good results [4]. Due to the large surface area and delocalized π electrons, graphene and graphene oxide (GO) are considered superior adsorbents with a high absorption capacity, and high affinity for aromatic compounds [5]. Graphene oxide has several polar groups in his chemical structure that can be modified with other materials to alter some of its characteristics, such as improved selectivity and analyte recovery.

Cyclodextrins (CD) are natural oligosaccharides produced by the digestion of starch by bacteria. The most well-known CDs are α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin; this class of compounds has a conical shape with a hydrophobic cavity and a hydrophilic outer surface, owing to the OH groups on its external wall [6]. They have been used as stationary phase ligands or mobile phase additives in separation sciences, especially chiral discrimination, and, more recently, they have been applied as sorbent materials for sample preparation [7,8]. The coupling of CD to GO has also been successfully employed as electrochemical sensors [9]. The composite produced in this way incorporates the advantages of both the cyclodextrin and the graphene oxide nanoparticles [10].

Flavonoids are non-steroidal compounds produced by plants. These compounds can be divided into subclasses such as flavones, flavonols, flavanones, and isoflavones, the latter being by far the best-known chemical group [11]. Most of their health benefits, such as lowering the risk of breast and prostate cancer, preventing heart disease, osteoporosis, and relieving menopausal symptoms, are attributed to phytoestrogens [12,13]. Once cyclodextrins and flavonoids have good affinity one to the other, the encapsulation of flavonoids by CD can improve their solubility and stability [14]. This fact suggests that CD coupled to GO is expected to present excellent sorbent material for sample preparation aiming to analyze isoflavones.

Fumes and Lanças reported for the first time the use of aminopropyl silica-supported graphene as a sorbent in a miniaturized sample preparation technique termed microextraction by packed sorbent (MEPS) and emphasized the importance of the development and investigation of innovative graphene-based new materials and their application to miniaturize sample preparation techniques [15]. In this study, we propose a selective and sensitive innovative analytical approach, employing cyclodextrin coupled to graphene as an extraction phase in the MEPS format, to identify and quantify the target isoflavones daidzein, genistein, formononetin, and biochanin A in soy-based juice samples.

2 | MATERIALS AND METHODS

2.1 | Reagents

High purity analytical standards (≥99%) of daidzein (DAI), genistein (GEN), formononetin (FOR), and biochanin A (BIO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The standard stock solutions (1000 mg/L) were prepared by weighing the required amount of each solid analytical standard, dissolving in HPLC grade methanol purchased from Tedia (Fairfield, OH, USA), and storing at −7°C until use. These stock solutions were diluted with ultrapure water (18.2 MΩ cm) obtained from a Milli-Q water purification system, from Millipore (Bedford, MA, USA), and used to prepare a mixed solution of isoflavones (work solution) responsible for providing spiked samples that were used for the method evaluation. All solutions were stored at −7°C until use; the work solutions were freshly prepared, immediately before their use.

Graphite powder, $KMnO_4$, dimethylformamide (DMF), amino-functionalized spherical silica gel (Si), *N*-ethyl-*N*'-(3-dimethyl aminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), and (3-aminopropyl) trietoxisilane (APTES) were all acquired from Sigma–Aldrich (St. Louis, MO, USA); H_2O_2 from Synth (Diadema, Brazil); H_2SO_4 from Tedia (Fairfield, OH, USA); HCl from Qhemis (Jundiai, Brazil), and β-cyclodextrin (> 97%) from Hewlett Packard (Palo Alto, CA, USA).

Acetonitrile (ACN), chromatographic analysis grade, was purchased from Tedia (Fairfield, OH, USA). Formic acid 96% was acquired from Sigma–Aldrich.

2.2 | Synthesis of graphene-based materials

GO was obtained based on the Hummers method, under optimized experimental conditions [16]. GO@Si functionalization with β-cyclodextrin was obtained based on an original report by Hou and co-workers [17]. Supporting Information Figure S1 summarizes the main steps involved in the synthesis process of the extraction phase β-CD@GO@Si.

2.3 | Instrumentation

A Shimadzu LC system (Kyoto, Japan) composed of a CBM-20A communication bus module, a DGU-20AS degasser, two LC 20-AD pumps, a CTO-20A column oven, and an SPD-20A UV-Vis detector, monitoring at 260 nm, was utilized during the evaluation of the sorbent materials and the MEPS optimization. Chromatographic analyses

were performed with a Poroshell 120 EC-C18 (2.1 mm × 100 mm × 2.7 μm) column from Agilent (Santa Clara, CA, USA). The column was operated at 42°C, employing water (A) and acetonitrile (B) as mobile phases, both containing 0.1% formic acid, using a gradient elution at a flow rate of 0.300 mL/min.

The evaluation of the figures of merit was performed using a UPLC–MS/MS system consisting in an ACQUITY UPLC (Milford, MA, USA) equipped with an ACQUITY UPLC® BEH C18 (2.1 mm × 100 mm × 1.7 μm) column, ACQUITY UPLC binary solvent pump, and ACQUITY UPLC sample manager, coupled to a mass spectrometer XEVO (tandem quadrupole) fitted with an ESI source. The column was operated at 42°C, employing water (A) and acetonitrile (B) as mobile phases, both containing 0.1% formic acid, using a gradient elution at a flow rate of 0.300 mL/min. The detailed information about the HPLC gradient conditions is described in Supporting Information Table S1. During the figures of merit evaluation, data were acquired in MRM mode, by monitoring two transitions that were previously optimized, applying a direct infusion of the analytical standards (0.5 mg/L). Supporting Information Table S2 summarizes the optimized conditions applied to the MS/MS system. The synthesized material's characterization is detailed in Supporting Information Figure S2.

2.4 | Performance of the sorbent materials synthetized

The device used in all MEPS experiments was designed, built, optimized, and further packed with the synthesized sorbent. The device body consisted of a polyethylene syringe purchased from Sigma–Aldrich. A 10 μL pipet tip was coupled to the syringe button, and 4 mg of the sorbent material was immobilized in this body by two polypropylene frits. The evaluation of the best sorbent material was performed in two steps.

In the first step, a comparison of the chromatographic peak area obtained for each analyte was obtained using each one of the three evaluated extraction phases: (1) non-functionalized silica particles with GO, (2) GO@Si, and (3) β-CD@GO@Si. For that purpose, the described MEPS–LC–UV system was used, all experiments being conducted under the same experimental conditions. The soybean juice matrix was spiked with 0.5 mg/L of the analytes. The samples were previously centrifuged at 4863 × *g* for 10 min to avoid obstructions, thus preserving the robustness of the MEPS hardware, and diluted 1:1 with ultrapure water. During this step, the MEPS procedure was performed employing 8 sampling cycles with 250 μL sample volume in each cycle; four wash cycles with 250 μL ultrapure water at pH 3.0; 20 (1 mL) suction cycles to dry the adsorbent from the material; and four desorption cycles using 100 μL of the elution solvent. For the regeneration/conditioning step, two cycles of 1 mL of ultrapure water and two cycles of 1 mL of elution solvent were used to avoid carryover.

In the second step of evaluation, the synthesized sorbent material was selectivity assessed by analyzing the matrix effect (ME) employing the described MEPS–UHPLC–ESI–MS/MS system. The sorption behavior of two sorbent materials synthesized in our laboratory (GO@Si and β-CD@GO@Si) was compared with a commercial phase Si-C-18. During this step, the MEPS procedure was performed as described before.

The ME was evaluated by determining the ratio between the peak area obtained for the matrix samples spiked after extraction and that obtained for a standard solution in the solvent ([spike after/solvent solution PA] ×100), under the same experimental conditions, according to the procedure previously reported by Matuszewski et al. [18]. Four different concentrations of 20, 50, 300, and 800 μg/L were evaluated in triplicate. Solutions of the analytical standards in water were prepared and the MEPS procedure was performed and the eluate was analyzed. The collected areas correspond to the "PA solvent solution." To obtain the areas corresponding to the "spike after" samples of soy-based juice were prepared and the MEPS extraction procedure was performed. The eluate was spiked with the analytes so that the final concentration was exactly equal to the "PA solvent solution" before MEPS.

2.5 | MEPS extraction performance evaluation

As the first step, we evaluated the contribution of the main experimental variables that might interfere with the method's performance, including the elution solvent, the elution solvent volume, and the sample pH. The solvents evaluated were methanol, ethanol, and acetonitrile; the elution solvent volumes evaluated were 50, 60, and 70 μL; the sample pH was evaluated at 3.0, 4.6 (unchanged sample), and 8.0. After choosing the best conditions for these variables, a factorial design 2^3 with the central point replicated three times was used to evaluate the number of sampling, washing, and desorption cycles that may influence the MEPS extraction procedure. The levels used for each variable are shown in Supporting Information Table S3.

2.6 | Method qualification

We based the determination and evaluation of the analytical figures of merit on the ICH (International Conference

on Harmonization) guidelines [19]. The validated parameters were: LOD, LOQ, linearity, accuracy, and precision. Details on the experimental procedure for this step are described in the Supporting Information.

3 | RESULTS AND DISCUSSION

3.1 | Materials characterization

The main results obtained during the characterization of the synthesized materials are summarized in Supporting Information Figure S2. Through the SEM images depicted in Supporting Information Figure S2a, it is possible to see the morphology of the synthesized material and the GO coating over a silica sphere. The silica particles (Si) act as a support for the deposition of the synthesized GO nanosheets onto its surface, giving it higher stability and forming the proposed GO@Si material.

The FT-IR spectrum corresponding to the GO nanosheets (Supporting Information Figure S2b), presents the following the most prominent peaks: (i) C–C vibrations observed at 1600 cm^{-1}, which are attributed to the graphitic skeleton; (ii) carboxyl groups at 1728 cm^{-1}, making a broad epoxy band at 1250 cm^{-1}; and a carbonyl vibration at 1050 cm^{-1}. The relatively broad peak ca. 3400 cm^{-1} is due to water molecules adsorbed onto the GO surface. Similar results were reported in the literature, suggesting successful graphite oxidation through the Hummer's method used in this work [20–22].

The β-CD TGA graph, depicted in Supporting Information Figure S2c, shows an abrupt mass weight loss occurring around 300°C, corresponding to β-CD, while Supporting Information Figure S2d displays the TGA graph of the composite β-CD@GO@Si. It can be seen a weight loss of approximately 7% occurring around 300°C through the examination of the derived weight (%/°C) curve. This graph shows a defined event of weight loss in this temperature range, corresponding to β-CD, and an approximately 17% weight loss around 600°C, corresponding to GO [8,23]. Based upon the obtained results, which confirmed their proposed chemical composition, the synthesized materials were then applied as a sorbent phase for the MEPS extraction studies, aiming for the development of the analytical method described in this work.

3.2 | Sorption performance of the sorbent materials synthetized

Figure 1 shows the results obtained for the first evaluation step of the sorbent material. The non-functionalized silica

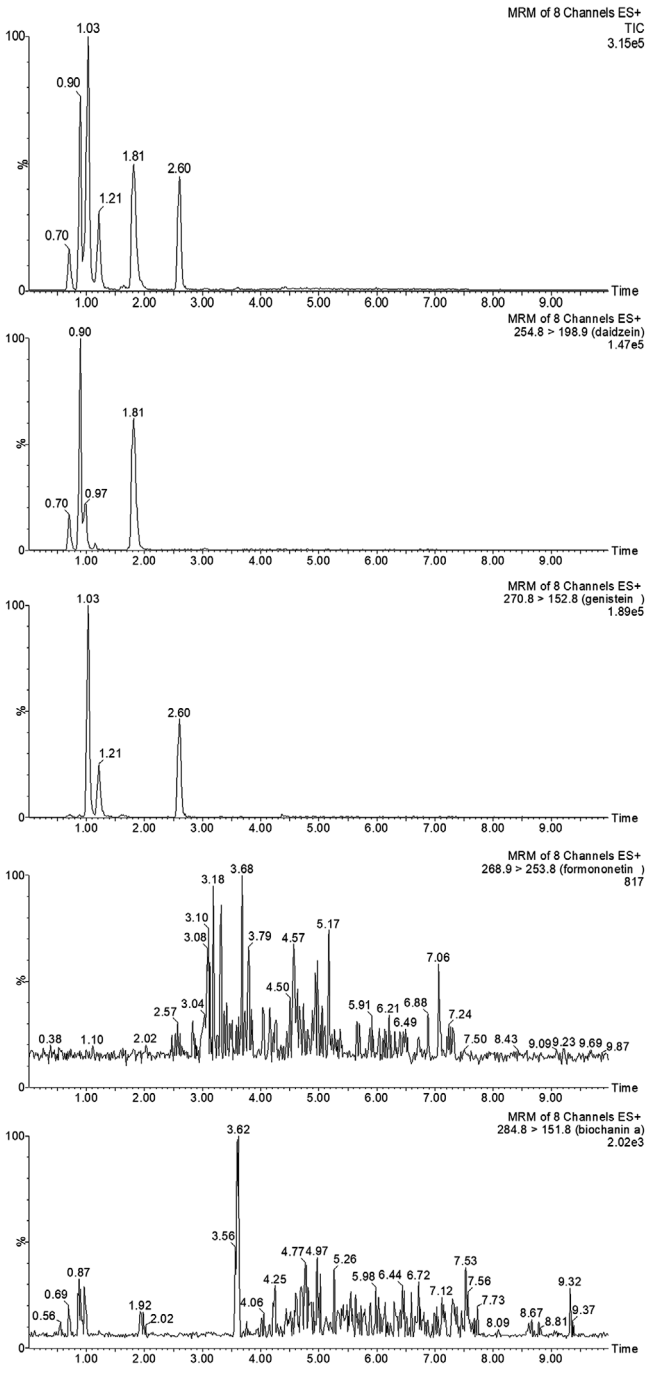

FIGURE 1 Comparative sorption characteristics obtained for non-functionalized silica particles; GO@Si; and β-CD@GO@Si. Evaluation was done through the chromatographic peak area obtained for each analyte in each sorbent material

showed the worst extraction results among the evaluated materials. The GO@Si synthesized material showed the highest peak area among the materials evaluated for all target analytes, which was expected according to the characteristics of the graphene-based materials. This result shows that the coupling of GO to silica improves the sorption capacity and extraction efficiency.

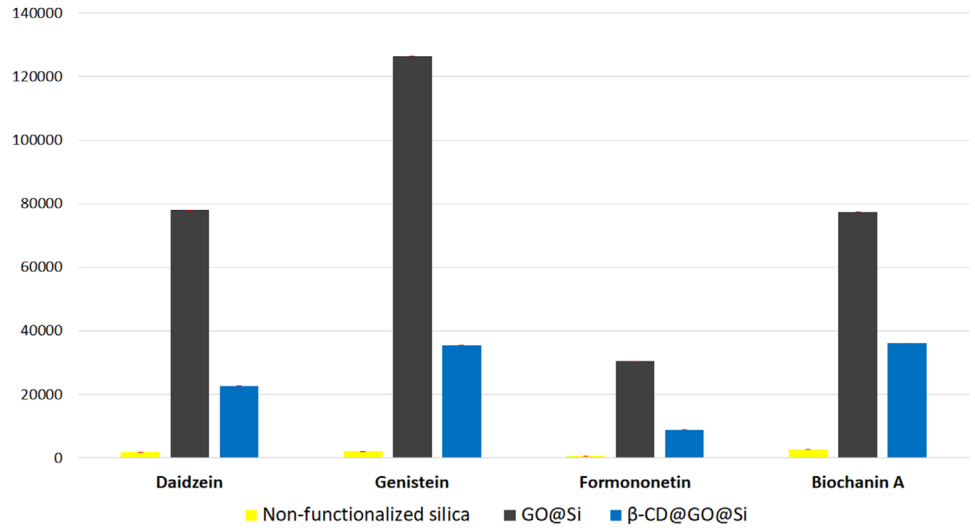

FIGURE 2 Results obtained from the matrix effect studies investigated in this work. (A) β-CD@GO@Si utilized as a sorbent material in different concentrations. (B) a comparison between β-CD@GO@Si, GO@Si, and C-18 ($n = 3$)

However, once our main objective was to improve material selectivity through the β-CD functionalization of GO, further experiments were designed to understand each sorbent's main features better. For this purpose, we evaluated the matrix effect using GO@Si, β-CD@GO@Si, and the commercial C18 material as extraction phases in MEPS sample preparation (Figure 2), employing the approach described by Matuszewski et al. [18].

Figure 2A depicts the matrix effect results obtained for β-CD@GO@Si as sorbent material in different spiking concentrations and Figure 4B depicts the comparison among β-CD@GO@Si, GO@Si, and C-18, each one spiked at 50 μg/L. These results show that although GO@Si presented an extraction yield higher than that obtained with β-CD@GO@Si in the first step evaluation, the latter material provided less matrix effects, which suggests that the functionalization of the GO with β-CD improves selectivity of the material. By comparing these results with those obtained for the commercial phase C18, we can see that GO improves the extraction efficiency, which can be explained by its higher sorption capacity due to the stronger affinity for aromatic compounds [5]. However, in spite of these properties, GO is a non-selective material. Although the GO functionalization by β-CD might cause a decrease in the peak area, β-CD@GO@Si presents a matrix effect between 86 - 106%, as shown in Figure 3A, where 100% corresponds to the total absence of matrix effect. Therefore, the ME presented by the β-CD@GO@Si extraction phase is in the 85–115% range accepted by agencies such as FDA and EMA for the matrix effect in analytical methods [24,25]. Figure 3B shows that the values

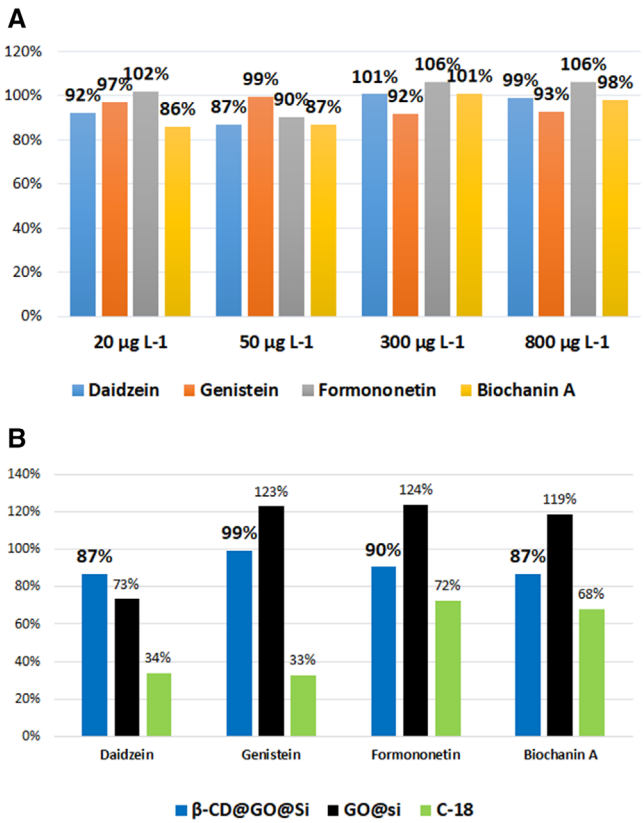

FIGURE 3 LC–MS/MS obtained after extraction/cleaning/desorption of MEPS- β-CD@GO@Si from a sample of soy juice. The total ion chromatograms (TIC) screen is displayed first and the MRM transitions monitored are displayed below. Note the abundance of ions on the right (for example, 1.89 e5 for genistein - third characteristic - and 817 for formononetine - fourth characteristic, showing the high abundance of the former and much less abundance of the later; Supporting Information Table S4)

obtained using GO@Si and C-18 are well outside the established range. The obtained results, consequently, indicate that the β-CD group adds selectivity to the GO@Si material.

Matrix effect is common in many HPLC methods, especially when using MS detection with ESI sources. Co-elution of analytes with matrix compounds causes competition between them and decreases the ionization efficiency, which can cause signal suppression or enhancement, elevated baseline, impact on extraction, or retention time [25]. Some strategies are used to overcome or at least decrease the matrix effect. Mobile-phase additives such as formic acid can improve sensitivity, sample dilution, injected sample reduction, extract dilution, sample extraction modification for better clean-up, internal standard, development of new sample preparation techniques, and development of new sorbent materials [26].

In this work, β-CD@GO@Si resulted in a lower matrix effect (see Section 2.4 for the experimental conditions) than the other evaluated materials. Thus, this material was selected to be further used in the method development step. The analysis of the areas resulting from the experiments to determine the ME showed a linear response both for the PA in solvent solution and for the increased sample area obtained after MEPS extraction. The Student's *t*-test comparing the slopes of the PA in solvent solution curves and the sample spiked after MEPS extraction curves showed a p-value > 0.05 confirming the parallelism between them (Supporting Information Table S4). These results show that cleaning the sample is so efficient that it does not affect the responses' linearity, and there is no increase or decrease in the analytical signals capable of configuring the matrix effect. Thus, it is possible to use an external standard calibration method instead of the method calibration by standard addition, which would be more laborious.

3.3 | Overall performance of the optimized MEPS extraction

When comparing methanol, ethanol, and acetonitrile as an elution solvent, methanol presented a higher peak area for the extracted analytes than the other solvents studied. Because of this, it was chosen as the elution solvent. The elution solvent volume shows very close results for 50 and 60 μL; although 50 μL might result in a slightly higher enrichment factor, we chose to work with 60 μL as the elution solvent volume. It was very labor-intensive and error-prone to work with 50 μL as the elution solvent volume due to the lab-made hardware set up developed for the MEPS technique. Because the isoflavones water solubility is affected by the pH of the solution, we evaluated three different pH values: (i) an acidic point; (ii) an alkaline point; and (ii) a point without any pH change. The maximum peak area was obtained at the point without sample change (pH 4.6).

In the Pareto chart (Supporting Information Figure S3), it can be seen that the wash and desorption cycles are the variables that most affect the extraction procedure at the tested level. The number of sampling cycles significantly affects the extraction process, except for biochanin A. Wash cycles have a negative influence: increasing the number of cycles results in loss of analyte. Desorption cycles have a positive influence, and the greater the number of cycles, the higher desorption of the analytes is achieved. The number of sampling cycles has a positive influence; the higher the number of cycles, the more analyte is extracted in all cases. Based on these results we adopted 12 sampling cycles, two washing cycles, and six desorption cycles. The regeneration/conditioning and drying step is described in Section 2.4

3.4 | Method qualification

By verifying the absence or presence of possible interfering peaks at the retention time of the target compounds, as well as by checking the MRM transitions for each analyte, the method selectivity was investigated. Supporting Information Figure S4 shows a representative MS/MS chromatogram of a water sample that was spiked with DAI, GEN, FOR, and BIO at a concentration of 150 μg/L before the MEPS extraction procedure (black trace), and of a blank (unspiked) water sample absent of isoflavones (red trace). All peaks were successfully separated, confirming the method's satisfactory selectivity. A typical MS/MS chromatogram of a soy-based juice sample is displayed in Figure 3, showing the absence of interference peaks.

The correlation coefficients obtained were always above 0.993. In intraday precision, RSD values ranged from 3.0 to 15.5%, while interday ranged from 5.0 to 12.8%. The values determined for accuracy were between 93.3 and 123.3%. The method showed LOQs varying from 1.0 to 3.0 μg/L and LODs from 0.5 to 1.5 μg/L. The results of merit figures are shown in Table 1.

3.5 | Comparisons with other methods

Several analytical approaches have been employed for the analysis of isoflavones in soy-derived products [27], as well as in plants [28], oils [29], fishes [30], rumen fluid and bovine milk [31], and yogurt [32]. When compared with them, the proposed method shows LOQs and LODs,

TABLE 1 Weight model, linear regression equation, and accuracy and precision ($n = 4$) of the intra- and interday assays

Compound	Regression equation	R^2	Weight	Range (μg/L)	LOD (μg/L)	Spiking level (μg/L)	Accuracy (%)	Precision CV(%)	
								Intraday	Interday
DAI	$y = 0.0759x + 387.51$	0.994	–	1–200	0.5	5	94.4	11.6	9.7
						50	118.5	3.0	5.0
						150	102.4	4.8	9.3
GEN	$y = 0.0777x + 253.57$	0.997	–	2–200	1.0	5	105.0	10.9	10.9
						50	119.0	6.8	6.4
						150	104.8	3.4	9.8
FOR	$y = 0.0691x + 254.09$	0.993	–	3–200	1.5	5	93.3	15.5	11.8
						50	123.3	3.2	9.3
						150	105.3	5.8	12.5
BIOC	$y = 0.0915x + 7.93$	0.994	$1/x$	2–200	1.0	5	117.1	6.7	12.8
						50	119.9	9.1	9.9
						150	105.0	3.5	9.3

similar to those reported. However, the distinct advantages of the proposed method consist of its low consumption of solvents and residues generation, very low amount of sample, acceptable matrix effect, very low cost, and a simple sample preparation step. In addition to the low cost of lab-made MEPS hardware, the extraction phase can be reused several times (over 50 times in this work) without presenting any perceived deterioration. Also, the entire extraction process can be carried out in 5 min. These results confirm the excellent performance of the developed method.

3.6 | Application of the optimized method to the analysis of soy-based juice samples

After the evaluation of the figures of merit, we applied the method to the analysis of the target isoflavones in soy-based juice samples to evaluate its applicability. Samples from three different brands were found and purchased at São Carlos SP - Brazil markets. MEPS, using the synthesized β-CD@GO@Si as a sorbent material, combined with LC–MS/MS, was applied for the analyses of the soy-based juice samples after centrifugation at 4863 × *g* for 10 min and dilution (3:1) with ultrapure water.

Two of the four target isoflavones investigated were found in all analyzed samples (Figure 3). Daidzein and genistein are considered as the principal isoflavones of soy and soy products [33]. On the other hand, formononetin and biochanin A are of much lower abundance in soy products. Therefore, the results obtained are consistent with those suggested in the literature.

4 | CONCLUDING REMARKS

In this work, an MEPS procedure combined with HPLC–MS/MS was described for the analysis of isoflavones in soy-based juice samples. The development of new nanomaterial for use as sorbents, especially the use of graphene-derived materials in sample preparation, is currently a significant trend together with miniaturized sample preparation techniques. The extraction phase synthesized for this study, composed of graphene oxide and β-cyclodextrin, keeps the high absorption characteristics of graphene-based materials while improving the extraction selectivity for the target analytes. This results in a good sample clean-up, and in the absence of matrix effects, which is especially relevant when working with ESI-MS ionization sources.

The method is reproducible, accurate, of low-cost, with an adequate detection limit (0.5–1.0 μg/L), and presenting high sensitivity and selectivity. We show a straightforward and cheap alternative for the MEPS sample preparation micro-technique that can be used in most laboratories having standard equipment.

The proposed method was successfully applied to the analysis of isoflavones in soy-based juice samples. The results suggest that this approach could be expanded to the determination of the investigated isoflavones in other complex soy-derived products and other matrices as biological fluids.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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REFERENCES

1. Núñez O, Lucci P. New Trends in Sample Preparation Techniques for Food Analysis. Nova Science, New York, NY 2016.
2. Nazario CED, Fumes BH, da Silva MR, Lanças FM. New materials for sample preparation techniques in bioanalysis. J Chromatogr, B: Anal Technol Biomed Life Sci 2017;1043: 81–95.
3. Novoselov KS, Geim AK, Morozov SV, Jiang D, Zhang Y, Dubonos SV, Grigorieva IV, Firsov AA. Electric field effect in atomically thin carbon films supplementary. Science (80-). 2004;5:1–12.
4. de Toffoli AL, Maciel EVS, Fumes BH, Lanças FM. The role of graphene-based sorbents in modern sample preparation techniques. J Sep Sci. 2018;41:288–302.
5. Zhang X, Chen S, Han Q, Ding M. Preparation and retention mechanism study of graphene and graphene oxide bonded silica microspheres as stationary phases for high performance liquid chromatography. J Chromatogr A. 2013;1307:135–43.
6. Leudjo Taka A, Pillay K, Yangkou Mbianda X. Nanosponge cyclodextrin polyurethanes and their modification with nanomaterials for the removal of pollutants from waste water: A review. Carbohydr Polym. 2017;159:94–107.
7. Gentili A. Cyclodextrin-based sorbents for solid phase extraction. J Chromatogr A. 2020;1609, 460654.
8. Song X-Y, Ha W, Chen J, Shi Y-P. Application of β-cyclodextrin-modified, carbon nanotube-reinforced hollow fiber to solid-phase microextraction of plant hormones. J Chromatogr A. 2014;1374:23–30.
9. Wang C, Li T, Liu Z, Guo Y, Li C, Dong C, Shuang S. An ultrasensitive sensor based on β -cyclodextrin modi fi ed magnetic graphene oxide for detection of tryptophan. J Electroanal Chem. 2016;781:363–70.
10. Xu J, Wang Q, Xuan C, Xia Q, Lin X, Fu Y. Chiral recognition of tryptophan enantiomers based on β-cyclodextrin-platinum nanoparticles/graphene nanohybrids modified electrode. Electroanalysis. 2016;28:868–73.
11. Yu J, Wu Q, Qiao S, Yu Z, Jin N, Yu B. Simultaneous determination of phytoestrogens and key metabolites in breast cancer patients' urine by liquid chromatography-tandem mass spectrometry. J Pharm Biomed Anal. 2009;50:939–46.
12. Bustamante-Rangel M, Delgado-Zamarreño M, Carabias-Martínez R, Domínguez-Álvarez J. Analysis of isoflavones in soy drink by capillary zone electrophoresis coupled with electrospray ionization mass spectrometry. Anal Chim Acta. 2012;709:113–9.
13. López-Biedma A, Sánchez-Quesada C, Delgado-Rodríguez M, Gaforio JJ. The biological activities of natural lignans from olives and virgin olive oils: A review. J Funct Foods. 2016;26:36–47.
14. Pinho E, Grootveld M, Henriques M. Cyclodextrins as encapsulation agents for plant bioactive compounds. Carbohydr Polym. 2014;101:121–35.
15. Fumes BH, Lanças FM. Use of graphene supported on aminopropyl silica for microextraction of parabens from water samples. J Chromatogr A. 2017;1487:64–71.
16. Xu S, Zhang Z, Liu J, Wang Y, Hu J. Facile preparation of reduced graphene by optimizing oxidation condition and further reducing the exfoliated products. J Mater Res. 2017;32:383–91.
17. Hou X, Lu X, Niu P, Tang S, Wang L, Guo Y. β-Cyclodextrin-modified three-dimensional graphene oxide-wrapped melamine foam for the solid-phase extraction of flavonoids. J Sep Sci. 2018;41:2207–13.
18. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem. 2003;75:3019–30.
19. Validation of Analytical Procedures: Text and Methodology Q2(R1). Int Conf Harmon Tech Requir Regist Pharm Hum USE. 2005, https://doi.org/10.1590/S1984-82502011000100012.
20. Krishnamoorthy K, Kim GS, Kim SJ. Graphene nanosheets: Ultrasound assisted synthesis and characterization. Ultrason Sonochem. 2013;20:644–9.
21. Krishnamoorthy K, Veerapandian M, Zhang LH, Yun K, Kim SJ. Antibacterial efficiency of graphene nanosheets against pathogenic bacteria via lipid peroxidation. J Phys Chem C. 2012;116:17280–7.
22. Kohantorabi M, Giannakis S, Gholami MR. Supported PtxPd1-x bimetallic nanoparticles on ionic liquid-functionalized SiO2@graphene oxide nanocomposite and its application as an effective multiphasic catalyst. Appl Catal A Gen. 2019, 30–43.
23. Ran X, Yang L, Qu Q, Li S, Chen Y, Zuo L, Li L. Synthesis of well-dispersive 2.0 nm Pd–Pt bimetallic nanoclusters supported on β-cyclodextrin functionalized graphene with excellent electrocatalytic activity. RSC Adv. 2017;7:1947–55.
24. Taylor PJ. Matrix effects: The Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry. Clin Biochem. 2005;38:328–34.
25. González O, Blanco ME, Iriarte G, Bartolomé L, Maguregui MI, Alonso RM. Bioanalytical chromatographic method validation according to current regulations, with a special focus on the non-well defined parameters limit of quantification, robustness and matrix effect. J Chromatogr A. 2014;1353:10–27.
26. George R, Haywood A, Khan S, Radovanovic M, Simmonds J, Norris R. Enhancement and suppression of ionization in drug analysis using HPLC-MS/MS in support of therapeutic drug monitoring: A review of current knowledge of its minimization and assessment. Ther Drug Monit. 2018;40:1–8.
27. Aresta A, Di Grumo F, Zambonin C. Determination of major isoflavones in soy drinks by solid-phase micro extraction coupled to liquid chromatography. Food Anal Methods. 2016;9:925–33.
28. Visnevschi-necrasov T, Barreira JCM, Cunha SC, Pereira G, Oliveira MBPP. Advances in isoflavone profile characterisation

using matrix solid-phase dispersion coupled to HPLC/DAD in Medicago species. Phytochem Anal. 2015;26:40–6.
29. Zhao X, Ma F, Li P, Li G, Zhang L, Zhang Q, Zhang W, Wang X. Simultaneous determination of isoflavones and resveratrols for adulteration detection of soybean and peanut oils by mixed-mode SPE LC-MS/MS. Food Chem. 2015;176:465–71.
30. Merlanti R, Lucatello L, Jesus LDe, Rita M, Laverda S, Capolongo F. Isoflavones quantification in rainbow trout muscle by QuEChERS tecnique and liquid chromatography coupled with mass spectrometry. J Food Compos Anal. 2018;70:114–24.
31. Kasparovska J, Krizova L, Lochman J, Dadakova K, Kasparovsky T. Soybean-Derived Isoflavone Determination in Rumen Fluid and Milk by LC-MS-(TOF). J Chromatogr Sci. 2016;54:997–1003.
32. Kašparovská J, Dadáková K, Lochman J, Hadrová S, Křížová L, Kašparovský T. Changes in equol and major soybean isoflavone contents during processing and storage of yogurts made from control or isoflavone-enriched bovine milk determined using LC–MS (TOF) analysis. Food Chem. 2017;222:67–73.
33. Matoša Kočar M, Vila S, Petrović S, Rebekić A, Sudarić A, Duvnjak T, Markulj Kulundžić A, Josipović A. Isoflavone concentrations in soybeans suitable for growing in Europe. Genetika. 2019;51:48–59.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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CAPÍTULO 4

DESENVOLVIMENTO DE UMA NOVA ABORDAGEM *ON-LINE* PARA O ACOPLAMENTO *ON-LINE* ENTRE MICRO EXTRAÇÃO EM FASE SÓLIDA (SPME) E CROMATOGRAFIA LÍQUIDA (LC).

[3] **Automated needle-sleeve based online hyphenation of solid-phase microextraction and liquid chromatography.**
Luis Felipe da Silva, Deyber Arley Vargas Medina, Fernando Mauro Lanças
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Automated needle-sleeve based online hyphenation of solid-phase microextraction and liquid chromatography

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ABSTRACT

A novel approach for the online coupling of solid-phase microextraction (SPME) and liquid chromatography (LC) is introduced. An innovative Si@GO@βCD coated needle-sleeve extractant device was developed and then employed in the automated online SPME-LC-UV determination of estrogen-like isoflavones from human urine samples. The extractant SPME device is easily attachable at the endpoint of an analytical syringe needle and operated by a lab-made autosampler. Fully automated online SPME-LC is accomplished by proper autosampler programming to perform the following steps: i) the analytes extraction by direct immersion of the extractant device into the stirred sample, ii) a rinsing step iii) the analytes desorption/enrichment, iv) the online transference of the extract to the LC injection valve. Besides allowing the online SPME hyphenation, this extraction modality efficiently addressed the drawbacks associated with the clogging and dispersion of graphene-based microextraction techniques performed in packed-bed and dispersive formats. The main extraction parameters and the performance of the automated online SPME-LC method developed were carefully studied. The results show a good sensitivity, reliability, and straightforward analytical strategy for the determination of organic compounds in complex samples. The detection limit of the method was 20 μg L^{1} for DAI and 10 μg L^{-1} for GEN, FOR and BIO. The intra-day RSD was below 10% and inter-day RSD was below 13%. The total analysis time was less than 17 min per sample.

1. Introduction

Solid-phase microextraction (SPME) is one of the most powerful and widely spread modern sample reparation techniques. Its characteristics towards the green chemistry, simplicity, versatility, and robustness, make this technique an advantageous analytical tool. These include not only its performance face to traditional solid and liquid phase extractions (SPE, LLE) but also compared with other modern miniaturized sample preparation techniques [1]. Since its introduction in the early '90s [2], SPME has been under continuous evolution and development, gaining considerable and spread application in diverse areas of the analytical chemistry [3–5]. Nowadays, a wide diversity of coating materials, geometries, extraction devices, and modalities of the technique are available for coupling with chromatography and/or mass spectrometry analysis [6–9].

In the most popular SPME configuration, the technique relies on the use of a fuse-silica/stableflex/steel fiber coated with a thin layer of sorbent phase, through which analytes are simultaneously isolated and enriches via a non-exhaustive adsorption/absorption process. Analytes migrate from the sample matrix to the extraction phase by passive diffusion until the equilibrium is reached. Hence, the trapped analytes are transferred to the analytical instrument by fiber heating into the injection port (GC) or by application of desorption solvent (LC) [10].

Under non-equilibrium conditions, manual SPME can be prone to errors, and low reproducibility, hence technique automation/hyphenation can be determinant in the accomplishment of improved robustness, precision, and sample-throughput [11]. Coupled with gas chromatography (GC), SPME can be easily automated through the autosampler system [12,13]. In this case, fiber transport, exposition (extraction), retraction, and injection are robotically performed in the at-line mode [14]. Although most current GC autosamplers hold SPME capabilities [15], they are restricted to specific functionalities, with limited flexibility for adaptation to new sample preparation methods. Also, their cost is still a limiting factor in many research laboratories.

On the other hand, the requirement of solvent desorption for non-volatile or thermally labile analytes has made the automated hyphenation of SPME with liquid chromatography (LC) more challenging and the development of automated interfacing strategies for this purpose is

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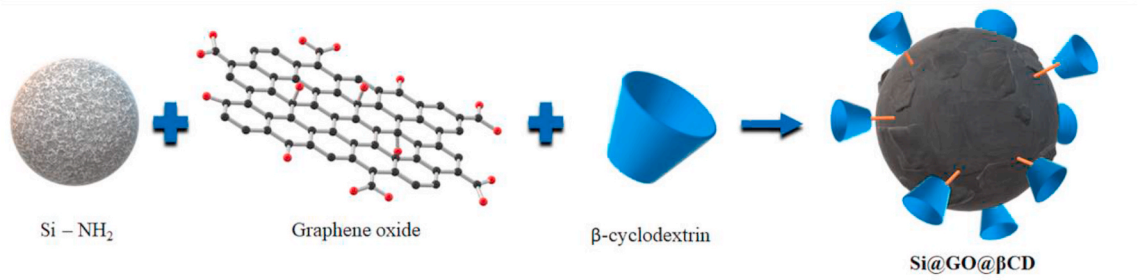

Fig. 1. Schematic representation of the synthesized extraction phase Si@GO@βCD.

scarce in the modern sample preparation literature [16,17]. All the previously reported attempts to interface fiber-based SPME with LC have involved the incorporation of solvent desorption interfaces, injection tees, and multi-port valves between the autosampler and the analytical column, requiring some additional manual steps [18–22]. Hitherto, the most advantageous automated coupling alternative, is the so-called in-tube SPME [23]. It is considered a multidimensional technique in which the HPLC injector is connected to a miniaturized extraction column, where the sample is injected and drawn in. Analytes are selectively extracted, preconcentrated in an SPME column, and sequentially transferred to the analytical column by valve switching [24]. Although in-tube SPME might be a very versatile strategy, the miniaturized extraction columns are frequently clogged and overpressurized, especially when packed with graphene-based sorbents [25,26].

Graphene (G) is a modern two-dimensional material, with a strong non-polar and hydrophobic character derived from its large delocalized *p*-electron system of sp^2-hybridized carbon atoms, which confers attractive retention capabilities for carbon-based ring structures [27, 28]. In recent years, G, GO, and its composites have widely been explored as sorbents in different SPME modalities, demonstrating applicability to the uptake of diverse organic compounds from environmental, clinical, forensic, and food-surveillance matrices [29–31]. Nevertheless, graphene-based sorbents are subject to drawbacks, such as i) irreversible material aggregation, which makes the packed-beds prone to clogging and high backpressures, and ii) excessive material dispersion, which makes difficult its recovery in dispersive extractions, even after filtration or centrifugation [31,32].

Diffeent Cylindrical, humanoid and Cartesian robots for automated liquid- and solid phase extraction are reported in the literature. This approach reduces operational workload, minimize the analyst exposure, possibility of working with small amounts of sample while maintaining or even improving reproducibility, and contributes to green chemistry with reduced waste [33–35]. In this paper, we presented an innovative approach for fully automated online coupling SPME and LC, fully compatible with the design and development of graphene-based coatings. A new needle-sleeve SPME device was developed. It is a propylene sleeve, attachable to the end of any analytical syringe needle so that after analyte extraction and solvent-desorption, the enriched extract can be easily aspirated/collected into the syringe barrel. The fiber-sleeve SPME device was coated a lab-made selectivity Si@GO@βCD [36].

As a model application, the developed automated needle-sleeve SPME setup was assessed in the determination of isoflavones (daidzein, genistein, formononetin, and biochanin A) in human urine samples. Isoflavones are weak estrogenic agonists, whose adverse or beneficial effects on human health are still controversial, so the development of fast and efficient methods for their determination in biological matrices allows for assessing the human exposure to these phytochemicals, is currently relevant and necessary [37,38].

Finally, all the proposed setup was operated by a lab-made autosampler [39] with high freedom capabilities for automated sample preparation [40–42], resulting in a versatile, simple, fast, and fully automated method, free of additional sample reconstitution steps for LC and or MS analysis.

2. Experimental

2.1. Reagents

High purity analytical standards (≥99%) of daidzein (DAI), genistein (GEN), formononetin (FOR), and biochanin A (BIO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The standard stock solutions (1000 mg L^{-1}) were prepared by weighing the required amount of each solid analytical standard and dissolving in HPLC grade methanol (Fairfield, OH, USA) and stored at −7 °C. These stock solutions were diluted with ultrapure water (18.2 MΩ cm) obtained from a Milli-Q water purification system from Millipore (Bedford, MA, USA) and used to prepare a mixed solution of isoflavones (work solution). The mixed solutions were used for spiking the samples for the method evaluation. All solutions were stored at -7 °C, and the work solutions were freshly prepared each week.

Graphite powder, potassium permanganate ($KMnO_4$), dimethylformamide (DMF), amino-functionalized silica gel spherical (Si) N-ethyl-N'-(3-dimethylamino propyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), (3-aminopropyl) trietoxisilane (APTES) were all acquired from Sigma-Aldrich (St Louis, EUA); H_2O_2 from Synth (Diadema, Brazil); H_2SO_4 from Tedia (Fairfield, OH, USA); HCl from Qhemis (Jundiai, Brazil), β-cyclodextrin (>97%) from Hewlett Packard (Palo Alto, CA, USA).

Methanol (MeOH), acetonitrile (ACN), chromatographic analysis grade, were purchased from Tedia (Fairfield, OH, USA). Formic acid 96% acquired from Sigma-Aldrich (St. Louis, MO, USA).

For the LC-UV method development, standard solutions of each isoflavone (100 μg mL^{-1}) were prepared in human urine; automated online SPME was carried out without any further sample pretreatment.

2.2. Synthesis of Si@GO@βCD

Si@GO@βCD sorbent was prepared following the procedure previously described by Silva and Lanças [36]. Graphite oxide was obtained based on the Hummers' method under optimized conditions [43]. Fine graphite oxide powder was dispersed in 50 mL of ultrapure water (1 mg mL^{-1}) and sonicated for 1 h up to obtain graphene oxide (GO). After sonication, the pH of the solution was adjusted to 4.0 with formic acid, and 0.4 mL of 10 mM EDC/5 mM NHS solution was added and stirred for 30 min at room temperature. Then 1.0 g of aminopropyl silica (Si) was added to the dispersion and stirred for 4 h at room temperature. Then, the dispersion was filtered, and the recovered Si@GO was washed with methanol and dried at 60 °C for 24 h. A solution with 1 g of β-cyclodextrin and 50 mL of DMF was stirred for 15 min at room temperature and then mix with 3 mL of (3-aminopropyl) trietoxisilane (APTES) under stirred conditions for 5 h and at room temperature. Finally, 1 g of Si@GO was added and stirred for 2 h at room temperature. The resulting Si@GO@βCD was filtered and dried at 60 °C for 48 h. Fig. 1 shows a schematic representation of the synthesis process.

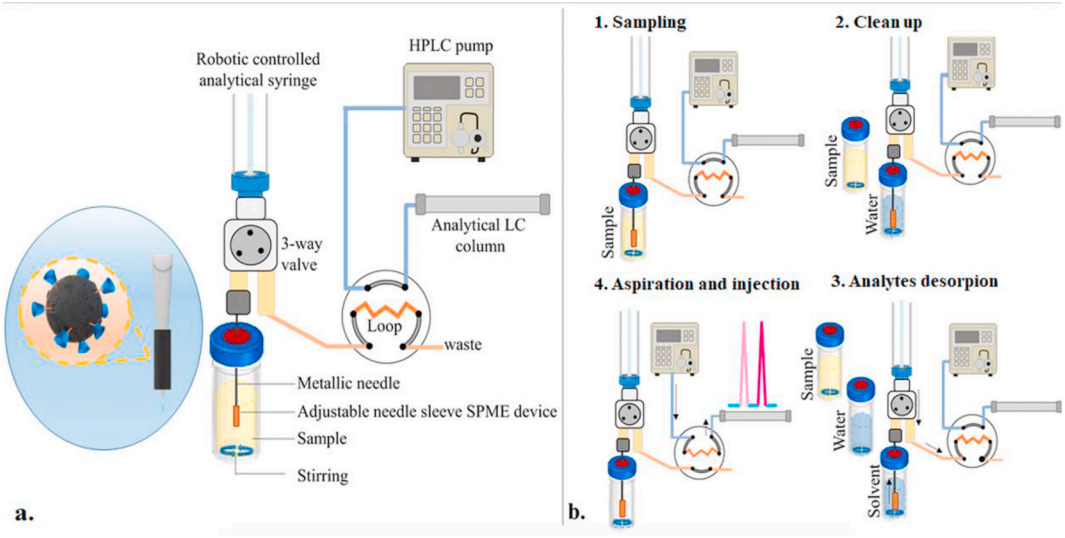

Fig. 2. Schematic representation of a) the developed automated SPME setup and, b) the automated online SPME procedure.

2.3. Adjustable needle sleeve SPME device fabrication

Removable needle-sleeve SPME devices were prepared from a disposable propylene pipette tip (5 μL). The endpoint of the pipette tip was rinsed with methanol and dried. A coating mixture was prepared by mixing 2 parts of Si@GO@βCD and 1 part of Araldite® glue (Tek Bond) up to obtain a homogenous dispersion and subsequently deposited with a spatula over the external surface of the pipette tip, covering a segment with 1 cm length and ~1 mm thickness layer. After dried by 24 h, the coated tip was rinsed by immersion in 20 mL methanol and water at an ultrasound bath for 1 h. Finally, for SPME sample preparation, the tip was cut, and the coated piece attached at the endpoint of a metallic needle of a robotically controlled analytical syringe (Fig. 2a).

2.4. Automated online needle-sleeve based SPME

All of the steps of the SPME procedure, including extraction, fiber clean up, solvent desorption, and injection into the HPLC system were auto-performed with a lab-made autosampler [39], controlled through a dedicate Arduino Sketch, developed for this specific purpose (Supplementary Material S1). The autosampler was hyphenated to the HPLC system through a three-way solenoid valve (NResearch Inc.). In the "off" position, it connects the syringe barrel to the metallic needle and in the "on" position with the HPLC injection Loop [41,42], as described in Fig. 2b.

Initially, equipped with an empty 100-μL gastight analytical syringe and the solenoid valve in the off position, the autosampler moves to a sampling position, introducing the needle equipped with the SPME sleeve device into a 25 mL beaker containing 20 mL of human urine. Extraction takes place under magnetic stirring, and once it is over, the system moves to the clean up position, rising the needle sleeve SPME device with 0.5 mL of water. Sequentially, the autosampler moves to the solvent desorption position, introducing the needle sleeve SPME device into 0.1 mL of MeOH for analytes desorption. Finally, the enriched extract is harvest into the syringe barrel, the valve commuted to the "on" position, and the extract injected into a 2-μL sampling loop. While the chromatographic separation occurs, the autosampler moves to the washing position and rinse the SPME device and the transference line with MeOH, preparing the system for the next extraction.

Clean up, desorption, and washing were carried out under vibrational shaking. For this purpose, the autosampler was equipped with a cell phone vibrational motor, commanded by a double H-bridge L298 N motor drive and controlled through the same automated SPME Arduino Sketch.

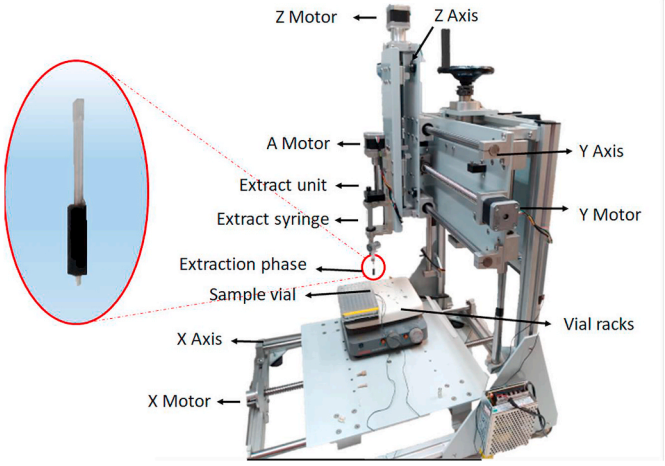

Fig. 3. Schematic representation of the developed Si@GO@βCD coated needle-sleeve device and the autosampler made in our lab.

2.5. Chromatographic conditions

For LC-UV analysis, chromatographic separation was performed using a Shimadzu LC system (Kyoto, Japan). It was composed of a CBM-20 A communication bus module, a DGU-20AS degasser, two LC 20-AD pumps, a CTO-20 A column oven, and an SPD-20 A UV–Vis detector, monitoring at 260 nm. Separation of the compounds after the SPME procedure was achieved in gradient elution mode. Water (phase A) and acetonitrile (phase B) were selected as the mobile phase, both with 0.1% formic acid. From 0.0 to 1.0 min, the mobile phase remains 30% B. From 1.0 to 4.0 min, the mobile phase changed linearly from 30% B to 50% B, and then maintained in that condition (50%) up to 9 min. From 9.0 to 9.5 min, the mobile phase changed linearly from 50% B to 90% B and then maintained in that condition (90%) up to 12.5 min returning to the 30% at 13.0 min. From 13 to 16.5 min, the column is conditioned for the next analysis. Separations were accomplished through a Kinetex Core-Shell C18 column (100 mm × 2.1 mm i. d., 2.7 μm particle size) from Phenomenex (Torrance, CA, EEUU) at a flow rate of 0.20 mL min^{-1} and the column temperature was set at 42 °C.

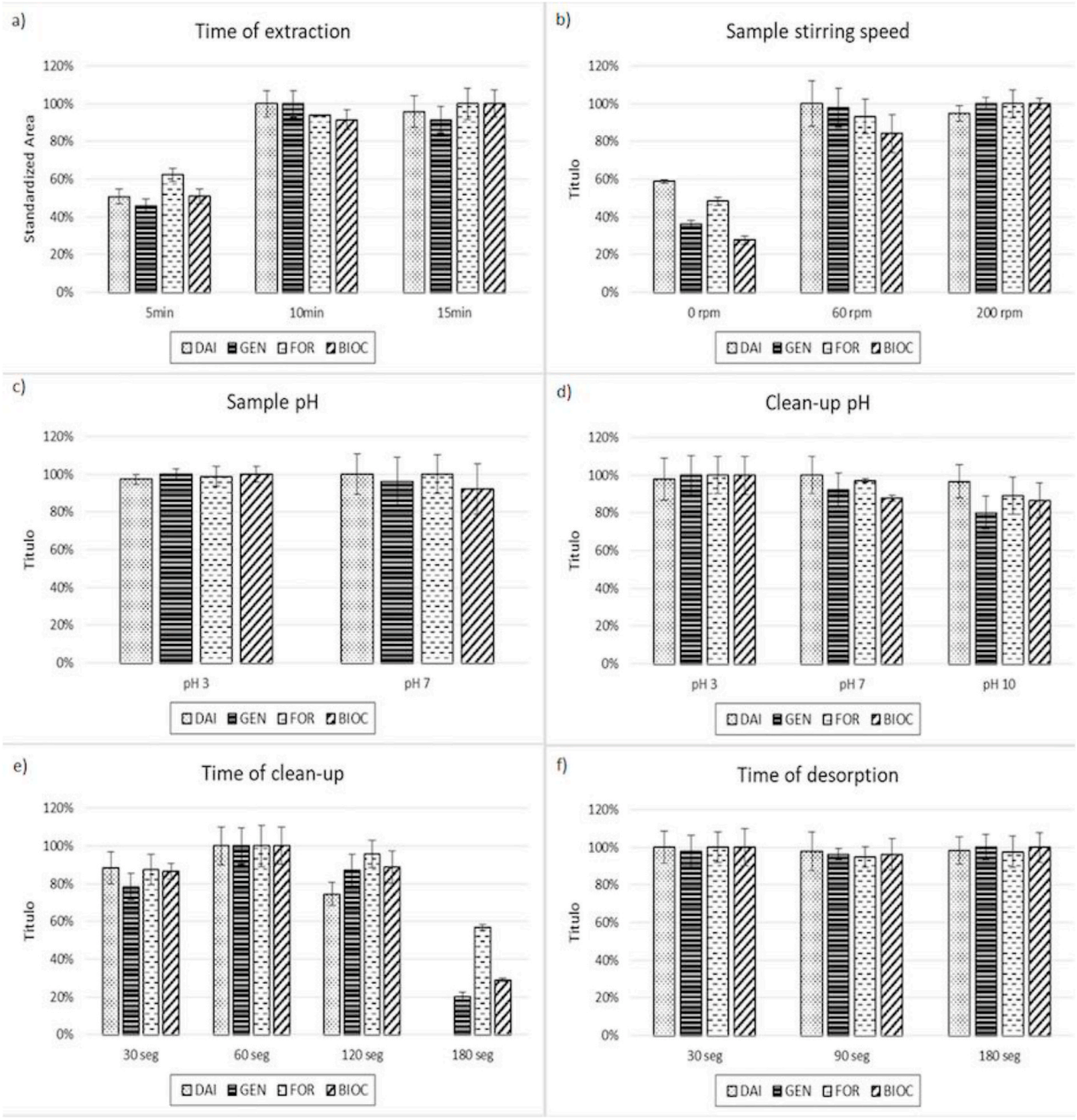

Fig. 4. Results of the study of the sample preparation step variables, where a) corresponds to the time that extraction phase has been in contact with the sample and b) sample stirring speed and c) pH of sample and d) pH of water of clean-up step and e) time that extraction phase has been in contact with the water to removal interferents and f) time that extraction phase has been in contact with MeOH to the desorption of analytes and response for each variable.

3. Results and discussion

3.1. Developed automated needle-sleeve SPME setup

Fig. 3 show a picture of the final appearance of the developed Si@GO@βCD coated needle-sleeve device and the lab-made autosampler. The synthesized extraction phase was studied by SEM image, FT-IR spectroscopy, and by thermogravimetric analysis as previously discussed by Silva and Lanças [36]. The Si@GO@βCD TGA graph, depicted in Supplementary Material as S2, shows a weight loss of approximately 7% occurring around 300 °C, through the examination of the derived weight (%/°C) curve. This graph shows a defined event of weight loss in this temperature range, corresponding to β-CD, and an approximately 17% weight loss around 600 °C, corresponding to GO [44].

The developed Si@GO@βCD coated needle-sleeve device presented suitable mechanical resistance and stability, even under the extensive use and 100% organic solvent conditions. No glue or residual interferences derived from the coating were observed in the chromatographic profiles after blank extractions. One single extractant device was used over 200 times without appreciable loss of its extraction capability.

The complete extraction procedure here developed was automated by a lab-made autosampler, whose flexibility allows easy adaptation of the overall online SPME process. Sampling was performed in a 25 mL beakers, where convection forces can be easily provided by magnetic stirring. Nevertheless, the clean-up and desorption stages were carried out in translucent polypropylene copolymer (PPCO) conical-bottom tubes of 2 mL and 250 μL, respectively. In this case, the convection force assistance was accomplished through the integration of a coin flag vibrational mini-motor, recovery from a recycled cell phone. The vibrational motor was installed under the conical-bottom tubes rack,

and integrated to the central control circuit through an L298 N dual H-bridge module being feed by the autosampler power supply and controlled by the central Arduino Mega 2560. A schematic design of the control circuit is presented in the supplementary information (Fig. S1).

3.2. Evaluation of the SPME influencing variables

SPME procedures typically involve steps as sorbent exposition, clean-up, analytes desorption, injection, and washing/conditioning for the next extraction. Here, to successfully apply automated needle-sleeve SPME for the determination of isoflavones in urine samples, the complete SPME procedure was programmed in the Arduino Integrated Development Environment (IDE). The developed Arduino® sketch and an illustrative video of the whole extraction process are attached as supplementary information. Later, the operational autosampler parameters and the experimental factors influencing the extraction efficiency —including time, stirring, and pH — at different stages were investigated using univariate improvement.

3.2.1. Effect of the extraction time

SPME is a non-exhaustive technique, so that the extraction time is an essential factor to be controlled, mainly when the extraction is performed under non-equilibrium conditions. The analytes uptake increases at longer extraction times until the equilibrium condition is reached. Suitable extraction time is an important parameter to obtain a practical compromise between method sensitivity and analytical throughput [15]. This study evaluated the influence of the extraction time in intervals of 5, 10, and 15 min. As a result, the longer the exposure time provided, the higher was the recovery of the analytes. The equilibrium was obtained after 10 min of extraction, as shown in Fig. 4 a), and selected for the subsequent experiments.

3.2.2. Sample stirring speed effect

The sample stirring can affect the velocity and efficiency of the extraction of the analytes. It is a physical matter for promoting the interaction between the analytes and the extraction phase. Theoretically, higher stirring rated could favor faster analytes transference, from the sample to the extraction phase. In this case, high stirring rates resulted in the formation of a vortex in the center of the sample container, hindering the effective contact between the sample and the needle-sleeve SPME device. To avoid that effect and boost the mass transference, we evaluated the efficiency of the extraction at 60 rpm, 200 rpm, and without magnetic stirring. The result shows that increasing the agitation of the sample increase the peak area of all analytes (Fig. 4 b). Then the stirring speed chosen was 200 rpm, the maximum possible without generating a vortex that would disturb the contact of the extraction phase and the sample.

3.2.3. Sample pH

The sample pH can modify the solubility of the analyte in the matrix and promote the interaction between the analytes and the extraction phase, increasing the extraction and decreasing the limits of detection and quantification. Isoflavones are slightly acidic compounds with multiple hydroxyl groups and estimated pKa values below 7.0. Hence, sample acidification should discourage isoflavones deprotonation, improving their affinity for hydrophobic extraction phases. For example, Satterfield et al. observed that the SPME efficiency improves for daidzein but worsens for genistein when the pH of the sample changed from pH 7 to pH 4, and a carbowax fiber was employed [45].

On the other hand, cyclodextrins are macromolecules with a hydrophobic inner cavity and a hydrophilic outer surface. Although their interaction with the analytes mainly depends on steric factors, the host-guest affinity also can be affected by electrostatic, dipole-dipole and H-bond interactions, so that the ionic form of the analyte can be a determinant factor [46]. Here, the influence of the urine sample pH was investigated in the pH range from 3 to 7 (adjusted with formic acid or NH_4OH). No statistically significant variations on the extraction efficiency were observed (Fig. 4 c). Therefore, we chose that the original sample pH (pH 6) would not be modified, maintaining sample preparation step as simple as possible.

3.2.4. Clean-up solution

The clean-up stage aims the selective of co-extracted matrix interferents removal, without significant analytes loss. Hence, the composition of the clean-up solution should be carefully selected.

In the same way, the pH of the clean-up solution can affect the solubility of interferers and analytes. The influence of the clean-up pH was evaluated at 3, 7, and 10 (adjusted with formic acid or NH_4OH). The result shows that at pH 3, the largest peak area was obtained for each analyte (Fig. 4 d).

3.2.5. Effect of the clean-up time

For matrix interferents removal, after extraction, the needle-sleeve SPME device was automated immersed in 0.1 mL of clean-up solution as stirred under vibrational conditions. Clean-up time was evaluated at 30, 60, 90, and 180 s. As a result, 60 s of clean-up step shows the best correlation of the elution of matrix interferents and retention of target analytes, resulting in the largest signal area of all analytes (Fig. 4 e). This result suggests that less exposure time is insufficient to obtain adequate cleaning, and matrix interferences disturb the analysis. On the other hand, a long cleaning period elutes the target analytes.

3.2.6. Desorption conditions

In a typical off-line SPME-HPLC method, the analytes are efficiently desorbed in a considerable amount of organic solvent. A further drying and resuspension in a reduced portion of the initial mobile phase usually follow this step to grants a maximum injection concentration and full compatibility with the chromatographic conditions. In online methods, not dried/resuspension is possible, so that desorption volume should attend the compromise between efficient analytes removal and a suitable concentration of the injection solution. Here, for compatibility with elution chromatographic conditions, 100% MeOH was chosen as the desorption/injection solvent. In the same way, for analyte desorption, the needle-sleeve device was fully immersed in 0.1 mL of desorption/injection solvent and stirred under vibrational conditions. The influence of the desorption time was evaluated at intervals of 30, 90, and 180 s. As a result, equilibrium was achieved in 30 s (Fig. 4 f). Then it was chosen as the method's desorption time.

After analytes desorption, the enriched extract was automatically collected into the syringe barrel, the solenoid valve switched and the enriched extract transferred to the HPLC injection valve, so that the chosen desorption volume also was enough to grants the effective filling of the transference Robot-HPLC lines (30 μL).

3.2.7. Needle-sleeve SPME device washing

To avoid carry-over between extractions, after analytes desorption and extract injection, the robot leads the extractant setup up to the washing position, where the needle-sleeve device was cleaned by immersion in 0.5 mL of methanol, under vibrational stirring conditions by 1 min. After, the robot leads the extractant setup up to the second washing position with 0.5 mL of methanol and draw/inject 3 times with the solenoid valve in looping charge position to clean the transference Robot-HPLC lines. Carry-over was assessed by blanc extraction between samples. No carry-over effect was observed in any case.

3.3. Analytical performance of the needle-sleeve based online SPME-LC-UV method

Under the selected experimental conditions, the analytical performance of the automated needle-sleeve based SPME-LC-UV method was evaluated (Table 1). Figures of merit including selectivity, the limit of detection (LOD), the limit of quantification (LOQ), linearity, accuracy,

Table 1
Analytical performance of the automated needle sleeve based SPME procedure.

Analyte	Linear range	Intercept	Slope	r^2	LOD		Accurace	Intraday RSD	Interday RSD	EF	ER %
	($\mu g\ L^{-1}$)		($\mu g\ L^{-1}$)		($\mu g\ L^{-1}$)	($\mu g\ L^{-1}$)	%	%	%		
Daidzein	40–300	−4337.72	130.529	0.995	20	40	119	9	13	5.26	2.63
						100	91	4	7		
						300	93	6	6		
Genistein	20–150	−4465.13	317.159	0.996	10	20	119	8	11	4.68	2.34
						50	93	6	6		
						150	98	2	4		
Formononetin	20–150	−284.525	95.519	0.996	10	20	114	10	9	3.86	1.93
						50	89	4	5		
						150	109	3	3		
Biochanin A	20–150	−3763.78	445.54	0.993	10	20	119	9	6	7.75	3.88
						50	91	3	4		
						150	110	3	5		

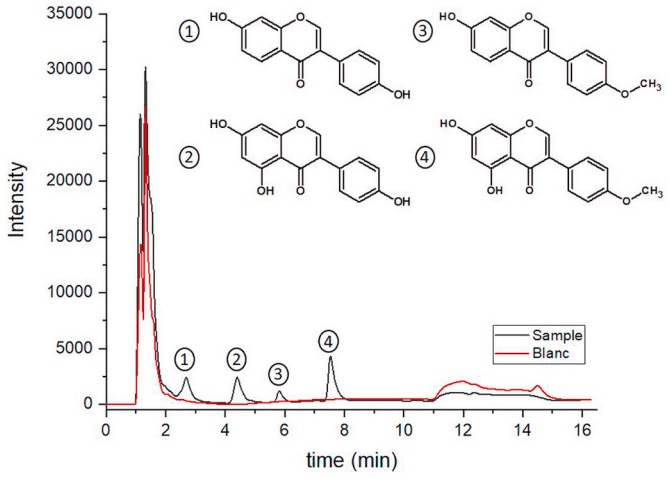

Fig. 5. Chromatogram obtained after needle-sleeve SPME extraction and enrichment of the target analytes spiked with 150 $\mu g\ L^{-1}$ (1) Daidzein; (2) Genistein; (3) Formononetin; (4) Biochanin A.

precision, enrichment factor (EF) and extraction recovery (ER), were determined to evaluate if the method would meet the requirements for routine isoflavones analysis according to international guidelines.

The limits of detection (LODs) were determined as the minimum concentration for which a signal-to-noise ratio ≥ 3 was obtained and ranged from 10 to 20 $\mu g\ L^{-1}$. Calibration curves were obtained from the quantification limits (signal/noise ≥ 10) up to 300 $\mu g\ L^{-1}$ for DAI and 150 $\mu g\ L^{-1}$ for GEN, FORM, and BIOC, by plotting the peak areas at five levels of concentration, in triplicate experiments. Linear responses with coefficients of determination (r^2) between 0.993 and 0.996 were obtained. The precision and accuracy were evaluated by the relative standard deviations (RSD (%)) for six replicates at three different isoflavones concentration. In intra- and inter-day precision were assessed at three levels of concentration and measured as relative standard deviation (% RSD), ranging between 2.0 - 10% and 2.0–13%, for In intra- and inter-day experiments, respectively. The values determined for accuracy were between 89 and 119%, showing the model's adequate adjustment to the experimental data. The enrichment factors (*EF*) were calculated as the ratio between the analyte concentration in the enriched final extract and the original sample ($EF = C_{elution\ solvent}/C_{sample}$). The concentration in the enriched final extract was obtained from the calibration curve obtained by direct injection (using the same online injection setup) of standard solutions of isoflavones in methanol. *EFs* between 3.86 and 7.75 were obtained under the selected conditions. The extraction recovery (*ER*), defined as the percentage of total analyte in the sample (n_{sample}) extracted by the drop ($n_{elution\ solvent}$), was calculated as the product of the *EF* by the phase ratio ($V_{elution\ solvent}/V_{sample}$) by 100, according to eq. (1)

$$ER = \left[\frac{n_{elution\ solvent}}{n_{sample}}\right] * 100 = \left[\frac{C_{e.solvent} * V_{e.solvent}}{C_{sample} * V_{sample}}\right] * 100 = \left[\frac{V_{e.solvent}}{V_{sample}}\right] EF * 100 \quad (1)$$

Finally, the absence of carry-over effects was established by using extraction on blank samples (20 mL of ultrapure water, under optimal extraction conditions). The experiments were carried out employing the same needle-sleeve device, performed at the beginning and end of each day, as well as between aleatory samples, during the overall method development. In any case, remaining analytes or sample interferences were observed in the blank samples.

Fig. 5 shows typical chromatograms obtained from the extraction of a sample spiked with 150 $\mu g\ L^{-1}$ of isoflavones. After the automated needle-sleeve based extraction, the extract is automatically injected into the online hyphenation SPME-LC-UV system. The developed SPME-LC-UV method allowed the extraction and online determination of the target analytes in 16.5 min, allowing the perfect synchronization between the sample preparation and chromatographic analysis.

Compared to previously reported methods using sorbent-based for sample preparation of isoflavones, the developed automated needle-sleeve SPME approach offers several advantages. They include its easiness, practicability, superior extraction-phase reuse, and high sample throughput, with competitive reliability and sensitivity.

Satterfield and coworkers developed an online SPME-HPLC-MS to determine the isoflavone aglycones genistein and daidzein in urine [45]. After manual extraction, the SPME fiber was desorbed in a commercially available desorption interface. Finally, after valve switching, the fiber was manually removed and cleaned to avoid further carry over. The total SPME method takes around 16 min without taking into account the fiber cleaning steps between extractions. Similarly, Aresta et al. developed an online SPME-HPLC method to determine major isoflavones in Soy drinks [47]. Also, in this case, extraction, injection, and fiber clean-up were manually performed, and the whole extraction method takes more than 55 min.

Mitani et al. developed a fully automated online method for the determination of daidzein and genistein in soybean foods by in-tube SPME coupled to HLC-DAD analysis [48]. Sample preparation was carried out by direct injection of 40 µL of the raw sample into a Supel-Q porous layer open tubular (PLOT) capillary column (60 cm × 0.32 mm ID, 12-µm film thickness, Supelco, Bellefonte, PA, USA). After column washing and conditioning with methanol and water and by two repeated draw/eject cycles (40 µL, each) with those solvents, isoflavones were extracted with 20 draw/eject cycles of the sample at 100 µL/min flow rate and then eluted to the analytical column with mobile phase by valve switching. According to the trap-column and load flow rate specifications, this method should take around 20 min for sample preparation plus an additiona l8 min for chromatography separation. Once in this type of setup it is not possible to prepare a new sample while

Table 2
Comparison between previously described sorbent-based extraction methods for isoflavones determination.

Method	Extraction phase	Matrix	Analysis	total analysis time (min)	LOD (ng mL^{-1})	Ref.
Manual SPME	Carbowax	human urine	HPLC-MS	>16	0.0027–0.025	[40]
Manual SPME	PDMS/DVB	Soy-based products	HPLC-DAD	>50	1.08–2.03	[42]
In-tube SPME	DVB	Soy-based products	HPLC-DAD	~28	0.41–0.48	[43]
Manual SPE	SiO_2MIP	human urine	HPLC-UV/DAD	–	0.04–0.06	[44]
Manual SPE	C-18	human urine	-UHPLC-PDA-FLR	–	1.21–4.10	[45]
Fully automated SPME	Si@GO@βCD	human urine	HPLC-UV	~17	10–20	This work

the previous one is being analyzed, the sample preparation and chromatographic stages can not be performed simultaneously.

Finally, some SPE-HPLC methods for determination on isoflavones in human urine have been reported too. For example, Chrzanowska et al. prepared a selective molecularly imprinted silica polymer (SiO_2MIP) for manual SPE of biochanin A, daidzein [49], and Redruello et al. employed conventional C18 sorbent [50]. Although provides good recovery, SPE methods are tedious, laborious, time-consuming, and requires a larger amount of extraction phases, solvents, and samples than its miniaturized versions.

The method here proposed has good sensitivity and precision with an adequate dynamic linear range. The main perceived advantage of the proposed method is the complete automation of analysis and the short time of all processes since sample preparation step, chromatographic separation, until the detection. Almost four analyses can be performed per hour, in a synchronized and uninterrupted way. The automated needle-sleeve based online hyphenation SPME-LC-UV is an approach that enables a wide range of applications in various liquid samples with varying sample volumes. When used in an automated process, it is possible to preform the whole sample preparation step with just one needle and syringe, being the extract directly injected into the HPLC loop. The synthesized extraction phase based on cyclodextrin coupled to a graphene composite shows outstanding durability, while improving the extraction power and selectivity. These characteristics allowed us to use just one needle-sleeve device 200 times without appreciably changing its extraction capabilities. Table 2 shows a comparison between previously described sorbent-based extraction methods for isoflavones determination.

4. Conclusion

An advantageous strategy for fully automated online coupling of SPME and liquid chromatography was developed, optimized, and validated. An innovative needle sleeve SPME device was introduced and applied to the simultaneous and reliable quantification of daidzein, genistein, formononetin, and biochanin A in human urine, in a fully automated setup, showing suitable sensitivity and analytical confidence. Although not as low as other previously reported SPME methods, the obtained LODs are low enough to detect the typically reported concentration levels of isoflavones in human urine, which could be improved by coupling of higher sensitivity detectors such as a mass spectrometer. Besides, the feasibility, simplicity, and efficiency of analysis, derived from the automation of the process and its synchronized and uninterrupted way, makes this approach a reliable and advantageous analytical tool for the determination of organic compounds in complex matrices via liquid chromatography. Finally, the several times reusable device and the few amounts of selective extraction phase and organic solvents employed confers to the needle-sleeve SPME technique green and low-cost characteristics.

Authorship contribution statement

Luis Felipe da Silva: Conceptualization, Methodology, Formal analysis, Writing - original draft. Deyber Arley Vargas Medina: Conceptualization, Resources, Methodology, Software, Writing - original draft. Fernando Mauro Lanças: Conceptualization, Funding acquisition, Supervision, Resources, Funding acquisition, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.121608.

References

[1] V. Jalili, A. Barkhordari, A. Ghiasvand, A comprehensive look at solid-phase microextraction technique: a review of reviews, Microchem. J. 152 (2020) 104319, https://doi.org/10.1016/j.microc.2019.104319.
[2] S. Huang, G. Chen, N. Ye, X. Kou, F. Zhu, J. Shen, G. Ouyang, Solid-phase microextraction: an appealing alternative for the determination of endogenous substances - a review, Anal. Chim. Acta 1077 (2019) 67–86, https://doi.org/10.1016/j.aca.2019.05.054.
[3] M. Sajid, M. Khaled Nazal, M. Rutkowska, N. Szczepańska, J. Namieśnik, J. Płotka-Wasylka, Solid phase microextraction: apparatus, sorbent materials, and application, Crit. Rev. Anal. Chem. 49 (2019) 271–288, https://doi.org/10.1080/10408347.2018.1517035.
[4] A. Naccarato, A. Tagarelli, Recent applications and newly developed strategies of solid-phase microextraction in contaminant analysis: through the environment to humans, Separations 6 (2019) 2–43, https://doi.org/10.3390/separations6040054.
[5] M. Lashgari, V. Singh, J. Pawliszyn, A critical review on regulatory sample preparation methods: validating solid-phase microextraction techniques, TrAC Trends Anal. Chem. (Reference Ed.) 119 (2019) 115618, https://doi.org/10.1016/j.trac.2019.07.029.
[6] G.A. Gómez-Ríos, J. Pawliszyn, Development of coated blade spray ionization mass spectrometry for the quantitation of target analytes present in complex matrices, Angew. Chem. Int. Ed. 53 (2014) 14503–14507, https://doi.org/10.1002/anie.201407057.
[7] D.M. Cárdenas-Soracá, V. Singh, E. Nazdrajić, T. Vasiljević, J.J. Grandy, J. Pawliszyn, Development of thin-film solid-phase microextraction coating and method for determination of artificial sweeteners in surface waters, Talanta 211 (2020) 120714, https://doi.org/10.1016/j.talanta.2020.120714.
[8] J. Xu, S. He, R. Jiang, F. Zhu, J. Ruan, H. Liu, T. Luan, G. Ouyang, Disposable solid-phase microextraction fiber coupled with gas chromatography-mass spectrometry for complex matrix analysis, Anal. Methods. 6 (2014) 4895–4900, https://doi.org/10.1039/C4AY00230J.
[9] R. Jiang, J. Pawliszyn, Thin-film microextraction offers another geometry for solid-phase microextraction, TrAC Trends Anal. Chem. (Reference Ed.) 39 (2012) 245–253, https://doi.org/10.1016/j.trac.2012.07.005.
[10] N. Reyes-Garcés, E. Gionfriddo, G.A. Gómez-Ríos, M.N. Alam, E. Boyacı, B. Bojko, V. Singh, J. Grandy, J. Pawliszyn, Advances in solid phase microextraction and perspective on future directions, Anal. Chem. 90 (2018) 302–360, https://doi.org/10.1021/acs.analchem.7b04502.

[11] I. Ali, V.K. Gupta, H.Y. Aboul-Enein, A. Hussain, Hyphenation in sample preparation: advancement from the micro to the nano world, J. Separ. Sci. 31 (2008) 2040–2053, https://doi.org/10.1002/jssc.200800123.
[12] C.L. Arthur, L.M. Killam, K.D. Buchholz, J. Pawliszyn, J.R. Berg, Automation and optimization of solid-phase microextraction, Anal. Chem. 64 (1992) 1960–1966, https://doi.org/10.1021/ac00041a034.
[13] R. Eisert, J. Pawliszyn, Design of automated solid-phase microextraction for trace analysis of organic compounds in aqueous samples, J. Chromatogr., A 776 (1997) 293–303, https://doi.org/10.1016/S0021-9673(97)00332-4.
[14] S. Risticevic, Y. Chen, L. Kudlejova, R. Vatinno, B. Baltensperger, J.R. Stuff, D. Hein, J. Pawliszyn, Protocol for the development of automated high-throughput SPME-GC methods for the analysis of volatile and semivolatile constituents in wine samples, Nat. Protoc. 5 (2010) 162–176, https://doi.org/10.1038/nprot.2009.181.
[15] S. Dugheri, N. Mucci, A. Bonari, G. Marrubini, G. Cappelli, D. Ubiali, M. Campagna, M. Montalti, G. Arcangeli, Solid phase microextraction techniques used for gas chromatography: a review, Acta Chromatogr. 32 (2019) 1–9, https://doi.org/10.1556/1326.2018.00579.
[16] C.G. Zambonin, Coupling solid-phase microextraction to liquid chromatography, A review, Anal. Bioanal. Chem. 375 (2003) 73–80, https://doi.org/10.1007/s00216-002-1623-1.
[17] H.L. Lord, Strategies for interfacing solid-phase microextraction with liquid chromatography, J. Chromatogr., A 1152 (2007) 2–13, https://doi.org/10.1016/j.chroma.2006.11.073.
[18] J. O'Reilly, Q. Wang, L. Setkova, J.P. Hutchinson, Y. Chen, H.L. Lord, C.M. Linton, J. Pawliszyn, Automation of solid-phase microextraction, J. Separ. Sci. 28 (2005) 2010–2022, https://doi.org/10.1002/jssc.200500244.
[19] J. Chen, J.B. Pawliszyn, Solid phase microextraction coupled to high-performance liquid chromatography, Anal. Chem. 67 (1995) 2530–2533, https://doi.org/10.1021/ac00111a006.
[20] L. Cárdenes, J.H. Ayala, A.M. Afonso, V. González, Solid-phase microextraction coupled with high-performance liquid chromatography for the analysis of heterocyclic aromatic amines, J. Chromatogr., A 1030 (2004) 87–93, https://doi.org/10.1016/j.chroma.2003.10.040.
[21] A.K. Malik, V. Kaur, N. Verma, A review on solid phase microextraction - high performance liquid chromatography as a novel tool for the analysis of toxic metal ions, Talanta 68 (2006) 842–849, https://doi.org/10.1016/j.talanta.2005.06.005.
[22] J.S. Aulakh, A.K. Malik, V. Kaur, P. Schmitt-Kopplin, A review on solid phase micro extraction - high performance liquid chromatography (SPME-HPLC) analysis of pesticides, Crit. Rev. Anal. Chem. 35 (2005) 71–85, https://doi.org/10.1080/10408340590947952.
[23] R. Eisert, J. Pawliszyn, Automated in-tube solid-phase microextraction coupled to high-performance liquid chromatography, Anal. Chem. 69 (1997) 3140–3147, https://doi.org/10.1021/ac970319a.
[24] M.E. Costa Queiroz, I. Donizeti de Souza, C. Marchioni, Current advances and applications of in-tube solid-phase microextraction, TrAC Trends Anal. Chem. (Reference Ed.) 111 (2019) 261–278, https://doi.org/10.1016/j.trac.2018.12.018.
[25] F.A. Hansen, S. Pedersen-Bjergaard, Emerging extraction strategies in analytical chemistry, Anal. Chem. 92 (2020) 2–15, https://doi.org/10.1021/acs.analchem.9b04677.
[26] J. Soares da Silva Burato, D.A. Vargas Medina, A.L. de Toffoli, E. Vasconcelos Soares Maciel, F. Mauro Lanças, Recent advances and trends in miniaturized sample preparation techniques, J. Separ. Sci. 43 (2020) 202–225, https://doi.org/10.1002/jssc.201900776.
[27] Z. Zhang, H.C. Schniepp, D.H. Adamson, Characterization of graphene oxide: variations in reported approaches, Carbon N. Y. 154 (2019) 510–521, https://doi.org/10.1016/j.carbon.2019.07.103.
[28] L. Sun, Structure and synthesis of graphene oxide, Chin. J. Chem. Eng. 27 (2019) 2251–2260, https://doi.org/10.1016/j.cjche.2019.05.003.
[29] A.L. de Toffoli, E.V.S. Maciel, B.H. Fumes, F.M. Lanças, The role of graphene-based sorbents in modern sample preparation techniques, J. Separ. Sci. 41 (2018) 288–302, https://doi.org/10.1002/jssc.201700870.
[30] H. Grajek, J. Jonik, Z. Witkiewicz, T. Wawer, M. Purchała, Applications of graphene and its derivatives in chemical analysis, Crit. Rev. Anal. Chem. (2019) 1–27, https://doi.org/10.1080/10408347.2019.1653165, 0.
[31] X. Hou, S. Tang, J. Wang, Recent advances and applications of graphene-based extraction materials in food safety, TrAC Trends Anal. Chem. (Reference Ed.) 119 (2019) 115603, https://doi.org/10.1016/j.trac.2019.07.014.
[32] F. Li, Y. Huang, K. Huang, J. Lin, P. Huang, Functional magnetic graphene composites for biosensing, Int. J. Mol. Sci. 21 (2020) 390, https://doi.org/10.3390/ijms21020390.
[33] V. Cerdà, Automation of radiochemical analysis by flow techniques – a review, TrAC Trends Anal. Chem. (Reference Ed.) 118 (2019) 352–367, https://doi.org/10.1016/j.trac.2019.06.001.
[34] R. Rodríguez, J. Avivar, L.O. Leal, V. Cerdà, L. Ferrer, Strategies for automating solid-phase extraction and liquid-liquid extraction in radiochemical analysis, TrAC Trends Anal. Chem. (Reference Ed.) 76 (2016) 145–152, https://doi.org/10.1016/j.trac.2015.09.009.
[35] M. Alexovič, Y. Dotsikas, P. Bober, J. Sabo, Achievements in robotic automation of solvent extraction and related approaches for bioanalysis of pharmaceuticals, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1092 (2018) 402–421, https://doi.org/10.1016/j.jchromb.2018.06.037.
[36] L.F. da Silva, F.M. Lanças, B-cyclodextrin coupled to graphene oxide supported on aminopropyl silica as a sorbent material for determination of isoflavones, J. Separ. Sci. (Unpublished results).
[37] S. Hüser, S. Guth, H.G. Joost, S.T. Soukup, J. Köhrle, L. Kreienbrock, P. Diel, D. W. Lachenmeier, G. Eisenbrand, G. Vollmer, U. Nöthlings, D. Marko, A. Mally, T. Grune, L. Lehmann, P. Steinberg, S.E. Kulling, Effects of isoflavones on breast tissue and the thyroid hormone system in humans: a comprehensive safety evaluation, Arch. Toxicol. 92 (2018) 2703–2748, https://doi.org/10.1007/s00204-018-2279-8.
[38] H. Zhang, X. Hu, M. Qiao, Y. Li, S. Cao, L. Ding, X. Feng, N. Kang, D. Zhang, F. Qiu, Simultaneous determination of five isoflavones in rat plasma by LC-MS/MS: comparative pharmacokinetic characteristics of Puerariae lobatae radix in normal and type 2 diabetic rats, J. Separ. Sci. 42 (2019) 2592–2601, https://doi.org/10.1002/jssc.201900341.
[39] D.A.V. Medina, L.F. Rodriguez Cabal, F.M. Lanças, Á.J. Santos-Neto, Sample treatment platform for automated integration of microextraction techniques and liquid chromatography analysis, HardwareX 5 (2019), https://doi.org/10.1016/j.ohx.2019.e00056.
[40] L.F. Rodríguez Cabal, D.A. Vargas Medina, A. Martins Lima, F.M. Lanças, Á. J. Santos-Neto, Robotic-assisted dynamic large drop microextraction, J. Chromatogr., A 1608 (2019), https://doi.org/10.1016/j.chroma.2019.460416.
[41] L.F.R. Cabal, D.A.V. Medina, J.L. Costa, F.M. Lanças, Á.J. Santos-Neto, Determination of ring-substituted amphetamines through automated online hollow fiber liquid-phase microextraction-liquid chromatography, Anal. Bioanal. Chem. 411 (2019) 7889–7897, https://doi.org/10.1007/s00216-019-02196-0.
[42] D.A.V. Medina, L.F. Rodríguez Cabal, G.M. Titato, F.M. Lanças, Á.J. Santos-Neto, Automated online coupling of robot-assisted single drop microextraction and liquid chromatography, J. Chromatogr., A 1595 (2019) 66–72, https://doi.org/10.1016/j.chroma.2019.02.036.
[43] S. Xu, Z. Zhang, J. Liu, Y. Wang, J. Hu, Facile preparation of reduced graphene by optimizing oxidation condition and further reducing the exfoliated products, J. Mater. Res. 32 (2017) 383–391, https://doi.org/10.1557/jmr.2016.476.
[44] X.-Y. Song, W. Ha, J. Chen, Y.-P. Shi, Application of β-cyclodextrin-modified, carbon nanotube-reinforced hollow fiber to solid-phase microextraction of plant hormones, J. Chromatogr., A 1374 (2014) 23–30, https://doi.org/10.1016/j.chroma.2014.11.029.
[45] M. Satterfield, D.M. Black, J.S. Brodbelt, Detection of the isoflavone aglycones genistein and daidzein in urine using solid-phase microextraction-high-performance liquid chromatography-electrospray ionization mass spectrometry, J. Chromatogr. B Biomed. Sci. Appl. 759 (2001) 33–41, https://doi.org/10.1016/S0378-4347(01)00200-6.
[46] A. Gentili, Cyclodextrin-based sorbents for solid phase extraction, J. Chromatogr., A 1609 (2020) 460654, https://doi.org/10.1016/j.chroma.2019.460654.
[47] A. Aresta, F. Di Grumo, C. Zambonin, Determination of major isoflavones in Soy drinks by solid-phase micro extraction coupled to liquid chromatography, Food Anal. Methods. 9 (2016) 925–933, https://doi.org/10.1007/s12161-015-0260-1.
[48] K. Mitani, S. Narimatsu, H. Kataoka, Determination of daidzein and genistein in soybean foods by automated on-line in-tube solid-phase microextraction coupled to high-performance liquid chromatography, J. Chromatogr., A 986 (2003) 169–177, https://doi.org/10.1016/S0021-9673(02)02014-9.
[49] A.M. Chrzanowska, A. Poliwoda, P.P. Wieczorek, Surface molecularly imprinted silica for selective solid-phase extraction of biochanin A, daidzein and genistein from urine samples, J. Chromatogr., A 1392 (2015) 1–9, https://doi.org/10.1016/j.chroma.2015.03.015.
[50] B. Redruello, L. Guadamuro, I. Cuesta, J.R. Álvarez-Buylla, B. Mayo, S. Delgado, A novel UHPLC method for the rapid and simultaneous determination of daidzein, genistein and equol in human urine, J. Chromatogr. B 1005 (2015) 1–8, https://doi.org/10.1016/j.jchromb.2015.09.029.

CAPÍTULO 5

ESTADO DA ARTE DO USO DE MATERIAIS À BASE DE GRAFENO EM PREPARO DE AMOSTRA.

[4] **The Current Role of Graphene-Based Nanomaterials in the Sample Preparation Arena.**
Edvaldo Vasconcelos Soares Maciel, Karen Mejía-Carmona, Marcela Jordan-Sinisterra, Luis Felipe da Silva, Deyber Arley Vargas Medina and Fernando Mauro Lanças
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REVIEW
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The Current Role of Graphene-Based Nanomaterials in the Sample Preparation Arena

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Since its discovery in 2004 by Novoselov et al., graphene has attracted increasing attention in the scientific community due to its excellent physical and chemical properties, such as thermal/mechanical resistance, electronic stability, high Young's modulus, and fast mobility of charged atoms. In addition, other remarkable characteristics support its use in analytical chemistry, especially as sorbent. For these reasons, graphene-based materials (GBMs) have been used as a promising material in sample preparation. Graphene and graphene oxide, owing to their excellent physical and chemical properties as a large surface area, good mechanical strength, thermal stability, and delocalized π-electrons, are ideal sorbents, especially for molecules containing aromatic rings. They have been used in several sample preparation techniques such as solid-phase extraction (SPE), stir bar sorptive extraction (SBSE), magnetic solid-phase extraction (MSPE), as well as in miniaturized modes as solid-phase microextraction (SPME) in their different configurations. However, the reduced size and weight of graphene sheets can limit their use since they commonly aggregate to each other, causing clogging in high-pressure extractive devices. One way to overcome it and other drawbacks consists of covalently attaching the graphene sheets to support materials (e.g., silica, polymers, and magnetically modified supports). Also, graphene-based materials can be further chemically modified to favor some interactions with specific analytes, resulting in more efficient hybrid sorbents with higher selectivity for specific chemical classes. As a result of this wide variety of graphene-based sorbents, several studies have shown the current potential of applying GBMs in different fields such as food, biological, pharmaceutical, and environmental applications. Within such a context, this review will focus on the last five years of achievements in graphene-based materials for sample preparation techniques highlighting their synthesis, chemical structure, and potential application for the extraction of target analytes in different complex matrices.

Keywords: graphene, graphene oxide, cyclodextrin, molecularly-imprinted polymer, magnetic, ionic liquid, sample preparation

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INTRODUCTION

Over the last decades, nanotechnology has become a promising tool in relevant scientific fields, allowing humanity to reach top levels of quality in several areas such as engineering, chemistry, medicine, and sports, among others (Lin et al., 2019). One of the most significant achievements in this context was the confirmation of the existence of a single-layered graphene sheet obtained

through mechanical exfoliation by Novoselov et al. in 2004 at Manchester University (Novoselov, 2004; Novoselov et al., 2005). The history of graphene (G) starts ∼70 years ago when Landau and Peierls stated that strictly 2D crystals were thermodynamically unstable with a slight likelihood even to exist (Peierls, 1935; Landau, 1937). At that time, the scientists thought that the melting point of a thin-film of atoms decreased proportionally to its thickness leading to structure decomposition or segregation (Peierls, 1935; Landau, 1937). Therefore, the atomic monolayers' existence would only be possible to exist as an epitaxially-grown part of 3D complex structures. Nonetheless, this theory was confronted by experimental observations reported in 2004, which preceded the discovery of more than one type of 2D atomic monolayers, highlighting graphene as the most important of them (Geim and Novoselov, 2007). For this reason, graphene theoretically represents a new class of materials possessing a one-atom thickness that, due to its intrinsic properties, are creating new possibilities of practical applications and becoming a hot topic in science.

Graphene can be defined as a carbon allotrope composed by a structure containing sp^2 hybridized atoms obeying a honeycomb pattern, which is the core for other widely-known allotropic forms (Grajek et al., 2019). In other words, graphene can be stacked to form graphite, rolled to form a carbon nanotube, or even wrapped to become a fullerene, as shown in **Figure 1**. In general, it is considered a wonder material due to its nanosheet structure, which has strong σ-orbitals in the 2D plane, ensuring its stiffness. At the same time, the un-hybridized π-orbitals are hinged outwards, superimposing one by one to form the long-range delocalized π electron system, responsible for its outstanding optical, and electrical properties (Grajek et al., 2019). In short, unmodified graphene sheets have a large theoretical surface area (ca. 2630 $m^2\ g^{-1}$) distributed along with the thinnest structure of the negligible mass, but high Young's modulus, as already discovered (Fumes et al., 2015). Likewise, its charge carriers have high mobility, possibly traveling micrometers without scattering, becoming an ideal material for producing electronic devices (Hou et al., 2019). Moreover, the excellent thermal and electrical conductivity (∼3,000 W mK^{-1} and 104 $\Omega^{-1}\ cm^{-1}$, respectively), transparency, and impermeability to gases must be underscored (Geim, 2009).

Although these top qualities suggest that graphene would be an ideal material with several different potential applications, the manufacturing (especially in industrial-scale) still represents a hurdle to its broad implementation. This occurs because the most utilized manufacturing approach is based on the graphite top-down mechanical exfoliation process by adhesive tape (Allen et al., 2010). Generally, this production method is laborious, non-reproducible, and highly dependent on human handling. It has attracted the chemists' interest in developing scalable alternative routes to produce significant amounts of high-quality graphene nanosheets. Including on this are the chemical exfoliation through liquid solutions, the bottom-up method to produce from organic precursors, among others (Allen et al., 2010; Vadivel et al., 2017). It must be noted that each of these alternative production methods has its proper characteristics. The chemical exfoliation provides interesting results regarding the quality of graphene nanosheets or even due to the existence of intermediary graphene-based compounds similarly attractive for such purposes, such as the graphite oxide or graphene oxide, for instance.

Even with the graphene existence being confirmed since 2004, its first application in sample preparation was only published in 2011 (Luo et al., 2011a; Zhang and Lee, 2011). The interest of analytical chemists on it and its derivatives is mostly due to the increasing demand for high-performance and selective materials to extract contaminants present in complex matrices containing a large number of interferents. The present outlook of our environment urges researchers to seek technological advances on sample preparation and analytical techniques to tackle even better the increasing use of chemicals by humans in many areas of life: agriculture, health-treatments, and abusive-drugs, among others. Within such a context, the necessity in performing a sample preparation step before the analytical techniques is mandatory due to the complexity related to the mostly analyzed matrices (biological fluids, food, plants, wastewaters, soil, and others). This step is crucial in the analytical workflow

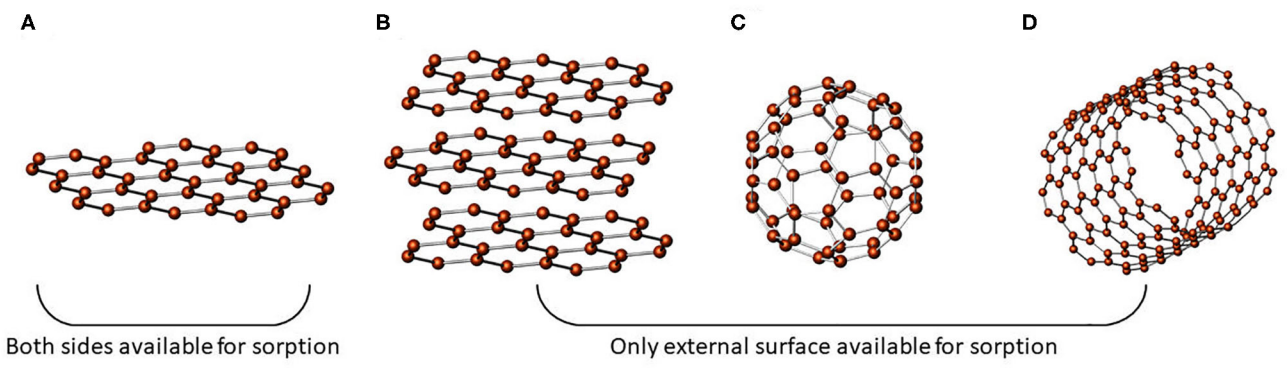

FIGURE 1 | Illustrative drawing of the primary carbon allotropes: **(A)** graphene; **(B)** graphite; **(C)** fullerene; **(D)** carbon nanotubes. Adapted from Maciel et al. (2019), copyright 2019, with permission from Elsevier.

responsible for eliminating matrix interferents, isolating, and pre-concentrating target analytes. For these reasons, several different sorption-based sample preparation techniques (e.g., microextraction by packed sorbent [MEPS], stir bar sorptive extraction [SBSE], magnetic solid-phase extraction [MSPE], among others) have been proposed. They are mostly derived from conventional SPE and its main miniaturized mode SPME (Fumes et al., 2015). Generally, they are performed by the employment of an extractive phase, usually named as sorbent. Ideally, this sorbent must present some essential characteristics such as good selectivity to the target compounds, high extraction capability, and even is desirable as a chemical inertia for those interferents present in the analyzed matrix (Toffoli et al., 2018).

Then, GBMs emerged as promising sorbents to be used in the extraction techniques, due to its chemical structure and properties, which favors the extraction performance by effectively removing the target analytes from complex matrices. Considering all the advantages herein presented, some of them are more interesting from the analytical chemistry standpoint. For example, the flat graphene structure allows potential target analytes to interact on both sides of it, which is advantageous for sorption-based sample preparation techniques. In the case of other carbon allotropes (e.g., carbon nanotubes, graphite, and fullerene), only the external surface is available for such interaction, which potentially diminishes extraction performance due to this steric hindrance associated. Additionally, the delocalized π-electron system favors electrostatic interaction between the graphene and molecules that possess aromatic rings in its structure.

For this reason, prevalent contaminants such as pesticides, preservatives, pharmaceuticals, and veterinary drugs can be remediated from the environment by using GBMs (Toffoli et al., 2018). Contrariwise, when the potential contaminant does not have aromatic rings, a functional intermediary produced from the chemical exfoliation, namely graphene oxide (GO), can be used instead of graphene. This is owing to its chemical structure that differs from G by the presence of oxygenated groups (e.g., hydroxyl, carbonyl, alkoxy) outside of its 2D-plane, possibly favoring interactions with polar active-sites in other molecules (Smith et al., 2019). Nonetheless, from an operational point of view, the fact that graphene is an ultra-light material makes difficult its deposition by, for example, centrifugation. In this way, some chemical modification or functionalization can be performed to overcome such drawbacks. Nowadays, other carbon-based compounds that possess one-atom planar structures are beginning to spur around, mainly due to the attention given to the scientific community's graphene in the last years. Including in this group are graphyne, graphdiyne, graphone, and graphane, all considered as graphene-derivative compounds (Peng et al., 2014). As an example, graphyne and graphdiyne are 2D-flat allotropic forms of graphene possessing the same honeycomb pattern, which suggests them as suitable for similar applications as its precursor (graphene).

Conversely, graphone and graphene emerged as hydrogenated graphene-derivative compounds susceptible to chemical modifications onto its surfaces. Despite these compounds already discussed in the literature, their synthesis remains a complicated process; for this reason, they have not yet been applied in sample preparation. However, considering the significant advances in graphene-based technologies since its discovery, these other allotropic forms might gain more attention from scientists throughout the years.

Following the background regarding the emerging of graphene, this review aims to present the state-of-art about this "wonder material" from a sample preparation viewpoint. In this way, several aspects such as synthesis and functionalization process, the main derivative classes, and its most suitable applications are divided among the next sections. In short, our primary goal was to present a review mostly covering the last 5 years' achievements of the still-evolving field of graphene-based materials in sample preparation and discuss the future trends and potential challenges that chemists should face in the years to come.

GRAPHENE AND GRAPHENE OXIDE

As mentioned, graphene (G) is a 2D monolayer of carbon atoms covalently bonded in a honeycomb pattern, displaying a flat sheet conformation (Solís-Fernández et al., 2017). Graphene and related materials are part of the graphene-based materials (GBMs), which comprises graphene (G) nanosheets (in mono, few, and multi-layers), graphene oxide (GO), and reduced graphene oxide (rGO) (De Marchi et al., 2018). A considerable variety of research articles about different synthesis methods, properties, and applications of GBMs are available (Papageorgiou et al., 2017; Lim et al., 2018; Liu and Zhou, 2019). Two different approaches can be used to obtain graphene: (i) the top-down, in which nanostructures are produced from larger dimensions, and (ii) the bottom-up, starting from atoms or small molecules to produce materials of larger dimensions.

In the top-down approach, graphene is prepared from graphite, by mechanical or chemical exfoliation, or chemical synthesis, separating the graphene thin layers parallelly stacked in the graphite and held together by weak van der Waals forces. Mechanical exfoliation is one of the simplest methods in which a simple direct contact with an adhesive tape (polymer) can take off the graphene layers from the surface of a graphite piece. One of the advantages of mechanical exfoliation is the possibility of different pile-up layers of other 2D materials with several graphene heterostructures (Solís-Fernández et al., 2017). However, it has only been implemented on a small scale and is highly susceptible to contamination. In the same way, organic solvents can be used to separate the graphite layers. Other exfoliation methods include the use of electric fields, sonication, and transfer printing technique (Lim et al., 2018). Several exfoliation methods, including different substrates, thermal released tape, and thermal approaches, have been proposed to improve the quality, size, and homogeneity of graphene (Solís-Fernández et al., 2017).

Chemical reduction of graphene oxide (GO) is the most popular method to obtain graphene. As shown in **Figure 2**, GO can be obtained by the oxidation of graphite powder and then exfoliated further to obtain single GO layers, which is

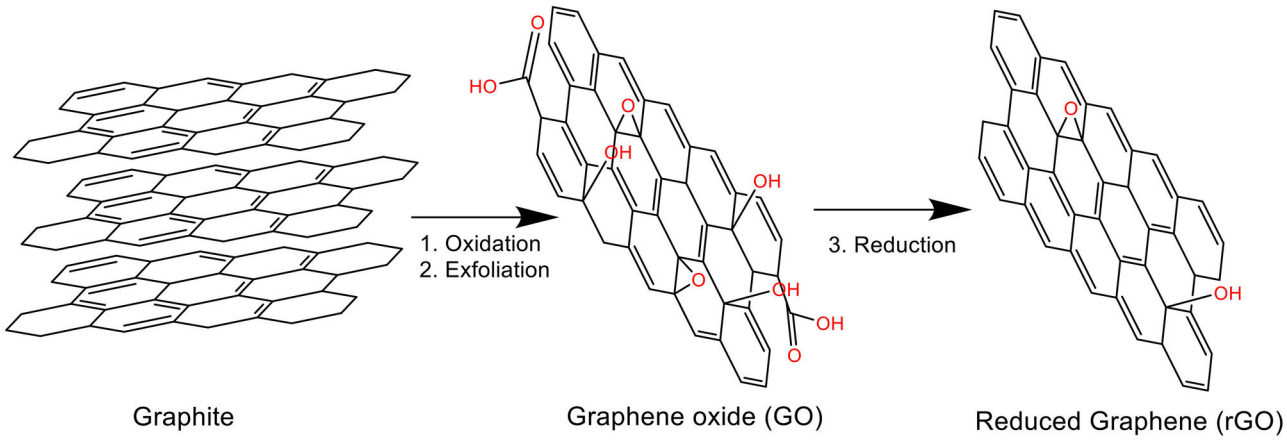

FIGURE 2 | Graphene chemical reduction pathway.

subsequently chemically reduced to obtain rGO. Although the final product obtained from this pathway is rGO, its properties are very similar to graphene but are structurally different (Dreyer et al., 2010; Singh et al., 2016). Chemical reduction of GO firstly involves exfoliation in water assisted by ultrasonication, followed by reduction of the oxygenated groups, hence precipitating the rGO from the solution due to its hydrophobicity (Singh et al., 2016). Among the wide variety of chemical reduction agents that can be employed, hydrazine is the most often used because of its high reductive efficiency, even though it is highly environmental toxic. As an alternative, the use of greener reduction agents (De Silva et al., 2017) and thermally-mediated or electrochemical reduction are also employed. Although chemical reduction of GO is a popular upscaling method, it yields a final product containing several structural defects on the sheets, which lead to low-quality materials with variable sizes and edges (Dreyer et al., 2010).

Conversely, the bottom-up approaches are another alternative to synthesize high-quality graphene layers. The leading methods used include pyrolysis, chemical vapor deposition (CVD), plasma synthesis, and epitaxial growth (Lim et al., 2018). Graphene sheets are prepared from small building blocks and assembled with dedicated precision, usually employing molecular modeling to build the layers. Among the bottom-up methods, CVD uses high temperatures for the decomposition of hydrocarbons, which are deposited on metal substrates, thus forming thin sheets of graphene (Papageorgiou et al., 2017). The main advantage of bottom-up methods is the production of high-quality graphene sheets. Nevertheless, these methods are not used for large-scale production.

Graphene oxide (GO) is like a graphene sheet functionalized on both sides with several oxygenated functions such as hydroxyl, carboxyl, and epoxy. These functions impose a hydrophilic character to GO; as a consequence, the interaction between layers is weaker compared to graphene, making GO an easily exfoliated material. GO structure depends principally on the purification methods (Singh et al., 2016). Compared to graphene, the GO structure is still ambiguous; thus, several structural models have been proposed to date (Dreyer et al., 2010; Sun, 2019). The graphene oxide can be obtained by the popular Hummer's method (Hummers and Offeman, 1958), which, until the date, has been subjected to multiple modifications and improvements (Shamaila et al., 2016). The original method proposes the oxidation of graphite powder by $KMnO_4$ and $NaNO_3$ in H_2SO_4 (Hummers and Offeman, 1958). Differences with other modified methods are principally on the type and toxicity of the oxidant reagents, and the quality of the obtained product (Lim et al., 2018).

A fascinating characteristic raised from the physical and chemical properties of graphene materials is the possibility to perform chemical functionalizations mainly to modify its reactivity yielding a large variety of graphene-based materials (GBMs). Thus, they can be currently used in several applications (Bottari et al., 2017; Mohan et al., 2018). Covalent or non-covalent pathways can functionalize GBMs. Non-covalent functionalization involves first the rupture of the van der Waals forces that stake together with the graphene layers with subsequent formation of non-covalent binding with the substrate by π-π, π-cation, and van der Waals interactions. For that, mechanical liquid exfoliation assisted by ultrasonication is used to overcome these forces; water, organic solvents, ionic liquids, surfactants, mixtures are employed to disperse, and stabilize the graphene sheets in the exfoliation process. For example, non-covalent functionalization can be obtained by forming a stable dispersion of graphene sheets in polymers (ionic, non-ionic, and polysaccharides), water solutions, and organic solvents as polyvinyl alcohol, chitosan, and alginate. They can be employed to obtain graphene aerogels and hydrogels (Dreyer et al., 2010; Bottari et al., 2017).

On the other hand, the covalent functionalization of graphene (G) yields a low substitution degree due to their stable carbon conjugation. However, covalent functionalization can be done by taking advantage of the oxygenated reactive groups of the graphene oxide (GO) sheets. Therefore, in the same way as the chemical reduction of graphene oxide, the hydroxyl, carboxyl,

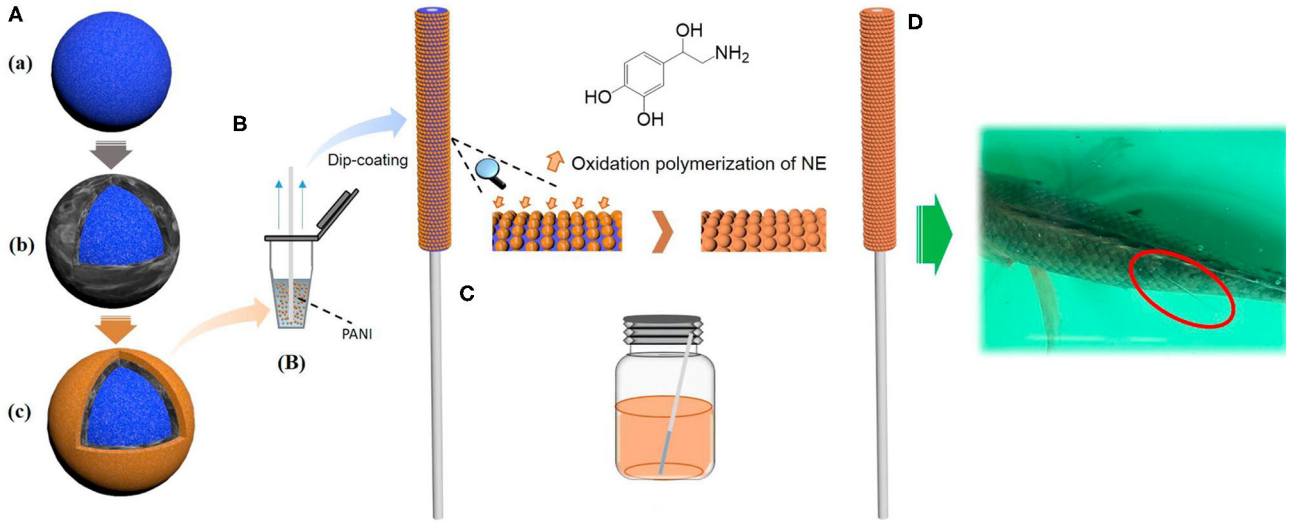

FIGURE 3 | Preparation and application scheme of C18@GO@PDDA SPME fiber. **(A)** Preparation of the particles (a. C18, b. C18@GO, and c. C18@GO@PDDA). **(B)** A home-made coated PANI fiber by the dip-coating method. **(C)** Bioinspired modification by the NE oxidation polymerization. **(D)** *In vivo* sampling in fish dorsal-epaxial muscle with the fiber. Reprinted with permission from Qiu et al. (2016), copyright 2016, American Chemical Society.

and epoxy groups can be covalently replaced by other functional groups. The GO surface modification with aliphatic amines to form an amide bond is one of the most common strategies (Dreyer et al., 2010). Undoubtedly, the derivatization of graphene materials improves their electrical, thermal, and mechanical properties as well as their dispersibility (Mohan et al., 2018). Some reasons for the use of functionalized graphene materials as sorbents in sample preparation include: (i) they present improved sorption capacity and recoveries; (ii) easy attachment of graphene onto surfaces to be reusable, and preventing sorbent losses; (iii) avoid the agglomeration of the graphene sheets; and (iv) favored sorbent isolation from the sample (Wang et al., 2014; Ye and Shi, 2015; Chen X. et al., 2016; González-Sálamo et al., 2016).

Considering the increasing use of GBMs in sample preparation techniques, the most representative and used materials in this arena are discussed in the following sections.

ANCHORED GRAPHENE-BASED MATERIALS

Alkyl and Aril Groups

Octadecylsilica particles (for short C18 or ODS) are by far the most commonly used sorbent in solid-phase extraction (SPE) and chromatographic separations. Apart from the conventional C8/C18 reversed phases, today mixed-mode polymeric sorbents are widely used in SPE because they present interactions with several compounds and better performance compared to the conventional ones, and they are also commercially available (Fontanals et al., 2020). Alkyl groups, in general, are commonly used to derivatize sorbents, including GO sheets, to modify their fundamental properties. As a consequence, C18 has also been employed to functionalize GO-based sorbents owing to the high surface area of the GO sheets. Their functionalization with octadecylsilane increases the surface load with C18 groups compared to silica particles (Liang et al., 2012; Xu et al., 2012). Subsequently, the extraction capacity is improved, and hydrophobic interactions increased. Therefore, being applied as a sorbet in reverse-phase, they show an improved extraction efficiency for the extraction of alkanes and PAHs, for instance (Xu et al., 2012). Recently, Qui et al. prepared a solid-phase microextraction (SPME) fiber with C18 particles (3.5 μm) coated with GO and poly(diallyl dimethylammonium chloride) (PDDA)–C18@GO@PDDA as shown in **Figures 3A,B**. Then, the surface of the fiber was modified by oxidative polymerization by polynorepinephrine (pNE) (**Figure 3C**), which plays the role of a bio-interface, compatible with *in-vivo* sampling (**Figure 3D**). The prepared SPME fibers showed higher efficiency than commercially available ones such as polydimethylsiloxane (PDMS) and polyacrylate (PA) for the monitoring of acidic drugs in fish samples. Additionally, the fiber exhibited excellent stability, sensitivity, and resistance for *in-vivo* matrices, showing potential for pharmacokinetics applications (Qiu et al., 2016). In another study, the same fiber type was successfully employed to analyze salicylic acid traces in plants *in-vivo* (Fang et al., 2018).

Although functionalization of GO occupies or replaces part of their original active sites, the sorption capacity of modified-GO materials can be lower or higher compared to GO, which mainly depends on the composite type formed and their specific interaction with the analytes. Even so, GBMs show superior sorptive properties compared to conventional sorbents (e.g., C18), which allows the use of graphene sorbents in small quantities (<100 mg) (Sitko et al., 2013). In this way, Ma et al. functionalized graphene oxide (GO) sheets with different amine-alkyl chains to obtain amine-rGO sorbents *via*

solvothermal synthesis. The sorption capacity of the different alkyl-amine-rGO materials was evaluated for the extraction of catechins and caffeine. Results showed that tributylamine-rGO has the highest sorption capacity (203.7 mg g^{-1}) for catechins being 11 times higher compared to GO sheets (18.7 mg g^{-1}) and other rGO-amino groups (ammonia, ethylenediamine, n-butylamine, tert-butylamine, dodecyl amine, and octadecyl amine). Hence, tributylamine-rGO was employed as a sorbent in a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method achieving a higher clean-up performance compared to traditional sorbents as PSA, C18, and graphitized carbon black (GCB), regularly used in QuERChERS (Ma et al., 2018). A similar comparison was performed by Fumes et al., which employed aminopropyl silica and PSA particles coated by graphene sheets. The extraction performance of the GO-coated particles, used as a sorbent in microextraction by packed sorbent (MEPS) method, were compared with conventional sorbents (C18, strata-X, PSA, amino silica) for the extraction of parabens in wastewater. Aminopropyl silica coated with GO (SiGO) and G (SiG) showed an improved extraction performance compared to conventional sorbents (Fumes and Lanças, 2017). Likewise, a recent work performed by the same research group showed improvements in the extraction capability of aminopropyl silica-GO particles when they are functionalized with C18 and further end-capped. The authors achieved low LODs and LOQs in a complex matrix (coffee samples) by using these particles in a packed in-tube SPME device (Mejía-Carmona and Lanças, 2020). Other interesting graphene-based applications carried out by the Lança's research group also includes tetracyclines' analysis in milk samples (Vasconcelos Soares Maciel et al., 2018) and the determination of triazines in environmental water samples (De Toffoli et al., 2018).

Several additional complex alkyl and aryl compounds have been used to functionalize GO. For example, Nurerk et al. synthesized a hybrid sorbent based on calix[4]arene-functionalized graphene oxide/polydopamine-coated cellulose acetate fiber (calix[4]arene-GO/PDA-CFs) for the extraction of aflatoxins in corn samples. Calix[4]arene is a macrocyclic molecule of four phenol units bonded by methylene bridges, which can favor the extraction of aflatoxins by H-bonding, hydrophobic, and π-π interactions. The recoveries of aflatoxins (AFs) obtained employing cellulose acetate CFs (35–41%), polydopamine coated CFs (PDA-CFs) (45–55%), calix[4]arene-GO-CFs (60–72%), GO/PDA-CFs (63–82%), and calix[4]arene-GO/PDA-CFs (86–94%) as SPE sorbents showed that together calix[4]arene and GO increased the efficiency of the sorbent phase (Nurerk et al., 2018). Recently, Zhou et al. synthesized a graphene oxide framework (GOF), a 3D nanoporous material, as coating sorbent for stir bar sorptive extraction (SBSE). Graphene oxide was covalently interconnected with a 1,4-phenylene diisocyanate (PPDI) to obtain three-dimensional GOF, which was immobilized onto the surface of stainless-steel wire (SSW) using polydopamine. The stir bar was applied successfully for the extraction of Sudan dyes in lake water and fruit juice (Zhou J. et al., 2019). Other recently published papers on alkyl and aryl modified graphene materials are shown in **Table 1**.

Cyclodextrins

Other emergent graphene-based materials for sample preparation are those combined with cyclodextrins (CD), which are cyclic oligosaccharides formed by starch enzymatic degradation, and are linked by several α-1,4-anhydroglucopyranose. In nature, they are made up of 6, 7, or 8 glucose units and categorized as α, β, and γ-CD, respectively. CDs have cone shapes with a hydrophobic cavity and a hydrophilic external surface. This specific structure allows them to form inclusion complexes with specific molecules such as polyphenolics compounds through hydrogen bonding, hydrophobic, and Van der Waals interactions (Pinho et al., 2014; Zhu et al., 2016). Reactive OH groups can also be replaced to modify their solubility, improve inclusion ability, or induce desired properties as functionalization for immobilization on a solid support. Consequently, more than 100 CDs are commercially available, and more than 1,500 derivatives have been synthesized for different purposes (Szejtli, 2004; Gentili, 2020).

The reliable recognition capacity of phenolic compounds, due to the excellent size match, becomes common to find works reporting a combination between graphene and cyclodextrins used as electrochemical detectors (Wang C. et al., 2016), electrocatalytic detector (Pham et al., 2016), and electrocatalytic material, for instance (Ran et al., 2017). Thus, there are many different strategies available in the literature to synthesize GBMs functionalized by cyclodextrins. GO functionalization with CD can be very simple, as reported by Cao et al. (2019). They prepared a suspension containing a graphene-based material and β-cyclodextrin, with it subsequently stirred under a heated water bath at a temperature of 60°C for 4 h. In this case, the resulting material was GO; if it is necessary to reduce graphene oxide to graphene, it can be done using hydrazine (Pham et al., 2016; Tan and Hu, 2017). As an example, modifications in the synthesis route can be made with (3-aminopropyl)triethoxysilane (APTES) to support an amino group to both graphene oxide nanosheets or cyclodextrins walls (Deng et al., 2017). This procedure results in an amido bonding between epoxy and –COOH groups of GO and $–NH_2$ from the APTES. **Figure 4** shows a scheme to exemplify the most common bond between GO and cyclodextrin, and how it is expected to be the structure of the graphene-based material functionalized by cyclodextrin.

The combination of graphene and cyclodextrins properties becomes the resulting material attractive to be employed in sample preparation techniques. An interesting application was carried out by Deng et al. The novel β-CD–GO-coated SPME fiber was prepared using a sol-gel technique and immobilizing onto a pre-functionalized stainless steel wire (Deng et al., 2017). They applied this material as a sorbent in a headspace technique (HS-SPME), aiming to extract organophosphate flame retardants in water samples to be analyzed by gas chromatography with nitrogen phosphorus detector (NPD). The method showed functional recovery (82.1–116.9%), linear range with correlation coefficients (R) ranging from 0.9955 to 0.9998. The LODs and LOQs for the nine analytes ranged from 1.1–60.4 to 2.7–170.5 ng L^{-1}, respectively; RSD was 2.2–9.6%, and enrichment factors obtained from 22.5 to 1307.5. This high enrichment

TABLE 1 | Recent applications (2015–2020) of alkyl and aryl functionalized graphene-based materials in sample preparation.

Sorbent	Analytes	Matrix	Sample preparation	Analysis	LOD	References
Diallyl dimethyl ammonium chloride-assembled GO-coated C18 (C18@GO@PDDA)	Salicylic acid and derivates	Aloe plants (*in-vivo* sampling)	SPME fiber	HPLC-DAD	1.8–2.8 $\mu g\ g^{-1}$	Fang et al., 2018
Aminopropyl silica coated GO-functionalized Octadecylsilane/end-capped (SiGOC18ecap)	Xanthines	Coffee	In-tube SPME	HPLC- MS/MS	0.1–0.2 $\mu g\ L^{-1}$	Mejía-Carmona and Lanças, 2020
Graphene derivatized silica	Fluoroquinolones	Water	SPE	HPLC-FLD	2 $ng\ L^{-1}$	Speltini et al., 2015
Guanidyl-functionalized GO-grafted silica (Guanidyl@GO@sil)	Herbicides	*Lycium barbarum*	SPE	HPLC-UV	0.5–2.0 $\mu g\ L^{-1}$	Hou et al., 2018b
Polypyrrole-coated GO and C18 incorporated in chitosan cryogel (PPY/GOx/C18/CS)	Carbamate pesticides	Fruit juices	SPE	HPLC-UV	0.5–2.0 $\mu g\ L^{-1}$	Klongklaew et al., 2018
Alkyl-NH_2/rGO	Pesticides	Tea	Modified QuEChERS	GC-MS/MS UHPLC-MS/MS	0.33–9.26 $\mu g\ kg^{-1}$	Ma et al., 2018
Calix[4]arene-functionalized GO/polydopamine-coated cellulose acetate fiber (calix[4]arene-GO/PDA-CFs)	Aflatoxins	Corn	SPE	HPLC-FLD	0.01–0.05 $\mu g\ kg^{-1}$	Nurerk et al., 2018
Graphene oxide supported on aminopropyl silica (Si-GO)	Tetracyclins	Bovine milk	MEPS	HPLC-MS/MS	0.03–0.21 $\mu g\ L^{-1}$	Vasconcelos Soares Maciel et al., 2018
Graphene oxide supported on aminopropyl silica (Si-GO)	Triazines	Water	In-tube SPME	HPLC-MS/MS	1.1–2.9 $ng\ L^{-1}$	De Toffoli et al., 2018
Graphene-C18 Reinforced Hollow Fiber (G-C18-HF)	Chlorophenols	Honey	HF-LPME	HPLC-UV	0.5–1.5 $\mu g\ kg^{-1}$	Sun et al., 2014
Poly(diallyldimethylammoniumchloride) assembled GO-coated C18 particles (C18@GO@PDDA)	Acidic pharmaceuticals	Fish (*in-vivo* sampling)	SPME fiber	HPLC-MS/MS	0.13–7.56 $\mu g\ kg^{-1}$	Qiu et al., 2016
Graphene oxide/silica modified with nitro-substituted tris(indolyl)methane	Organic acids	Honey and nongfu spring drink	SPE	HPLC-DAD	0.5–1.0 $\mu g\ L^{-1}$	Wang N. et al., 2016
Graphene supported on aminopropyl silica (Si-G) and primary-secondary amine (PSA) silica (PSA-G)	Parabens	Water	MEPS	UHPLC-MS/MS	0.06–0.09 $\mu g\ L^{-1}$	Fumes and Lanças, 2017
Acrylamide-functionalized graphene	Monoamine acidic metabolites	Urine and plasma	μSPE	HPLC-UV	0.08–0.25 $\mu g\ L^{-1}$	Yang et al., 2015
Tannic acid functionalized graphene	Beryllium	Wastewater and street dust	d-SPE	Atomic absorption	0.84 $ng\ L^{-1}$	Yavuz et al., 2018
GO framework interconnected by 1,4-phenylene diisocyanate (PPDI)	Sudan dyes (G, I, II, and III)	Lake water and fruit juice	SBSE	HPLC-UV	0.15–0.3 $\mu g\ L^{-1}$	Zhou J. et al., 2019

factor is attributed to the combined advantages of β-CD and GO. When compared with the commercial fibers and some published methods, the GO/β-CD sol-gel coating fiber showed a higher extraction efficiency, except for those organophosphate flame retardants containing a benzene ring. Similarly, Cao et al. combined the advantages of graphene and cyclodextrins with ionic liquids and ILs (Cao et al., 2019). They synthesized a $VOIm^+$ $AQSO_3^-$ functionalized β-cyclodextrin/magnetic graphene oxide material (Fe_3O_4@SiO_2/GO/β-CD/IL), which was used as a sorbent to extract plant growth regulators from vegetable samples using magnetic solid-phase extraction (MSPE) followed by UHPLC-MS/MS analysis. This approach showed fast separation, high surface area, high adsorption capability, and environmental friendliness. The comparison between Fe_3O_4@SiO_2/GO, Fe_3O_4@SiO_2/GO/β-CD, and Fe_3O_4@SiO_2/GO/IL showed that Fe_3O_4@SiO_2/GO/β-CD/IL had higher extraction efficiency and selective adsorption capacity.

For food analysis, β-CD combined with GO were used in the sample preparation during the analysis of organochlorine pesticide residues in honey (Mahpishanian and Sereshti, 2017). The prepared material was applied as a sorbent in vortex-assisted magnetic solid-phase extraction (MSPE) before

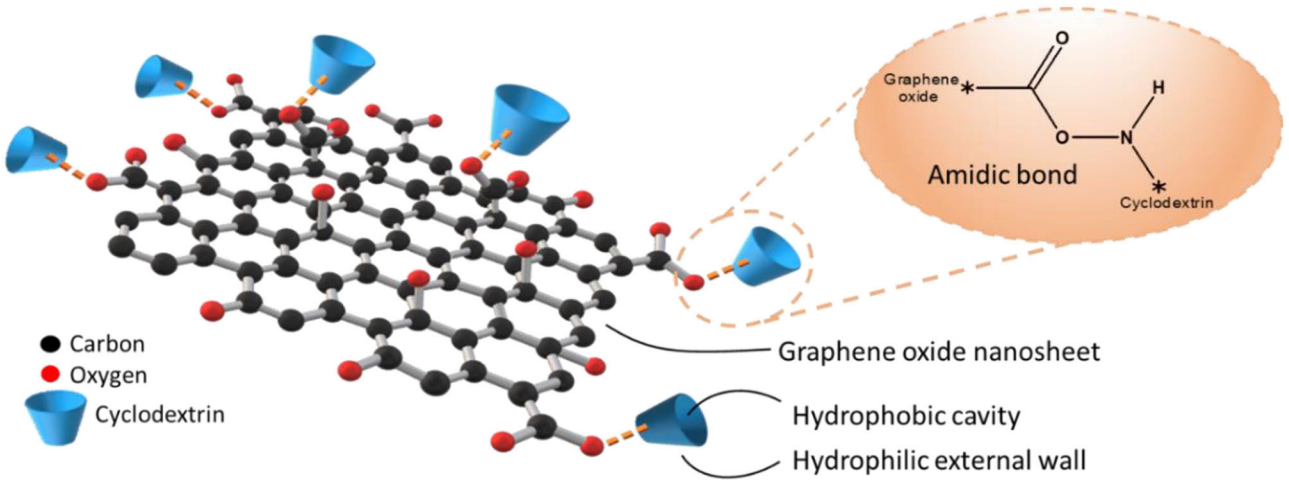

FIGURE 4 | Scheme illustrating a graphene-based sorbent functionalized with cyclodextrins through peptide bonds.

TABLE 2 | Recent applications (2017–2020) of graphene-based materials functionalized by cyclodextrins to sample preparation.

Sorbent	Analytes	Matrix	Sample preparation	Analysis	LOD	References
G-Fe_3O_4-β-CD	Bisphenol-A	Water	MSPE	UV-vis	**	Ragavan and Rastogi, 2017
GNS/β-CD	Phenolphthalein	Water	d-SPE	UV-vis	**	Tan and Hu, 2017
Fe_4O_3-GO-β-CD	Neonicotinoid pesticide	Water	MSPE	HPLC-MS/MS	**	Liu G. et al., 2017
β-CD/MrGO	Organochlorine pesticides	Honey	MSPE	GC-ECD	0.52–3.21 ng kg^{-1}	Mahpishanian and Sereshti, 2017
GO/β-CD sol-gel coating fiber	Organophosphate flame retardants	Environmental water	HS-SPME	GC-NPD	1.1–60.4 ng L^{-1}	Deng et al., 2017
Fe_3O_4@SiO_2/GO/β-CD/IL	Plant growth regulators	Vegetables	MSPE	UHPLC-MS/MS	0.01–0.18 μg kg^{-1}	Cao et al., 2019

***Not specified.*

gas chromatography-electron capture detection (GC-ECD) analysis. The method was optimized and evaluated, showing linearity ranging from 2 to 10,000 ng kg^{-1} and $R^2 > 0.9966$, RSDs < 7.8%, LODs from 0.52–3.21 ng kg^{-1}, and LOQ from 1.73–10.72 ng kg^{-1}. For the real samples, the proposed sorbent showed good recoveries in the range of 78.8–116.2% with RSDs ($n = 3$) below 8.1%.

These works demonstrated that cyclodextrins' functionalized GBMs possess great supramolecular recognition, high extraction efficiency, good recoveries, and enrichment capability. It is noteworthy that in all reported works, the chosen graphene-based material is actually the graphene oxide (GO). This trend is justified by the presence of epoxy and -COOH groups on the GO surface, favoring the bonding with the CDs. Although some works reported the employment of graphene combined with cyclodextrins as sorbent, these materials were obtained by graphene oxide reduction (Ragavan and Rastogi, 2017; Tan and Hu, 2017). In this strategy, the reduction stage can be performed before or after the support between graphene-based and CDs. In this way, considering that the oxygenated groups present in the GO structure can improve interaction with molecules of β-CD (Tan and Hu, 2017), it is presumable that the reduction of graphene oxide after β-CD coupling is the best synthesis route to maximize the amount of cyclodextrin coupled.

To complement this topic, **Table 2** presents recently published works using graphene-based materials combined with cyclodextrins for sample preparation. It must be highlighted that all applications employed β-cyclodextrin. Considering the existence of over 100 commercially available and more than 1,500 derivative materials already described, it is clear that sorbents based on graphene functionalized with cyclodextrins are a broad research field to be still explored. Finally, the GBMs/CD's excellent characteristics for supramolecular recognition, high extraction efficiencies, good recoveries, and enrichment capability should contribute to its widespread development in the coming years.

Magnetic Materials

Although graphene and its derivatives are considered to be cutting-edge materials in modern sorbent-based sample preparation (Toffoli et al., 2018; Grajek et al., 2019; Hou et al., 2019), their use can be related to some drawbacks in both bed-packed and dispersive microextraction. Their strong van der Waals interactions may cause irreversible aggregation of the material, causing graphene swelling, which often occurs due to the continuous water/solvent deposition between the graphene nanosheets (Zheng et al., 2017; Iakunkov et al., 2019). For these reasons, columns and microextraction devices packed with GBMs are usually susceptible to clogging and high backpressures. Likewise, for dispersive techniques, graphene nanosheets are well-suspended in solution, creating difficulty for the sorbent recovery, even after filtration and centrifugation (Hou et al., 2019; Li F. et al., 2020).

Within such a context, a modern, and advantageous strategy to overcome those drawbacks is the magnetic solid-phase extraction (MSPE) which is considered an efficient and environment-friendly sample preparation technique (Šafariková and Šafarik, 1999). MSPE extraction mechanism relies on the use of extraction sorbents supported over magnetic materials (Laura et al., 2019). In general, MSPE is a dispersive technique—thin-films or blocks format are also possible—in which the sorbent collection from the sample bulk is easily performed by application of an external magnetic field (Ibarra et al., 2015; Evrim et al., 2019). The use of graphene-based materials for MSPE not only efficiently eliminates the clogging problems from the packed-dispositive but can also enhance the extraction capacity due to GBMs' properties. Also, MSPE possibly eliminates additional centrifugation and filtration steps (Li et al., 2018). For these reasons, the use and development of magnetic sorbents incorporating GBMs have become a key-point in sample preparation in recent years. Nowadays, this combination has been applied in the MSPE of a wide diversity of organic and inorganic analytes from several complex samples, including the treatment of solid matrices (Feriduni, 2019).

These graphene-based magnetic sorbents are currently obtained by physical or chemical anchoring of the magnetic carries onto the graphene sheets. The most common carries include iron (Fe), cobalt, (Co), and nickel (Ni) oxides, highlighting the magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) as the magnetic nanoparticles (MNPs) more frequently used (Laura et al., 2019). The Fe_3O_4 and γ-Fe_2O_3 have superparamagnetic properties, are easy to prepare, and disperse very well in aqueous solutions. Besides, those are MNPs feasible to be modified and functionalized (Yu M. et al., 2019). More popular methods for the preparation of Fe_3O_4 and γ-Fe2O3 based magnetic sorbents include chemical co-precipitation, hydrothermal synthesis, sol-gel reactions, solvothermal synthesis, thermal decomposition, microemulsion, and sonochemical approaches (Filik and Avan, 2019).

The most straightforward type of graphene magnetic sorbents is prepared by direct immobilization of the MNPs on the surface of the G. In this context, two synthetic routes can be employed for obtaining them: (i) the chemical co-precipitation and (ii) the hydrothermal synthesis. The chemical co-precipitation is based on the deposition of iron ions over the nanosheets by adding an alkaline solution to an aqueous dispersion of graphene Fe^{3+}/Fe^{2+} salts, at elevated temperature and vigorous stirring. For example, this method was employed by Yang et al. to prepare superparamagnetic GO/Fe_3O_4 nanoparticles (Yang et al., 2009). After primary treatment of GO sheets, a dispersion of GO, $FeCl_3$ was stirred under inert atmosphere by several hours. After that, Fe^{3+}/Fe^{2+} ions were coordinated by the carboxylate anions on the GO sheets, and then GO/Fe_3O_4 nanoparticles were precipitated by the addition of an aqueous NaOH solution (**Figure 5**). As Fe^{3+} shows higher affinity than Fe^{2+} for carboxylic groups, the ratio of those ions should be controlled—generally, 2:1 is used—and the content of the carboxylic acid groups on the GO sheets needs to be previously determined by acid-base titration (Yang et al., 2009). The chemical co-precipitation method can also be employed to prepare magnetic reduced graphene oxide sorbents (rGO/Fe_3O_4). As an example, Chandra et al. prepared an rGO/Fe_3O_4 for arsenic removal from surface water samples (Chandra et al., 2010). In this case, after dispersion and precipitation with ammonia, GO/Fe_3O_4 particles were reduced to rGO/Fe_3O_4 by slowly adding hydrazine hydrate under stirring conditions 90°C.

An important issue is that the morphology of graphene magnetic sorbents prepared via chemical co-precipitation can be challenging to control. Thus, the resulting magnetic material sometimes presents low absorptivity due to the uneven distribution and agglomeration of the Fe_3O_4 particles on the nanosheets. Within such a context, hydrothermal synthesis has been proposed as an alternative to yield sorbents with better Fe_3O_4 particles distribution with more G exposed adsorption sites and then, improved adsorption capacity. This method is based on the reduction of Fe^{3+} and GO in sodium acetate and polyethyleneglycol in an autoclave at elevated temperature (Li et al., 2018). In this way, Wu and cookers prepared rGO/Fe_3O_4 particles (Wu et al., 2013), sonicating GO first in ethylene glycol, and then in the presence of $FeCl_3$. After complete dispersion, the obtained clear solution was spiked with sodium acetate, and the mixture was sealed in a Teflon-lined stainless-steel autoclave and maintained at 200°C for 8 h. The authors reported regular morphology particles.

Also, more reproducible, stable, and versatile graphene magnetic sorbents can be prepared from silica-coated magnetite particles (Fe_3O_4@SiO2). In this case, before graphene anchoring, Fe_3O_4 particles are modified with a silane coupling agent, consisting of tetraethyl orthosilicate (TEOS) and (3-aminopropyl) triethoxysilane (APTES) (Li et al., 2018). Sequentially, the graphene can be coupled to the particles by physical adsorption or by covalent bonding. Luo et al. prepared Fe_3O_4@SiO_2/G particles for extraction of sulfonamides from water samples, by physical immobilization of graphene nanosheets on silica-coated magnetite (Luo et al., 2011b). The procedure obtained by pure dispersion under sonication for several hours renders particles not stable enough to continue reusing. Therefore, the chemical bonding of the graphene to the silanol groups is the preferred synthetic method. Amino groups are introduced on the surface of the Fe3O4@SiO2 particles and GO, anchored via an amidation

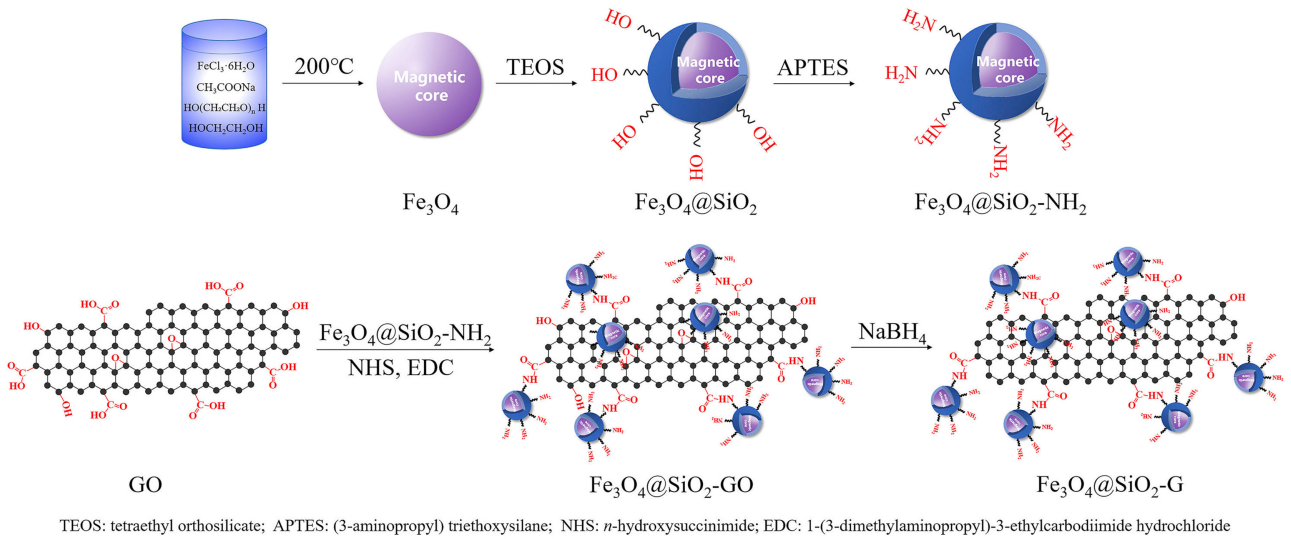

FIGURE 5 | Schematic representation of GO loaded with Fe3O4 nanoparticles. Reprinted with permission from Yang et al. (2009), copyright 2009, Royal Society of Chemistry.

reaction with the aid of cross-linking agents such as 1-(3-dimethyl aminopropyl)-3-ethyl carbodiimide hydrochloride (EDC) and -hydroxysuccinimide (NHS). This process is schematically represented in **Figure 6** (Li et al., 2018). For example, Zhang et al. prepared Fe_3O_4@SiO_2/GO particles by mixing Fe_3O_4@SiO_2 and 3-aminopropyltriethoxysilane in isopropanol, under N_2 atmosphere at 70°C, followed by addition of a GO solution containing NHS and EDC, stirring overnight (Zhang et al., 2014). Like Fe_3O_4/rGO composites, Fe_3O_4@SiO_2/rGO particles can be obtained by the posterior reduction of GO with hydrazine.

The adsorption capacity of these magnetic particles is mostly based on the hydrophobic interactions of the honeycomb-like lattice into the carbon atoms. Consequently, bare graphene magnetic particles are not suitable MSPE sorbents for polar or ionic compounds. The main advantage of the Fe_3O_4@SiO_2/G particles is the possibility to covalently bond additional functional moieties, which improve the adsorption capacity, selectivity, and applicability of them. In this manner, Fe_3O_4@SiO_2/G have been covalently functionalized with ionic surfactants, ionic liquids (ILs), deep eutectic solvents, boronate affinity materials (BAM), supramolecules (crown ethers, cyclodextrins, calixarenes, cucurbiturils, and pillararenes), aptamers, polymers, and metal-organic frameworks (MOFs). For those magnetic GBMs, their preparation and applications were recently comprehensively reviewed by Li et al. They provided a summary of the state of the art of them, highlighting application as MSPE sorbents of organic compounds, biomolecules, and metal ions (Li et al., 2018). For this reason, herein, we provide an updated overview of the reported magnetic graphene sorbents between 2019 and 2020, and their applications in the determination of small organic molecules by chromatographic analysis.

In this context, magnetic graphene sorbents have found spread applications in the extraction of biomolecules organic compounds and metal ions. An assessment in the Scopus database, using the keywords "graphene" and "magnetic solid-phase extraction," yield 248 results from 2010. Among them, 26 correspond to review papers and the rest to research papers mainly dedicated to describing the application of magnetic graphene sorbent in different areas of the analytical chemistry. As mentioned previously, Li et al. recently published a summary of the applications of graphene-based MSPE. In **Table 3**, we provide a summary of the magnetic graphene sorbent applications published from 2019 to date. It is noteworthy that a vast number of researchers using graphene-derived magnetic materials focus their efforts in areas as environmental surveillance and food security applications. This includes a wide diversity of analytes such as drug residues, pesticides, hormones, food additives, and active plant ingredients.

Molecularly-Imprinted Polymers

Another group of materials explored for sample preparation involves the molecularly-imprinted polymers (MIPs) combined with GBMs. In this context, MIPs are prepared from a template (mold) which behaves structurally similarly as the target analytes, to achieve a selective interaction through a template-complementary binding site (Pan et al., 2018). These materials are traditionally prepared by copolymerization of the complex formed between the template and a functional monomer. This can occur through either covalent (hydrogen bonds) or non-covalent bonds (ionic and hydrophobic interactions) with a cross-linking agent in the presence of a suitable porogenic solvent (Zhou T. et al., 2019). After that, the molecular template is eliminated, resulting in a rigid three-dimensional cavity selective to the target analytes. As an example, **Figure 7** depicts the

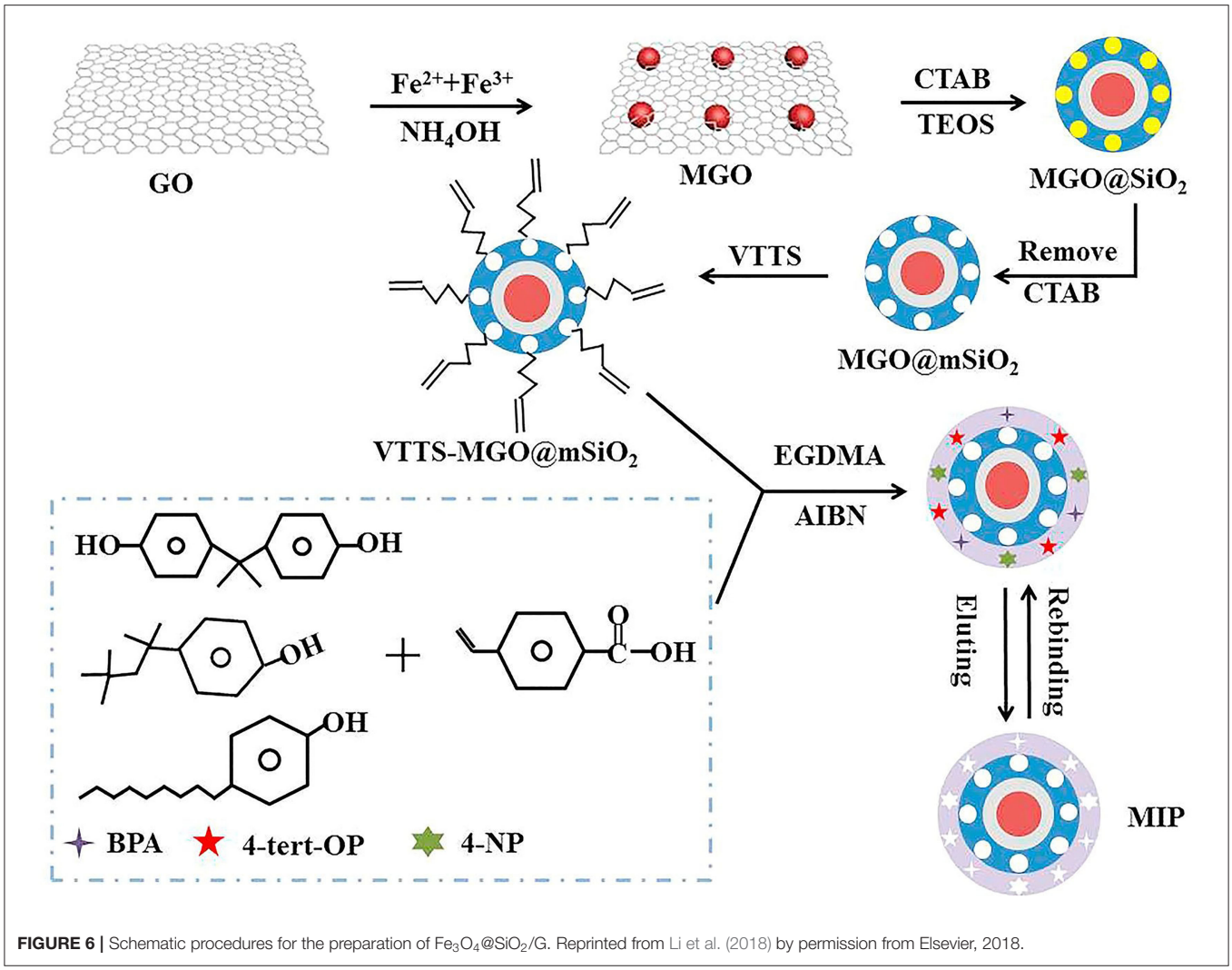

FIGURE 6 | Schematic procedures for the preparation of $Fe_3O_4@SiO_2/G$. Reprinted from Li et al. (2018) by permission from Elsevier, 2018.

synthesis steps employed by Xie et al. (2019). The authors selected 4-vinylbenzoic acid as a functional monomer to prepare the template taking advantage of the presence of the carboxyl ring and benzene, which can bind to BPA, 4- tert-OP, and 4-NP through hydrogen bonds and π-π interactions in the polymerization process.

Due to this high selectivity and relatively easy preparation, these materials have been widely used for molecular recognition and separation in different fields (sensors, drug delivery, protein recognition, and chromatography). In this section, their application in the field of sample preparation combined with GBMs is covered, highlighting preparation strategies of aqueous-recognition MIPs cleverly to achieve such functionalization (Zhou T. et al., 2019). For example, Luo et al. (2017) synthesized boronic acid-functionalized with graphene oxide, with a subsequent immobilization of ovalbumin as MIP-template, to obtain a high-selective sorbent, namely GO-APBA/MIPs. This strategy was chosen to overcome such difficulties related to specific recognition and separation of glycoproteins in complex biological samples. A comparison between the resulting material (GO-APBA/MIPs) and a bare GO-MIPs, without insertion of boronic acid, showed more extraction performance for the hybrid obtained sorbent. In this way, the outstanding recognition capacity by linking boronic acid and MIP cavities together with a high surface area of GO can represent a promising strategy to produce high performative sorbent material for biological glycoproteins.

Another interesting example was reported by Cheng et al. (2017), consisting of a more straightforward strategy using GO combined with MIP to extract and efficiently pre-concentrate bis(2-ethylhexyl) phthalate (DEHP) in environmental water samples. Contrary to the prior study (Luo et al., 2017), considering the smaller complexity of the target compound and water samples, the employment of only GO-MIP was already enough to achieve excellent extraction performance. In this case, dispersive solid-phase microextraction combined with HPLC-UV reported enrichment factors of more than 100-fold compared to the directly injected extract, highlighting this simple GO-MIP as a suitable sorbent in such cases.

TABLE 3 | Recent applications (2019–2020) of graphene-based MSPE to the determination of organic compound by chromatography and mass spectrometry.

Sorbent	Analytes	Matrix	Analysis	LOD	References
Magnetic N-doped 3D graphene-like framework carbon (Fe3O4@N-3DFC)	Cephalosporin antibiotics	River water and zebrafish samples	HPLC-UV	0.20–0.45 $\mu g\ L^{-1}$	Niu et al., 2020
Fluorine and nitrogen functionalized magnetic graphene (G-NH-FBC/Fe2O3)	Perfluoroalkyl and polyfluoroalkyl substances	Water and functional beverage	HPLC-Orbitrap HRMS	3 ng L^{-1}	Xian et al., 2020
Graphene-like MoS2-modified magnetic carbon-dot nanoflowers (MoS2@Fe3O4@C-dot NFs)	Ibuprofen	Pharmaceutical, environmental water and synthetic urine samples	HPLC-DAD	11 × 10^{-6} $\mu g\ mL^{-1}$	Yilmaz and Sarp, 2020
Oxide/lanthanum phosphate nanocomposite (MGO@LaP)	Chlorpyrifos pesticides	Water and fruit samples	GC–ECD	0.67 $\mu g\ L^{-1}$	Asadi et al., 2020
Fe3O4/rGO	Oral anticoagulants	Human plasma	HPLC-DAD	0.003 $\mu g\ mL^{-1}$	Ferrone et al., 2020
Iron crosslinked alginate encapsulated magnetic graphene oxide (Fe/alginate/Fe3O4/rGO)	Endocrine-disrupting compounds	Superficial water	HPLC-UV	8–14 ng L-1	Shah and Jan, 2020
chitosan functionalized magnetic graphene oxide nanocomposite (Fe_3O_4@SiO_2@CS/GO)	Alkaloids	Chinese herb (*Pericarpium papaveris*)	UHPLC-MS/MS	0.016–0.092 $\mu g\ kg^{-1}$	Tang et al., 2020
Fe_3O_4/rGO	Oral anticoagulants	Human plasma	UHPLC-DAD	0.003 $\mu g\ mL^{-1}$	Tang et al., 2020
Magnetic carbon nanodot/graphene oxide hybrid material (Fe_3O_4@C-nanodot@GO)	Ibuprofen	Human blood	HPLC-DAD	8.0 ng mL^{-1}	Yuvali et al., 2020
Fe_3O_4/GO	Psychoactive drugs	Urine	UHPLC-MS/MS	0.02–0.2 $\mu g\ L^{-1}$	Lu et al., 2020
Fe_3O_4/GO	Melamine	Water and dairy products	HPLC-UV	0.03 $\mu g\ L^{-1}$	Abdolmohammad-zadeh, 2020
Three-dimensional graphene aerogel combined with Fe_3O_4 nanoparticles (3DG-Fe_3O_4@Sp)	Cholecalciferol (vitamin D3)	Bovine milk	HPLC-UV	3.01 $\mu g\ L^{-1}$	Sereshti et al., 2020
Fe3O4/GO	45 multi-class pesticides	Vegetables (cabbage, leek, and radicchio)	GC-MS	0.4–4.0 μg kg−1	Chatzimitakos et al., 2019
Aptamer-functionalized Fe_3O_4/graphene oxide (Fe_3O_4/GO/Apt)	Chloramphenicol	Honey and Milk	HPLC-DAD	0.24 $\mu g\ L^{-1}$	Tu et al., 2019
Flower-like hybrid material composed of Fe_3O_4, graphene oxide and CdSe nanodots (Fe_3O_4/GO/CdSe)	Ibuprofen	Pharmaceuticals, water, and urine	HPLC-DAD	0.36 ng mL^{-1}	Sarp and Yilmaz, 2019
Fe_3O_4/rGO	Aflatoxin B1 and B2	Vegetable Oils	HPLC-PCD-FLD	0.01–0.02 $\mu g\ kg^{-1}$	Yu L. et al., 2019
Fe_3O_4/rGO	N-nitrosamines	Mainstream cigarette smoke	HPLC-MS/MS	0.018–0.057 ng $cigarette^{-1}$	Pang et al., 2019
GO@NH_2@Fe_3O_4	Quinolones	Water	MALDI-TOF MS	0.010 mg L^{-1}	Tang H. et al., 2019
GO@NH_2@Fe_3O_4	Triazines	Water	DART-MS	1.6–152.1 ng L^{-1}	Jing et al., 2019
Magnetic nitrogen-doped reduced graphene oxide (Fe_3O_4@N-rGO)	Endocrine disruptors	Carbonated beverages	HPLC-DAD	0.1–0.2 $\mu g\ L^{-1}$	Li et al., 2019b
Molecular imprinted polymer (MIP) material combined with magnetic graphene oxide (Fe_3O_4/GO-MIP)	Quercetin and luteolin	Green tea and serum samples	HPLC-UV	0.09–4.5 ng mL^{-1}	Dramou et al., 2019
Magnetic graphene-like molybdenum disulfide nanocomposite	Triazines and sulfonylurea herbicides	Water	UHPLC-MS	20 and 170 ng L^{-1}	Zhou Y. et al., 2019
Ionic liquid magnetic graphene (IL@MG)	Microcystins	Water	UHPLC-MS/MS	0.414 ng L^{-1} and 0.216 ng L^{-1}	Liu X. et al., 2019
Covalent organic framework-derived hydrophilic magnetic graphene Composite (magG@PDA@TbBd)	Phthalate esters	Milk	CapillaryLC-MS	0.004–0.02 ng mL^{-1}	Lu et al., 2019a

(Continued)

TABLE 3 | Continued

Sorbent	Analytes	Matrix	Analysis	LOD	References
Curcumin loaded magnetic graphene oxide	Parabens	Toothpaste and mouthwash	HPLC-DAD	0.4–1.0 ng mL^{-1}	Razavi and Es, 2019
Fe_3O_4/rGO	Non-steroidal Anti-inflammatory Drugs	Animal food	HPLC-MS/MS	0.1–0.5 μg kg^{-1}	Wang et al., 2019
Fe_3O_4/rGO	Phenolic compounds	Oil seeds	LC-MS/MS	0.02–90.00 μg kg^{-1}	Lang et al., 2019
Fe_3O_4/rGO	Pesticides	Water	HPLC-UV	0.2–1.6 ng mL^{-1}	Madej et al., 2019
Three-dimensional hierarchical frameworks based on molybdenum disulfide-graphene oxide-supported magnetic nanoparticles (Fe_3O_4/GO/MoS_2)	Fluoroquinolone antibiotics	Water	HPLC-UV	0.25–0.50 ng mL^{-1}	Xiao et al., 2019
Magnetic nanoparticles/graphene oxide (TPN/Fe_3O_4NPs/GO) nanocomposite	Pesticides	Water	HPLC-UV	0.17–1.7 μg L^{-1}	Moradi et al., 2019
Silver-modified Fe_3O_4/graphene nanocomposite (Ag@Fe_3O_4@G)	Aromatic amines	Water	HPLC-UV	0.10–0.20 μg L^{-1}	Alasl et al., 2019
Fe_3O_4/GO	Chlorophenols	Sewage water	GC-ECD	3.0–28.4 ng L^{-1}	Esfandiarnejad and Sereshti, 2019
Ternary nano-composite, magnetite/reduced graphene oxide/silver (Fe_3O_4/rGO/Ag)	Morphine and codeine	Blood and urine	HPLC-UV-VIs	1.8–2.1 ng L^{-1}	Abdolmohammad-zadeh et al., 2019
Magnetic amino-functionalized zinc metal-organic framework based on a magnetic graphene oxide composite (M-IRMOF/Fe_3O_4/GO)	Heterocyclic fungicides	Lettuce	HPLC-MS/MS	0.21–1.0 μg L^{-1}	Liu G. et al., 2019
Fe_3O_4/rGO	Chiral pesticides	Cucumber, tomato, cabbage, grape, mulberry, apple, and pear	Chiral HPLC-MS/MS	0.02–10.0 μg g^{-1}	Zhao et al., 2019
Reduced graphene oxide-carbon nanotubes composite (Fe_3O_4/rGO-CNTs)	Sulfonamides	Milk	HPLC-UV	0.35–1.32 μg L^{-1}	Feng et al., 2019
Magnetic graphene oxide/multiwalled carbon nanotube core-shell (GO/MWCNT/Fe_3O_4/SiO_2)	Paracetamol and caffeine	Synthetic urine and wastewater	HPLC-UV	0.48–3.32 ng mL^{-1}	Ibrahim et al., 2019
Magnetized graphene oxide functionalized with hydrophilic phytic acid and titanium(IV) (magGO@PEI@PA@Ti^{4+})	Nucleobases, nucleosides, and nucleotides	Medicinal mushroom *C. Sinensis,* and natural foods	HPLC-DAD	1.8–2.8 ng mL^{-1}	Zhang Q. et al., 2019
MagG@SiO_2@ZIF-8 composites	Phthalate Easers	Human Plasma	GC-MS	0.003–0.01 ng mL^{-1}	Lu et al., 2019b
Multi-templates molecularly imprinted polymer (MIP) on the surface of mesoporous silica-coated magnetic graphene oxide (MGO@mSiO_2),	Alkylphenols	Water	HPLC-DAD	0.010–0.013 μg L^{-1}	Xie et al., 2019
GO	Phthalate esters	Bottled, injectable and tap waters	HPLC-UV	0.004–0.013 mg L^{-1}	Abdelghani et al., 2019
Fe_3O_4/rGO	Non-steroidal anti-inflammatory drugs and bisphenol-A	Tap water	HPLC-DAD	0.031 mg L^{-1} and 0.023 mg L^{-1} 0.1785 mg L^{-1}	Ungku Abdullah et al., 2019
rGO/$ZnFe_2O_4$ nanocomposite	Estrogens	Water, soil, and fish	UHPLC-QTOF-MS	0.01–0.02 ng mL^{-1}	Li W. et al., 2020
Polydopamine functionalized magnetic graphene (PDA@MG)	Triazole fungicides	Water	HPLC-UV	4.8–8.4 ng L^{-1}	Xiong et al., 2019
zeolitic imidazolate framework-7@graphene oxide (mag-ZIF-7@GO)	Fungicides	Water and soil samples	HPLC-Orbitrap HRMS	0.58–2.38 ng L^{-1}	Zhang S. et al., 2019

(Continued)

TABLE 3 | Continued

Sorbent	Analytes	Matrix	Analysis	LOD	References
Magnetic polyethyleneimine modified reduced graphene oxide (Fe_3O_4@PEI-rGO)	Polar non-steroidal anti-inflammatory drugs	Water	HPLC-DAD	0.2 $\mu g\ L^{-1}$	Li et al., 2019a
Guanidinium ionic liquid modified magnetic chitosan/graphene oxide nanocomposites (GIL-MCGO)	DNA	Human whole blood and *E. coli* cell lysate	**	**	Liu M. et al., 2019

***Not specified.*

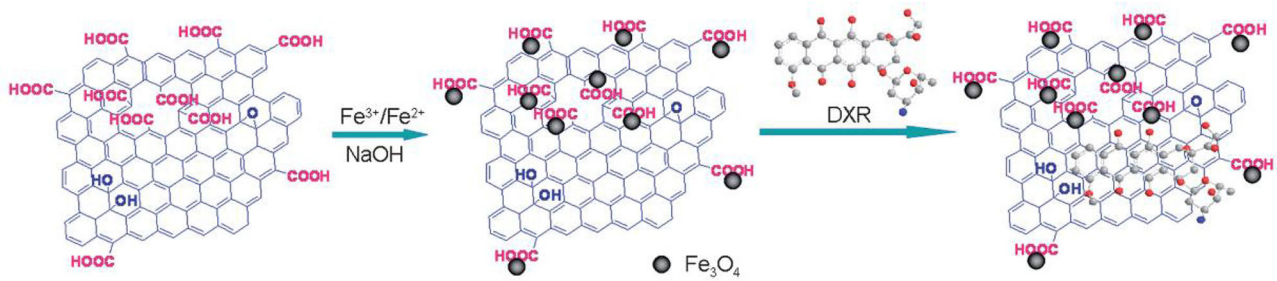

FIGURE 7 | Schematic diagram representing the synthesis of VTTS-MGO@mSiO2@MIP. Reprinted with permission from Xie et al. (2019), copyright 2019, Elsevier.

An attractive approach to obtain high performative material involves the use of the graphene-based MIP functionalized with a magnetic particle to favor the extraction and sorbent isolation. Within such a context, Ning et al. (2014) proposed a novel nanosized substrate imprinted polymer (GO-MIP-Fe_3O_4) on magnetic graphene oxide (GO-Fe_3O_4) surface to remove 17β-estradiol (17β-E_2) from food samples. The resulting sorbent has shown a functional extraction recovery of 84.20% at a low concentration level of 0.5 $\mu mol\ L^{-1}$. Furthermore, due to the magnetic properties of the GO-MIP-Fe_3O_4, a simple, fast, and efficient separation of 17β-E_2 were achieved, suggesting the combination between these materials as an excellent way to obtain hybrid sorbents. Following the same trend, Barati et al. (2017) synthesized a MIP based on magnetic-chitosan functionalize with GO to extract fluoxetine from environmental and biological samples. From our viewpoint, the outstanding characteristic of this work is the excellent pre-concentration factor of 500 related to such sorbent, which reinforces the combination of MIP, GBMs, and magnetic materials as an excellent way to improve sample preparation performance. Finally, another study based on a similar approach was presented by Fan et al., who prepared, through a chemical co-precipitation method, a novel hybrid sorbent based on MIP, GO, and superparamagnetic Fe_3O_4 particles (GO-MIP-Fe_3O_4). In this case, the author worked with natural samples, specifically alkaloids (evodiamine and rutaecarpine) extract from *Evodiae Fructus*, suggesting a great versatility of such magnetic GO-MIP sorbent. Also, its analytes' recovery achieves values over 82% considering as good values from our viewpoint.

Moreover, **Table 4** presents other published studies to complement the discussion regarding hybrid sorbents combining MIP with GBMs.

Ionic Liquids

In 1914, a work with ionic liquids (ILs) was reported for the first time, although at that time, the author had no idea of the importance that these would take in the scientific area almost a century later (Walden, 2003). From the 1980's to the present, the interest in studying ionic liquids grew exponentially (Evans et al., 1981; Shi et al., 2015; Welton, 2018). Although they have been applied in several scientific areas, in the analytical chemistry the studies have focused mainly on the use of them for extraction, and separation purposes (Rodríguez-Sánchez et al., 2014; Tang et al., 2014; García-Alvarez-Coque et al., 2015; Marcinkowski et al., 2015; Hu et al., 2016; Yu et al., 2016; Nawała et al., 2018; Rykowska et al., 2018).

The ILs are compounds with a dual nature acting as nonpolar for nonpolar analytes and, inversely, for those with a strong proton donor group, depending on the separation mechanism that it presents (Berthod et al., 2018). Therefore, their excellent properties (high thermal stability, good solubility, and easily functionalization) have been modified in several different ways. One approach consists of replacing the anionic or cationic part with another material, of automatically regulating the IL property or nature (hydrophobicity, hydrophilicity, viscosity, and among others) (Ho et al., 2014; An et al., 2017). In this way, improvements in their sensitivity and selectivity to the extraction of the different analytes can be achieved. One of these desired enhancements is obtained by combining the ILs with GBMs, owing to their different types of chemical interactions with the analytes (e.g., n-π, π-π, hydrogen bonding, dipolar, ionic charge/charge) (Chen Y. et al., 2016; Feng et al., 2020). For these reasons, IL-GBMs present excellent extraction efficiency for a wide variety of analytes in several complex matrices (e.g., environmental, food, drinks, biological,

TABLE 4 | Applications of MIPs-GO composite in sample pretreatment.

Sorbent	Analytes	Matrix	Sample preparation	Analysis	LOD	References
GO-APBA/MIP	Ovalbumin	Egg white	SDS	Gel-Electrophoresis	**	Luo et al., 2017
bis(2-ethylhexyl) phthalate (DEHP)	Trace DEHP phthalate	Water	SPME	HPLC-UV	0.92 ng mL^{-1}	Cheng et al., 2017
MIPs-GO-Fe_3O_4	17β-estradiol	Milk powder	External magnet	**	0.035 and 0.10 μmol L^{-1}	Ning et al., 2014
GO-QDs-MIPs	p-t-octylphenol	Water	SPE	UHPLC-UV	0.15 μmol L^{-1}	Han et al., 2015
(TFMAA)-GO (EGDMA)-GO	Cefadroxil	Water	d-SPE	UPLC-DAD	0.01 μg mL^{-1}	Chen and Ye, 2017
MGR@MIPs	4-nitrophenol	Lake water	MIP	HPLC-UV	**	Luo et al., 2016
MIP-GO/Chm	Fluoxetine	Tap, well and spring water, and urine	MSPE	UV–Vis spectrophotometry	0.03 μg L^{-1}	Barati et al., 2017
MIP@Fe_3O_4@GO	Evodiamine and rutaecarpine	*Evodiae fructus*	External magnet	HPLC-UV	**	Fan et al., 2017

***Not specified.*

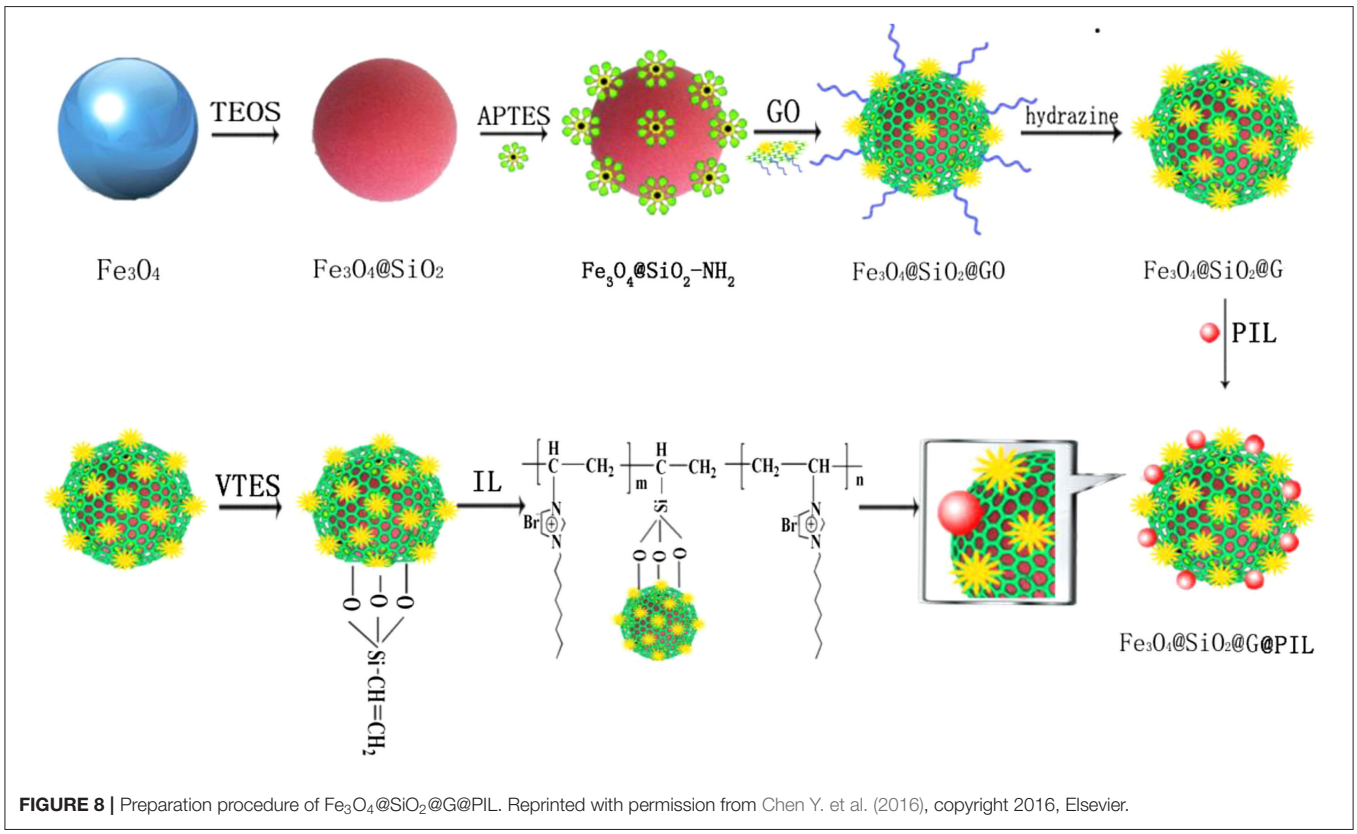

FIGURE 8 | Preparation procedure of Fe_3O_4@SiO_2@G@PIL. Reprinted with permission from Chen Y. et al. (2016), copyright 2016, Elsevier.

and among others), as presented. Additionally, **Figure 8** illustrates a typical synthesis process performed to achieve such hybrid sorbents.

The employment of ILs combined with GMBs includes the work reported by Liu X. et al. (2019) to determine the environmentally-dangerous monocyclic heptapeptides (microcystins, MC) in natural water samples. They synthesized the IL-G by the co-precipitation route. For the analytes' extraction, the MSPE technique was used while for its separation, determination, and quantification, the authors employed UHPLC-MS/MS. For the optimization of the experimental parameters, univariate analysis, and orthogonal screening were used. The analysis time was 18 min, with excellent linearity. The LODs were 0.414 and 0.216 ng L^{-1} for MC-LR and MC-RR, respectively, reporting traces of these two compounds in natural water samples, and it can be concluded that the method used is promising for the study of other types of microcystins in water samples.

Other interesting applications based on IL-GBMs include work published by Chen Y. et al. (2016). They synthesized magnetite nanoparticles (Fe_3O_4) of controlled size by a co-precipitation method to obtain the Fe_3O_4-SiO_2-G-PIL hybrid sorbent; the obtained material was used to determine preservatives in vegetables by QuEChERS following GC-MS analysis. In this study, it was possible to take advantage of the functional properties related to magnetic nanoparticles and also by its coupling to the GO with the polymeric ILs (e.g., high surface area, and solvent effects). Moreover, the method's detection limits varied between 0.82 and 6.64 $\mu g\ kg^{-1}$ for the 20 preservatives studied. Similarly, Tashakkori et al. (2019) synthesized a series of ionic liquids grafted onto stainless steel wires, which were previously coated with GO, using a sol-gel technique. The authors used this hybrid material as a sorbent in an on-line DI-SPME-GC-MS approach to determine phthalate esters (PAE) in several samples such as tap water and seawater and coffee. They reported low detection limits (5–30 $ng\ L^{-1}$) and the lab-made SPME fibers being used more than 120 times, which is a useful feature when compared with the commercially available ones such as PA and CAR/PDMS. These results reinforce the great interest in developing hybrid GBMs combined with ILS.

Additionally, biological samples were analyzed by Ding et al. (2015). They functionalized magnetic chitosan with a series of ionic liquids of guanidinium and graphene oxide, aiming to extract trypsin, lysozyme, ovalbumin, and albumins from bovine serum. In this case, the analytical results obtained by this hybrid guanidinium-IL functionalized with MCGO were compared with those achieved by employing just GO, magnetic chitosan, or MCGO. In this case, the hybrid guanidinium-IL-MCGO exhibits higher extraction performance compared to the other sorbents, which suggests the combination of GBMs and ILs as a suitable strategy to achieve more performative extractive phases. Also, Hou et al. (2016) proposed a poly(1-vinyl-3-hexylimidazolium bromide)-GO-grafted silica [poly($VHIm^+Br^-$)-GO-Sil] as a hybrid IL sorbent to extract flavonoids in urine samples. They synthesized the material by an interesting process consisting of an *in situ* radical chain-transfer polymerization and then *in situ* anion exchange. In this case, the procedure started with the silica coating by GO, using a manufacturing layer by layer fabrication method. Then, the poly ($VHIm^+Br^-$)-GO-Sil anion was transformed into hexafluorophosphate (PF^{6-}) by *in situ* anion exchange. The method based on SPE-HPLC-UV showed acceptable extraction recoveries for four flavonoids, with limits of detection in the range of 0.1–0.5 $\mu g\ L^{-1}$. The proposed material showed ecological and cost-effective advantages. It can be applied successfully to the extraction and enrichment of flavonoids in human samples, even allowing the study of metabolic kinetics.

Finally, considering other types of samples, an interesting study published by Zhou et al. (2016) was carried out to analyze phthalates (PAE) in eraser samples. The Office of Quality and Technology Supervision of the province of Jiangsu in China required PAE analyses in several samples to maintain concentrations inside the accepted limits registered by law. For this reason, it is necessary to monitor residues of this compound not only in food but also in everyday objects used by people (Zhou et al., 2016). Within such a context, a new graphene oxide compound modified with ionic liquids (GO-[AEMIM][Br]) was synthesized through an amidation reaction between the amino groups of the ILs and the carboxyl groups of GO. The high extraction capacity reported by this hybrid sorbent suggests the combination of the properties of ILs and GBMs (tunability and high surface area) as a positive relationship that assisted the PAE extraction in such a non-common sample.

Apart from the interesting application herein discussed, other relevant studies based on hybrid sorbents combining IL and GBMs are shown in **Table 5**.

Miscellaneous

This section aims to present some alternative graphene-based materials that could be an alternative for the sample preparation in the future. For this reason, there are not many publications apart from the materials already discussed in the previous sections. An original example recently published by Tahmasebi (2018) consisted of intercalating aluminum polyoxocations (Al_{30}) between the graphene oxide (GO) nanosheets and further support this onto polycaprolactone nanofibers, aiming to enhance temperature, and pH resistance to the sorbent phase. This novel material was applied in SPE extraction of four statin drugs showing acceptable analytical performance. The author emphasized the excellent ability of this sorbent to interact with acidic polar species, possibly due to the resistance to pH variations. Another interesting application was performed by Li et al. (2014) whose synthesized a magnetic ionic liquid/chitosan/GO (MCGO-IL) as a biodegradable/bio-sorbent for the mitigation of Cr (IV) in the treatment of residual in environmental water samples. A solid-liquid separation was performed in the presence of an external magnetic field, involving a pseudo-second-order kinetic sorption step. The maximum adsorption capacity was 145.35 $mg\ g^{-1}$, obtained thanks to the intermolecular hydrogen bond between MCGO-IL and Cr(IV), due to the hydroxy and amine groups to which the ions bind metallic. This result demonstrates the potential of this hybrid sorbent material in the cleaning processes of contaminating metal ions in complex matrices such as water. Additionally, the authors emphasize its low producing cost as a useful characteristic since commercially available sorbents often are expensive and non-reusable.

Additionally, the more significant interlayer distance between GO sheets, due to Al_{30} insertions, can enhance the interaction with the π-electron system onto the GO surface, resulting in enhanced extraction performance. Similarly, Amiri et al. (2019) synthesized another hybrid sorbent but this time supporting GO nanosheets with polyoxotungstate (POT), instead of Al, to enhance chemical stability and pH resistance once POT act as charge-compensating and space-filling compound. This strategy is proposed to enhance the POT water dispersibility and surface area of the sorbent, possibly favoring the extraction performance while ensuring its high thermal and pH stability. Another strategy, underscored by Farajvand et al. (2018), was to covalently-bond an electrically conducting polymers onto the GO surface to diminish its self-aggregation in aqueous as well as enhance adsorption capacity. In this case, polyaniline was used

TABLE 5 | Applications of Ils-GO composite in sample pretreatment.

Sorbent	Analyte	Matrix	Sample preparation	Analysis	LOD	References
IL-GO@Silica	Chlorophenols	Water	SPE	HPLC-UV	**	Wang et al., 2017a
IL@MG	Microcystins	Water	MSPE	UHPLC-MS/MS	0.414 ng L^{-1} and 0.216 ng L^{-1}	Liu X. et al., 2019
$[C_4C_{12}im]$@GO	Hg	Water	SPE	AAS	14 ng L^{-1}	Sotolongo et al., 2018
[BMim]@MGO	Heavy metal ions	Water	ICP-OES	ICP-OES	0.1–1.0 μg L^{-1}	Rofouei et al., 2017
MGONPs-C_{16}mimBr	Chlorophenols	Water	MSPE	HPLC-UV	0.10–0.13 μg L^{-1}	Liu W. et al., 2017
PGO-MILN	Chlorophenols	Water	MSPE	LC-MS/MS	0.2–2.6 ng L^{-1}	Cai et al., 2016
GO-1-butyl-3-aminopropyl imidazolium chloride.	Anabolic steroids β-blockers	Water	SPE	HPLC-DAD	7–23 ng L^{-1}	Serrano et al., 2016
GO-PILs monolith	Phenolic	Water	SPME	HPLC-UV	0.2–0.5 μg L^{-1}	Sun et al., 2016
Fe_3O_4@SiO_2@G@PIL	Preservatives	Vegetables	QuEChERS	GC-MS	0.82–6.64 μg kg^{-1}	Chen Y. et al., 2016
rGO/ILN-ETD	Estrogens	Milk	ETD	HPLC-DAD	0.09–0.30 μg L^{-1}	Chu et al., 2019
1-(3-aminopropyl)-3-vinyl imidazolium bromide/tetrafluoroborate) grafted	Phthalate Esters	Coffee Tap Water Seawater	SPME	GC-MS	5–30 ng L^{-1}	Tashakkori et al., 2019
PANI-MWCNTs-rGO-IL	Alcohols	Tea	SPME	GC-FID	2.2–28.3 ng L^{-1}	Li et al., 2016
IL-TGO	Auxins	Soybean sprouts	PT-SPE	HPLC-DAD	0.004–0.026 μg g^{-1}	Zhang H. et al., 2018
MGO-C_{16}MIM-DMG	Trace nickel	Spinach Cacao powder Tea Cigarette	MSPE	FAAS	0.16 μg L^{-1}	Aliyari et al., 2016
MCGO@guanidinium IL	Protein	Bovine serum	MSPE	UV-vis	**	Ding et al., 2015
poly(VHIm$^+$Br$^-$)@GO@Sil	Flavonoids	Urine	SPE	HPLC-UV	0.1–0.5 μg L^{-1}	Hou et al., 2016
IL-coated Fe_3O_4/GO	Hemin	Serum	SPE	FAAS	3.0 μg L^{-1}	Farzin et al., 2016
1-(3-aminopropyl)imidazole chloride modified MGO	Polysaccharides	Brown alga	MSPE	HPLC-UV	**	Wang et al., 2017b
PILs@GO@Sil	Phenolic acids	Black wolfberry yogurt and urine	SPE	HPLC-UV	0.20–0.50 μg L^{-1}	Hou et al., 2018a
Magnetic GO/PPy	Methotrexate	Urine	d-SPE	HPLC-UV	7 ng mL^{-1}	Hamidi et al., 2019
3D-IL-Fe_3O_4-GO	PAHs	Human blood	PT-SPE	GC-MS	0.002–0.004 μg L^{-1}	Zhang Y. et al., 2018
PIL(Br)-G/SiO_2	Human serum albumin	Human whole blood	SPE	HPLC-UV	**	Liu et al., 2018
Fe_3O_4/GO NPs	Cephalosporins	Urine	MSPE	HPLC-UV	0.6 and 1.9 ng mL^{-1}	Wu et al., 2016
GO-[AEMIM][Br]	Phthalates	Eraser	SPE	HPLC-UV	0.02–0.88 ng mL^{-1}	Zhou et al., 2016

***Not specified.*

considering its various oxidation states possessing distinct charge carriers which allow chemical interactions with several heavy metals. In this case, this sorbent was tested for Cd isolation from environmental water samples by SPE and subsequent dispersive liquid-liquid extraction.

In general, all these modifications performed in the G or GO chemical structures aim to improve its performance. However, a work published by Ashori et al. seems to follow the reverse trend focusing on using the graphene oxide as a reinforcement for glass fiber and epoxy composites. For this reason, the primary goals were to improve chemical reactivity, toughness, and adhesion to polymeric matrices, including the GO's widely-known properties. Although this material had not been applied for sample preparation yet, its high mechanical strength might be a promising tool particularly for miniaturized sample preparation techniques once they often demand high-pressure procedures causing clogging problems when GO or G are applied as sorbents. Recently, another interesting compound,

namely graphene-aerogel, has gained attention mainly due to its superior and tunable volume as well as surface area as compared to graphene. The aerogel only itself exhibits poor extraction performance for water-soluble analytes, thus demanding some modification on it, as the functionalization of graphene-based compounds. In this way, graphene-based aerogels are suitable for sample preparation since they can relate the great qualities of graphene with the impressive compressibility of aerogels. Therefore, high-performance sorbents packed into sample preparation hardware can be reusable many times, considering the compressibility factor, which can help unpack and recover these extractive phases. As examples, Maggira et al. (2019) and Tang S. et al. (2019) reported two self-recoverable graphene-aerogels which were successfully applied to the analysis of sulfonamides and phenolic compounds in complex matrices, respectively.

Considering the impressive arising of graphene-based sorbents throughout the last years, herein, we aimed to pinpoint some of the unusual approached to perform such modifications and production of extractive phase. However, novel sorbent phases can be expected to be developed daily, considering the great qualities of nanomaterials for analytical chemistry purposes, highlighting the graphene.

CONCLUDING REMARKS

Bearing in mind the great importance of sample preparation to clean-up samples, extract, and pre-concentrate target analytes become easier to understand the increasing interest of the analytical chemistry in developing modern strategies to optimize such a critical stage. In this context, one of the most relevant and promising fields is the development of more performative and environmentally-friend sorption-based techniques and, by consequence, the sorbents commonly used on them. As it is known, a good sorbent material must have some essential characteristics, including (i) selectivity for specific analytes and thus present chemical inertia for matrix interferents; (ii) good recovery and enrichment factors; and (iii) simple and non-expensive production. Once fulfilling these requirements, graphene-based materials have increasingly seemed to be the right candidate since their first application in sample preparation around 2011. Its large surface area, together with the π-π delocalized electron system, aid in improving so much the extraction performance of target compounds possessing aromatic rings as several chemical classes (e.g., pesticides, pharmaceuticals, and others) as herein discussed on section Graphene and Graphene Oxide.

Although the successful application in its "bare" form (G and GO), sometimes the functionalization of them seems to enhance even more the performance of such sorbents. For this reason, in this work, we have shown several different applications based on the use of hybrid materials consisting of GBMs anchored to other extractive sorbents such as alkyl and aryl groups, cyclodextrins, magnetic particles, molecularly imprinted polymers, ionic liquids, and among others. The functionalization is often achieved by forming covalent or hydrogen bondings, using sol-gel or polymerization router, or even electrochemical deposition. This interesting approach is encouraged by the possibility to ally the advantages of each class in only one. Apart from this goal, the clogging and backpressure problems often related to G and GO when packed in sorbent-based sample preparation techniques are overcome by the addition of other compounds. For example, several works underscored in sections Alkyl and Aril Groups, Cyclodextrins, and Magnetic Materials based on magnetic particles, or anchoring *in silica* reported this result.

Similarly, increasing on extraction selectivity and by consequence, performance is observed when GBMs were functionalized with both MIPS or the tunable-ILs, as can be seen in sections Molecularly-Imprinted Polymers and Ionic Liquids. This background explains the significant tendency to work with hybrid graphene-based materials instead of its bare form. GBMs are an excellent sorbent, often surpassing the commercially available phases such as C8 and C18.

After all, by assessing the recent literature and considering the vast number of applications involving graphene in the sample preparation arena, as herein discussed, an increasing tendency to expand the footprint of GBMs functionalized with several classes must continue in the years to come. This conclusion is mainly supported by the unique favorable GBMs physical-chemical properties, which—together with the advancements on the materials synthesis routes, extraction techniques, and related subjects—evidenced this field as one of the most critical developments in the sample preparation area nowadays. Also, an increasing number of papers reporting the employment of hybrid GBMs and miniaturized sample preparation techniques must be expected. This trend can be projected considering the high potential obtained by combining the well-established benefits of automation/miniaturization with the use of more selective and performative materials, possibly leading to greener sample preparation techniques by following the Green Chemistry concept.

AUTHOR CONTRIBUTIONS

EM wrote sections Introduction, Miscellaneous, and Concluding Remarks, as well as edited the whole manuscript. KM-C wrote sections Graphene and Graphene Oxide and Alkyl and Aril Groups, built **Table 1**, and revised the whole manuscript. MJ-S wrote sections Molecularly-Imprinted polymers and Ionic Liquids, as well as built **Tables 4**, **5**. LS wrote section Cyclodextrins and built **Table 2**. DV wrote section Magnetic Materials and built **Table 3**. FL conceptualized, supervised, and edited all versions of the manuscript, as well as provide all required facilities. All authors contributed to the article and approved the submitted version.

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REFERENCES

Abdelghani, J. I., Freihat, R. S., and El-sheikh, A. H. (2019). Magnetic solid phase extraction of phthalate products from bottled, injectable and tap waters using graphene oxide : effect of oxidation method of graphene. *J. Environ. Chem. Eng.* 8:103527. doi: 10.1016/j.jece.2019.103527

Abdolmohammad-zadeh, H., Zamani, A., and Shamsi, Z. (2019). Preconcentration of morphine and codeine using a magnetite/reduced graphene oxide/silver nano-composite and their determination by high-performance liquid chromatography. *J. Chromatogr. A* 1590, 2–9. doi: 10.1016/j.chroma.2018.12.064

Abdolmohammad-zadeh, H. (2020). A simple magnetic solid-phase extraction method based on magnetite/graphene oxide nanocomposite for pre-concentration and determination of melamine by high-performance liquid chromatography. *Environ. Sci. Pollut. Res.* 27, 9826–9834. doi: 10.1007/s11356-020-07681-z

Alasl, M. R. K., Sohrabi, M. R., and Davallo, M. (2019). Determination of trace amounts of aromatic amines after magnetic solid-phase extraction using silver-modified Fe3O4/graphene nanocomposite. *J. Sep. Sci.* 42, 1777–1785. doi: 10.1002/jssc.201800896

Aliyari, E., Alvand, M., and Shemirani, F. (2016). Modified surface-active ionic liquid-coated magnetic graphene oxide as a new magnetic solid phase extraction sorbent for preconcentration of trace nickel. *RSC Adv.* 6, 64193–64202. doi: 10.1039/C6RA04163A

Allen, M. J., Tung, V. C., and Kaner, R. B. (2010). Honeycomb carbon: a review of graphene. *Chem. Rev.* 110, 132–145. doi: 10.1021/cr900070d

Amiri, A., Mirzaei, M., and Derakhshanrad, S. (2019). A nanohybrid composed of polyoxotungstate and graphene oxide for dispersive micro solid-phase extraction of non-steroidal anti-inflammatory drugs prior to their quantitation by HPLC. *Microchim. Acta* 18:6534. doi: 10.1007/s00604-019-3694-0

An, J., Rahn, K. L., and Anderson, J. L. (2017). Headspace single drop microextraction versus dispersive liquid-liquid microextraction using magnetic ionic liquid extraction solvents. *Talanta* 167, 268–278. doi: 10.1016/j.talanta.2017.01.079

Asadi, M., Sereshti, H., and Rashidi Nodeh, H. (2020). Development of magnetic dispersive microsolid-phase extraction using lanthanum phosphate nanoparticles doped on magnetic graphene oxide as a highly selective adsorbent for pesticide residues analysis in water and fruit samples. *Res. Chem. Intermed.* 46, 2789–2803. doi: 10.1007/s11164-020-04121-y

Barati, A., Kazemi, E., Dadfarnia, S., and Haji Shabani, A. M. (2017). Synthesis/characterization of molecular imprinted polymer based on magnetic chitosan/graphene oxide for selective separation/preconcentration of fluoxetine from environmental and biological samples. *J. Ind. Eng. Chem.* 46, 212–221. doi: 10.1016/j.jiec.2016.10.033

Berthod, A., Ruiz-Ángel, M. J., and Carda-Broch, S. (2018). Recent advances on ionic liquid uses in separation techniques. *J. Chromatogr. A* 1559, 2–16. doi: 10.1016/j.chroma.2017.09.044

Bottari, G., Ángeles Herranz, M., Wibmer, L., Volland, M., Rodríguez-Pérez, L., Guldi, D. M., et al. (2017). Chemical functionalization and characterization of graphene-based materials. *Chem. Soc. Rev.* 46, 4464–4500. doi: 10.1039/C7CS00229G

Cai, M. Q., Su, J., Hu, J. Q., Wang, Q., Dong, C. Y., Pan, S. D., et al. (2016). Planar graphene oxide-based magnetic ionic liquid nanomaterial for extraction of chlorophenols from environmental water samples coupled with liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* 1459, 38–46. doi: 10.1016/j.chroma.2016.06.086

Cao, S., Chen, J., Lai, G., Xi, C., Li, X., Zhang, L., et al. (2019). A high efficient adsorbent for plant growth regulators based on ionic liquid and β-cyclodextrin functionalized magnetic graphene oxide. *Talanta* 194, 14–25. doi: 10.1016/j.talanta.2018.10.013

Chandra, V., Park, J., Chun, Y., Lee, J. W., Hwang, I., and Kim, K. S. (2010). Water-dispersible magnetite-reduced graphene oxide composites for arsenic removal. *ACS Nano* 4, 3979–3986. doi: 10.1021/nn1008897

Chatzimitakos, T. G., Karali, K. K., and Stalikas, C. D. (2019). Magnetic graphene oxide as a convenient nanosorbent to streamline matrix solid-phase dispersion towards the extraction of pesticides from vegetables and their determination by GC–MS. *Microchem. J.* 151:104247. doi: 10.1016/j.microc.2019.104247

Chen, X., Hai, X., and Wang, J. (2016). Graphene/graphene oxide and their derivatives in the separation/isolation and preconcentration of protein species: a review. *Anal. Chim. Acta* 922, 1–10. doi: 10.1016/j.aca.2016.03.050

Chen, X., and Ye, N. (2017). A graphene oxide surface-molecularly imprinted polymer as a dispersive solid-phase extraction adsorbent for the determination of cefadroxil in water samples. *RSC Adv.* 7, 34077–34085. doi: 10.1039/C7RA02985C

Chen, Y., Cao, S., Zhang, L., Xi, C., Li, X., Chen, Z., et al. (2016). Preparation of size-controlled magnetite nanoparticles with a graphene and polymeric ionic liquid coating for the quick, easy, cheap, effective, rugged and safe extraction of preservatives from vegetables. *J. Chromatogr. A* 1448, 9–19. doi: 10.1016/j.chroma.2016.04.045

Cheng, L., Pan, S., Ding, C., He, J., and Wang, C. (2017). Dispersive solid-phase microextraction with graphene oxide based molecularly imprinted polymers for determining bis(2-ethylhexyl) phthalate in environmental water. *J. Chromatogr. A* 1511, 85–91. doi: 10.1016/j.chroma.2017.07.012

Chu, F., Gao, M., Wang, H., Li, J., Zheng, Z., Wang, X., et al. (2019). Development of an effervescent reaction-enhanced microextraction method for preconcentration/extraction of trace estrogens in milk using a reduced graphene oxide-assisted ionic liquid-based nanofluid. *Anal. Methods* 11, 3608–3618. doi: 10.1039/C9AY00803A

De Marchi, L., Pretti, C., Gabriel, B., Marques, P. A. A. P., Freitas, R., and Neto, V. (2018). An overview of graphene materials: properties, applications and toxicity on aquatic environments. *Sci. Total Environ.* 631–632, 1440–1456. doi: 10.1016/j.scitotenv.2018.03.132

De Silva, K. K. H., Huang, H. H., Joshi, R. K., and Yoshimura, M. (2017). Chemical reduction of graphene oxide using green reductants. *Carbon N. Y.* 119, 190–199. doi: 10.1016/j.carbon.2017.04.025

De Toffoli, A. L., Fumes, B. H., and Lanças, F. M. (2018). Packed in-tube solid phase microextraction with graphene oxide supported on aminopropyl silica: determination of target triazines in water samples. *J. Environ. Sci. Heal. B* 53, 434–440. doi: 10.1080/03601234.2018.1438831

Deng, J., Zhang, P., Jin, T., Zhou, H., and Cheng, J. (2017). Graphene oxide/β-cyclodextrin composite as fiber coating for high efficiency headspace solid phase microextraction of organophosphate ester flame retardants in environmental water. *RSC Adv.* 7, 54475–54484. doi: 10.1039/C7RA07903F

Ding, X., Wang, Y., Wang, Y., Pan, Q., Chen, J., Huang, Y., et al. (2015). Preparation of magnetic chitosan and graphene oxide-functional guanidinium ionic liquid composite for the solid-phase extraction of protein. *Anal. Chim. Acta* 861, 36–46. doi: 10.1016/j.aca.2015.01.004

Dramou, P., Itatahine, A., Fizir, M., and Ait, Y. (2019). Preparation of novel molecularly imprinted magnetic graphene oxide and their application for quercetin determination. *J. Chromatogr. B* 1124, 273–283. doi: 10.1016/j.jchromb.2019.06.007

Dreyer, D. R., Park, S., Bielawski, C. W., and Ruoff, R. S. (2010). The chemistry of graphene oxide. *Chem. Soc. Rev.* 39, 228–240. doi: 10.1039/B917103G

Esfandiarnejad, R., and Sereshti, H. (2019). Designing an absolutely solvent-free binary extraction system as a green strategy for ultra-trace analysis of chlorophenols. *Microchem. J.* 146, 701–707. doi: 10.1016/j.microc.2019.01.072

Evans, D. F., Chen, S.-H., Schriver, G. W., and Arnett, E. M. (1981). Thermodynamics of solution of nonpolar gases in a fused salt. Hydrophobic *bonding behavior in a nonaqueous system. J. Am. Chem. Soc.* 103, 481–482. doi: 10.1021/ja00392a049

Evrim, S., Tekkeli, K., and Durmus, Z. (2019). Magnetic solid phase extraction applications combined with analytical methods for determination of drugs in different matrices review. *J. Chil. Chem. Soc.* 2, 4448–4458. doi: 10.4067/S0717-97072019000204448

Fan, J.-P., Liao, D.-D., Xie, Y.-L., Zheng, B., Yu, J.-X., Cao, Y.-H., et al. (2017). A molecular imprinted polymer on the surface of superparamagnetic Fe 3 O 4 -graphene oxide (MIP@Fe 3 O 4 @GO) for simultaneous recognition and enrichment of evodiamine and rutaecarpine in Evodiae fructus. *J. Appl. Polym. Sci.* 134, 1–9. doi: 10.1002/app.44465

Fang, X., Chen, G., Qiu, J., Xu, J., Wang, J., Zhu, F., et al. (2018). Determination of four salicylic acids in aloe by *in vivo* solid phase microextraction coupling with liquid chromatography-photodiode array detection. *Talanta* 184, 520–526. doi: 10.1016/j.talanta.2018.03.043

Farajvand, M., Farajzadeh, K., and Faghani, G. (2018). Synthesis of graphene oxide/polyaniline nanocomposite for measuring cadmium(II) by solid phase

extraction combined with dispersive liquid-liquid microextraction. *Mater. Res. Express* 5:075017. doi: 10.1088/2053-1591/aace3a

Farzin, L., Shamsipur, M., and Sheibani, S. (2016). Solid phase extraction of hemin from serum of breast cancer patients using an ionic liquid coated Fe3O4/graphene oxide nanocomposite, and its quantitation by using FAAS. *Microchim. Acta* 183, 2623–2631. doi: 10.1007/s00604-016-1906-4

Feng, J., Loussala, H. M., Han, S., Ji, X., Li, C., and Sun, M. (2020). Recent advances of ionic liquids in sample preparation. *TrAC Trends Anal. Chem*. 125:115833. doi: 10.1016/j.trac.2020.115833

Feng, Y., Hu, X., Zhao, F., and Zeng, B. (2019). Fe 3 O 4 /reduced graphene oxide-carbon nanotubes composite for the magnetic solid-phase extraction and HPLC determination of sulfonamides in milk. *J. Sep. Sci*. 42, 1058–1066. doi: 10.1002/jssc.201801177

Feriduni, B. (2019). Magnetic graphene oxide—based solid-phase extraction combined with dispersive liquid—liquid microextraction for the simultaneous preconcentration of four typical pesticide residues in fruit juice and *pulp. Food Analytical Methods* 12, 2742–2752. doi: 10.1007/s12161-019-01635-z

Ferrone, V., Todaro, S., Carlucci, M., Fontana, A., Ventrella, A., Carlucci, G., et al. (2020). Optimization by response surface methodology of a dispersive magnetic solid phase extraction exploiting magnetic graphene nanocomposite coupled with UHPLC-PDA for simultaneous determination of new oral anticoagulants (NAOs) in human plasma. *J. Pharm. Biomed. Anal*. 179:112992. doi: 10.1016/j.jpba.2019.112992

Filik, H., and Avan, A. A. (2019). Magnetic nanostructures for preconcentration, speciation and determination of chromium ions : a review. *Talanta* 203, 168–177. doi: 10.1016/j.talanta.2019.05.061

Fontanals, N., Marcé, R. M., and Borrull, F. (2020). "Porous polymer sorbents," in *Solid-Phase Extraction*, ed C. F. Poole (Amsterdam: Elsevier), 55–82. doi: 10.1016/B978-0-12-816906-3.00003-0

Fumes, B. H., and Lanças, F. M. (2017). Use of graphene supported on aminopropyl silica for microextraction of parabens from water samples. *J. Chromatogr. A* 1487, 64–71. doi: 10.1016/j.chroma.2017.01.063

Fumes, B. H., Silva, M. R., Andrade, F. N., Nazario, C. E. D., and Lanças, F. M. (2015). Recent advances and future trends in new materials for sample preparation. *TrAC Trends Anal. Chem*. 71, 9–25. doi: 10.1016/j.trac.2015.04.011

García-Alvarez-Coque, M. C., Ruiz-Angel, M. J., Berthod, A., and Carda-Broch, S. (2015). On the use of ionic liquids as mobile phase additives in high-performance liquid chromatography. *a review. Anal. Chim. Acta* 883, 1–21. doi: 10.1016/j.aca.2015.03.042

Geim, A. K. (2009). Graphene: status and prospects. *Science* 324, 1530–1534. doi: 10.1126/science.1158877

Geim, A. K., and Novoselov, K. S. (2007). The rise of graphene. *Nat. Mater*. 6, 183–191. doi: 10.1038/nmat1849

Gentili, A. (2020). Cyclodextrin-based sorbents for solid phase extraction. *J. Chromatogr. A* 1609:460654. doi: 10.1016/j.chroma.2019.460654

González-Sálamo, J., Socas-Rodríguez, B., Hernández-Borges, J., and Rodríguez-Delgado, M. Á. (2016). Nanomaterials as sorbents for food sample analysis. *TrAC* 85, 203–220. doi: 10.1016/j.trac.2016.09.009

Grajek, H., Jonik, J., Witkiewicz, Z., Wawer, T., and Purchała, M. (2019). Applications of graphene and its derivatives in chemical analysis. *Crit. Rev. Anal. Chem*. 7, 1–27. doi: 10.1080/10408347.2019.1653165

Hamidi, S., Azami, A., and Mehdizadeh Aghdam, E. (2019). A novel mixed hemimicelles dispersive micro-solid phase extraction using ionic liquid functionalized magnetic graphene oxide/polypyrrole for extraction and pre-concentration of methotrexate from urine samples followed by the spectrophotometric method. *Clin. Chim. Acta* 488, 179–188. doi: 10.1016/j.cca.2018.11.006

Han, S., Li, X., Wang, Y., and Chen, S. (2015). Multifunctional imprinted polymers based on CdTe/CdS and magnetic graphene oxide for selective recognition and separation of p-t-octylphenol. *Chem. Eng. J*. 271, 87–95. doi: 10.1016/j.cej.2015.02.080

Ho, T. D., Zhang, C., Hantao, L. W., and Anderson, J. L. (2014). Ionic liquids in analytical chemistry: fundamentals, advances, and perspectives. *Anal. Chem*. 86, 262–285. doi: 10.1021/ac4035554

Hou, X., Liu, S., Zhou, P., Li, J., Liu, X., Wang, L., et al. (2016). Polymeric ionic liquid modified graphene oxide-grafted silica for solid-phase extraction to analyze the excretion-dynamics of flavonoids in urine by box-behnken statistical design. *J. Chromatogr. A* 1456, 10–18. doi: 10.1016/j.chroma.2016.05.096

Hou, X., Lu, X., Tang, S., Wang, L., and Guo, Y. (2018a). Graphene oxide reinforced ionic liquid-functionalized adsorbent for solid-phase extraction of phenolic acids. *J. Chromatogr. B* 1072, 123–129. doi: 10.1016/j.jchromb.2017.11.013

Hou, X., Tang, S., Guo, X., Wang, L., Liu, X., Lu, X., et al. (2018b). Preparation and application of guanidyl-functionalized graphene oxide-grafted silica for efficient extraction of acidic herbicides by Box-Behnken design. *J. Chromatogr. A* 1571, 65–75. doi: 10.1016/j.chroma.2018.08.030

Hou, X., Tang, S., and Wang, J. (2019). Recent advances and applications of graphene-based extraction materials in food safety. *TrAC* 119:115603. doi: 10.1016/j.trac.2019.07.014

Hu, H., Liu, B., Yang, J., Lin, Z., and Gan, W. (2016). Sensitive determination of trace urinary 3-hydroxybenzo[a]pyrene using ionic liquids-based dispersive liquid-liquid microextraction followed by chemical derivatization and high performance liquid chromatography-high resolution tandem mass spectrometry. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci*. 1027, 200–206. doi: 10.1016/j.jchromb.2016.05.041

Hummers, W. S., and Offeman, R. E. (1958). Preparation of graphitic oxide. *J. Am. Chem. Soc*. 80, 1339–1339. doi: 10.1021/ja01539a017

Iakunkov, A., Sun, J., Rebrikova, A., Korobov, M., Klechikov, A., Vorobiev, A., et al. (2019). Swelling of graphene oxide membranes in alcohols: effects of molecule size and air ageing. *J. Mater. Chem. A* 7, 11331–11337. doi: 10.1039/C9TA01902B

Ibarra, I. S., Rodriguez, J. A., Galán-vidal, C. A., Cepeda, A., and Miranda, J. M. (2015). Magnetic solid phase extraction applied to food *analysis. J. Chem*. 2015, 1–13. doi: 10.1155/2015/919414

Ibrahim, H., Yilmaz, E., and Soylak, M. (2019). Magnetic solid phase extraction of trace paracetamol and caffeine in synthetic urine and wastewater samples by a using core shell hybrid material consisting of graphene oxide/multiwalled carbon nanotube/Fe3O4/SiO2. *Microchem. J*. 145, 843–851. doi: 10.1016/j.microc.2018.11.056

Jing, W., Zhou, Y., Wang, J., Ni, M., Bi, W., Da, D., et al. (2019). Dispersive magnetic solid-phase extraction coupled to direct analysis in real time mass spectrometry for high-throughput analysis of trace environmental contaminants. *Anal. Chem*. 91, 11240–11246. doi: 10.1021/acs.analchem.9b02197

Klongklaew, P., Naksena, T., Kanatharana, P., and Bunkoed, O. (2018). A hierarchically porous composite monolith polypyrrole/octadecyl silica/graphene oxide/chitosan cryogel sorbent for the extraction and pre-concentration of carbamate pesticides in fruit juices. *Anal. Bioanal. Chem*. 410, 7185–7193. doi: 10.1007/s00216-018-1323-0

Landau, L. (1937). Zur theorie der phasenumwandlungen II. *Phys. Zeitschrift der Sowjetunion* 11, 26–35.

Lang, H., Yang, R., Dou, X., Wang, D., Zhang, L., and Li, J. (2019). Simultaneous determination of 19 phenolic compounds in oilseeds using magnetic solid phase extraction and LC-MS/MS. *LWT Food Sci. Technol*. 107, 221–227. doi: 10.1016/j.lwt.2019.03.018

Laura, A., Chiara, C., Giorgia, C., Barbera, L., Maria, C., Susy, M., et al. (2019). Recent applications of magnetic solid—phase extraction for sample preparation. *Chromatographia* 82, 1251–1274. doi: 10.1007/s10337-019-03721-0

Li, F., Huang, Y., Huang, K., Lin, J., and Huang, P. (2020). *Functional magnetic graphene composites for* biosensing. *Int. J. Mol. Sci*. 21:390. doi: 10.3390/ijms21020390

Li, L., Luo, C., Li, X., Duan, H., and Wang, X. (2014). Preparation of magnetic ionic liquid/chitosan/graphene oxide composite and application for water treatment. *Int. J. Biol. Macromol*. 66, 172–178. doi: 10.1016/j.ijbiomac.2014.02.031

Li, L., Wu, M., Feng, Y., Zhao, F., and Zeng, B. (2016). Doping of three-dimensional porous carbon nanotube-graphene-ionic liquid composite into polyaniline for the headspace solid-phase microextraction and gas chromatography determination of alcohols. *Anal. Chim. Acta* 948, 48–54. doi: 10.1016/j.aca.2016.11.020

Li, N., Chen, J., and Shi, Y. (2019b). Magnetic nitrogen-doped reduced graphene oxide as a novel magnetic solid- phase extraction adsorbent for the separation of bisphenol endocrine disruptors in carbonated beverages. *Talanta* 201, 194–203. doi: 10.1016/j.talanta.2019.04.002

Li, N., Chen, J., and Shi, Y.P. P. (2019a). Magnetic polyethyleneimine functionalized reduced graphene oxide as a novel magnetic sorbent for the separation of polar non-steroidal anti-inflammatory drugs in waters. *Talanta* 191, 526–534. doi: 10.1016/j.talanta.2018.09.006

Li, N., Jiang, H., Wang, X., Wang, X., Xu, G., Zhang, B., et al. (2018). Recent advances in graphene-based magnetic composites for magnetic solid-phase extraction. *TrAC* 102, 60–74. doi: 10.1016/j.trac.2018.01.009

Li, W., Zhang, J., Zhu, W., Qin, P., Zhou, Q., Lu, M., et al. (2020). Facile preparation of reduced graphene oxide/ZnFe2O4 nanocomposite as magnetic sorbents for enrichment of estrogens. *Talanta* 208:120440. doi: 10.1016/j.talanta.2019.120440

Liang, X., Wang, S., Liu, S., Liu, X., and Jiang, S. (2012). A novel octadecylsilane functionalized graphene oxide/silica composite stationary phase for high performance liquid chromatography. *J. Sep. Sci*. 35, 2003–2009. doi: 10.1002/jssc.201200258

Lim, J. Y., Mubarak, N. M., Abdullah, E. C., Nizamuddin, S., Khalid, M., and Inamuddin (2018). Recent trends in the synthesis of graphene and graphene oxide based nanomaterials for removal of heavy metals—a review. *J. Ind. Eng. Chem*. 66, 29–44. doi: 10.1016/j.jiec.2018.05.028

Lin, L., Peng, H., and Liu, Z. (2019). Synthesis challenges for graphene industry. *Nat. Mater*. 18, 520–524. doi: 10.1038/s41563-019-0341-4

Liu, B., and Zhou, K. (2019). Recent progress on graphene-analogous 2D nanomaterials: properties, modeling and applications. *Prog. Mater. Sci*. 100, 99–169. doi: 10.1016/j.pmatsci.2018.09.004

Liu, G., Huang, X., Lu, M., Li, L., Li, T., and Xu, D. (2019). Facile synthesis of magnetic zinc metal-organic framework for extraction of nitrogen-containing heterocyclic fungicides from lettuce vegetable samples. *J. Sep. Sci*. 42, 1451–1458. doi: 10.1002/jssc.201801169

Liu, G., Li, L., Xu, D., Huang, X., Xu, X., Zheng, S., et al. (2017). Metal–organic framework preparation using magnetic graphene oxide–β-cyclodextrin for neonicotinoid pesticide adsorption and removal. *Carbohydr. Polym*. 175, 584–591. doi: 10.1016/j.carbpol.2017.06.074

Liu, J., Liang, Y., Shen, J., and Bai, Q. (2018). Polymeric ionic liquid-assembled graphene-immobilized silica composite for selective isolation of human serum albumin from human whole blood. *Anal. Bioanal. Chem*. 410, 573–584. doi: 10.1007/s00216-017-0758-z

Liu, M., Ding, X., Wang, X., Li, J., Yang, H., and Yin, Y. (2019). Extraction of DNA from complex biological sample matrices using guanidinium ionic liquid modi fied magnetic nanocomposites. *RSC Adv*. 9, 23119–23128. doi: 10.1039/C9RA01505A

Liu, W., Zha, J., Meng, X., Wu, J., Sun, C., Qiu, P., et al. (2017). Efficient removal and trace determination of chlorophenols from water using mixed hemi-/ad-micelle ionic liquid-coated magnetic graphene oxide and the adsorption mechanism. *Anal. Methods* 9, 4581–4591. doi: 10.1039/C7AY01241A

Liu, X., Gao, S., Li, X., Wang, H., Ji, X., and Zhang, Z. (2019). Determination of microcystins in environmental water samples with ionic liquid magnetic graphene. *Ecotoxicol. Environ. Saf*. 176, 20–26. doi: 10.1016/j.ecoenv.2019.03.063

Lu, Q., Guo, H., Zhang, Y., Tang, X., Lei, W., Qi, R., et al. (2020). Graphene oxide-Fe 3 O 4 nanocomposite magnetic solid phase extraction followed by UHPLC-MS/MS for highly sensitive determination of eight psychoactive drugs in urine samples. *Talanta* 206:120212. doi: 10.1016/j.talanta.2019.120212

Lu, Y., Wang, B., Wang, C., Yan, Y., Wu, D., Liang, H., et al. (2019a). A covalent organic framework—derived hydrophilic magnetic graphene composite as a unique platform for detection of phthalate esters from packaged milk samples. *Chromatographia* 82, 1089–1099. doi: 10.1007/s10337-019-03741-w

Lu, Y., Wang, B., Yan, Y., Liang, H., and Wu, D. (2019b). Silica protection—sacrifice functionalization of magnetic graphene with a metal—organic framework (ZIF-8) to provide a solid-phase extraction composite for recognization of phthalate easers from human plasma samples. *Chromatographia* 82, 625–634. doi: 10.1007/s10337-018-3673-3

Luo, J., Gao, Y., Tan, K., Wei, W., and Liu, X. (2016). Preparation of a magnetic molecularly imprinted graphene composite highly adsorbent for 4-nitrophenol in aqueous medium. *ACS Sustain. Chem. Eng*. 4, 3316–3326. doi: 10.1021/acssuschemeng.6b00367

Luo, J., Huang, J., Cong, J., Wei, W., and Liu, X. (2017). Double recognition and selective extraction of glycoprotein based on the molecular imprinted graphene oxide and boronate affinity. *ACS Appl. Mater. Interfaces* 9, 7735–7744. doi: 10.1021/acsami.6b14733

Luo, Y.-B., Cheng, J.-S. S., Ma, Q. A., Feng, Y.-Q. Q., and Li, J.-H. H. (2011a). Graphene-polymer composite: extraction of polycyclic aromatic hydrocarbons from water samples by stir rod sorptive extraction. *Anal. Methods* 3, 92–98. doi: 10.1039/C0AY00624F

Luo, Y.-B., Shi, Z., Gao, Q., and Feng, Y. (2011b). Magnetic retrieval of graphene : extraction of sulfonamide antibiotics from environmental water samples. *J. Chromatogr. A* 1218, 1353–1358. doi: 10.1016/j.chroma.2011.01.022

Ma, G., Zhang, M., Zhu, L., Chen, H., Liu, X., and Lu, C. (2018). Facile synthesis of amine-functional reduced graphene oxides as modified quick, easy, cheap, effective, rugged and safe adsorbent for multi-pesticide residues analysis of tea. *J. Chromatogr. A* 1531, 22–31. doi: 10.1016/j.chroma.2017.11.044

Maciel, E. V. S., de Toffoli, A. L., Neto, E. S., Nazario, C. E. D., and Lanças, F. M. (2019). New materials in sample preparation: recent advances and future trends. *TrAC* 119:115633. doi: 10.1016/j.trac.2019.115633

Madej, K., Jonda, A., Borcuch, A., Piekoszewski, W., Chmielarz, L., and Gil, B. (2019). A novel stir bar sorptive-dispersive microextraction in combination with magnetically modified graphene for isolation of seven pesticides from water samples. *Microchem. J*. 147, 962–971. doi: 10.1016/j.microc.2019.04.002

Maggira, M., Deliyanni, E. A., and Samanidou, V. F. (2019). Synthesis of graphene oxide based sponges and their study as sorbents for sample preparation of cow milk prior to HPLC determination of sulfonamides. *Molecules* 24:2086. doi: 10.3390/molecules24112086

Mahpishanian, S., and Sereshti, H. (2017). One-step green synthesis of β-cyclodextrin/iron oxide-reduced graphene oxide nanocomposite with high supramolecular recognition capability: application for vortex-assisted magnetic solid phase extraction of organochlorine pesticides residue from honey sam. *J. Chromatogr. A* 1485, 32–43. doi: 10.1016/j.chroma.2017.01.035

Marcinkowski, Ł., Pena-Pereira, F., Kloskowski, A., and Namieśnik, J. (2015). Opportunities and shortcomings of ionic liquids in single-drop microextraction. *TrAC* 72, 153–168. doi: 10.1016/j.trac.2015.03.024

Mejía-Carmona, K., and Lanças, F. M. (2020). Modified graphene-silica as a sorbent for in-tube solid-phase microextraction coupled to liquid chromatography-tandem mass spectrometry. Determination *of xanthines in coffee beverages. J. Chromatogr. A* 1621:461089. doi: 10.1016/j.chroma.2020.461089

Mohan, V. B., Lau, K., Hui, D., and Bhattacharyya, D. (2018). Graphene-based materials and their composites: a review on production, applications and product limitations. *Compos. B Eng*. 142, 200–220. doi: 10.1016/j.compositesb.2018.01.013

Moradi, S., Saber-tehrani, M., and Faraji, M. (2019). Simultaneous magnetic solid phase extraction of acidic and basic pesticides using triazine-based polymeric network modified magnetic nanoparticles/graphene oxide nanocomposite in water and food samples. *Microchem. J*. 146, 630–639. doi: 10.1016/j.microc.2019.01.047

Nawała, J., Dawidziuk, B., Dziedzic, D., Gordon, D., and Popiel, S. (2018). Applications of ionic liquids in analytical chemistry with a particular emphasis on their use in solid-phase microextraction. *TrAC* 105, 18–36. doi: 10.1016/j.trac.2018.04.010

Ning, F., Peng, H., Li, J., Chen, L., and Xiong, H. (2014). Molecularly imprinted polymer on magnetic graphene oxide for fast and selective extraction of 17β-estradiol. *J. Agric. Food Chem*. 62, 7436–7443. doi: 10.1021/jf501845w

Niu, P., Nie, X., Li, Y., Liang, X., Wang, L., and Guo, Y. (2020). Magnetic N-doped 3D graphene-like framework carbon for extraction of cephalexin monohydrate and ceftiofur hydrochloride. *Talanta* 215:120932. doi: 10.1016/j.talanta.2020.120932

Novoselov, K. S. (2004). Electric field effect in atomically thin carbon films. *Science* 306, 666–669. doi: 10.1126/science.1102896

Novoselov, K. S., Jiang, D., Schedin, F., Booth, T. J., Khotkevich, V. V., Morozov, S. V., et al. (2005). Two-dimensional atomic crystals. *Proc. Natl. Acad. Sci. U. S. A.* 102, 10451–10453. doi: 10.1073/pnas.0502848102

Nurerk, P., Bunkoed, W., Kanatharana, P., and Bunkoed, O. (2018). A miniaturized solid-phase extraction adsorbent of calix[4]arene-functionalized graphene oxide/polydopamine-coated cellulose acetate for the analysis of aflatoxins in corn. *J. Sep. Sci*. 41, 3892–3901. doi: 10.1002/jssc.201800440

Pan, J., Chen, W., Ma, Y., and Pan, G. (2018). Molecularly imprinted polymers as receptor mimics for selective cell recognition. *Chem. Soc. Rev*. 47, 5574–5587. doi: 10.1039/C7CS00854F

Pang, Y. Q., Chen, X. J., Li, X. Y., Zhang, H. F., Jiang, X.Y., Zhu, F. P., et al. (2019). Magnetic solid-phase extraction of tobacco-specific N-nitrosamines using magnetic graphene composite as sorbent. *J. Sep. Sci*. 42, 3119–3125. doi: 10.1002/jssc.201900438

Papageorgiou, D. G., Kinloch, I. A., and Young, R. J. (2017). Mechanical properties of graphene and graphene-based nanocomposites. *Prog. Mater. Sci*. 90, 75–127. doi: 10.1016/j.pmatsci.2017.07.004

Peierls, R. (1935). Quelques propriétés typiques des corps solides. *Ann. l'institut Henri Poincaré* 5, 177–122.

Peng, Q., Dearden, A. K., Crean, J., Han, L., Liu, S., Wen, X., et al. (2014). New materials graphyne, graphdiyne, graphone, and graphane: review of properties, synthesis, and application in nanotechnology. *Nanotechnol. Sci. Appl*. 7, 1–29. doi: 10.2147/NSA.S40324

Pham, T. S. H., Fu, L., Mahon, P., Lai, G., and Yu, A. (2016). Fabrication of β-cyclodextrin-functionalized reduced graphene oxide and its application for electrocatalytic detection of carbendazim. *Electrocatalysis* 7, 411–419. doi: 10.1007/s12678-016-0320-3

Pinho, E., Grootveld, M., and Henriques, M. (2014). Cyclodextrins as encapsulation agents for plant bioactive compounds. *Carbohydr. Polym*. 101, 121–135. doi: 10.1016/j.carbpol.2013.08.078

Qiu, J., Chen, G., Liu, S., Zhang, T., Wu, J., Wang, F., et al. (2016). Bioinspired polyelectrolyte-assembled graphene-oxide-coated C18 composite solid-phase microextraction fibers for *in vivo* monitoring of acidic pharmaceuticals in fish. *Anal. Chem*. 88, 5841–5848. doi: 10.1021/acs.analchem.6b00417

Ragavan, K. V., and Rastogi, N. K. (2017). β-Cyclodextrin capped graphene-magnetite nanocomposite for selective adsorption of bisphenol-A. *Carbohydr. Polym*. 168, 129–137. doi: 10.1016/j.carbpol.2017.03.045

Ran, X., Yang, L., Qu, Q., Li, S., Chen, Y., Zuo, L., et al. (2017). Synthesis of well-dispersive 2.0 nm Pd–Pt bimetallic nanoclusters supported on β-cyclodextrin functionalized graphene with excellent electrocatalytic activity. *RSC Adv*. 7, 1947–1955. doi: 10.1039/C6RA24893D

Razavi, N., and Es, Z. (2019). Curcumin loaded magnetic graphene oxide solid-phase extraction for the determination of parabens in toothpaste and mouthwash coupled with high performance liquid chromatography. *Microchem. J*. 148, 616–625. doi: 10.1016/j.microc.2019.04.057

Rodríguez-Sánchez, S., Galindo-Iranzo, P., Soria, A. C., Sanz, M. L., Quintanilla-López, J. E., and Lebrón-Aguilar, R. (2014). Characterization by the solvation parameter model of the retention properties of commercial ionic liquid columns for gas chromatography. *J. Chromatogr. A* 1326, 96–102. doi: 10.1016/j.chroma.2013.12.020

Rofouei, M. K., Jamshidi, S., Seidi, S., and Saleh, A. (2017). A bucky gel consisting of Fe3O4 nanoparticles, graphene oxide and ionic liquid as an efficient sorbent for extraction of heavy metal ions from water prior to their determination by ICP-OES. *Microchim. Acta* 184, 3425–3432. doi: 10.1007/s00604-017-2370-5

Rykowska, I., Ziemblinska, J., and Nowak, I. (2018). Modern approaches in dispersive liquid-liquid microextraction (DLLME) based on ionic liquids: a review. *J. Mol. Liq*. 259, 319–339. doi: 10.1016/j.molliq.2018.03.043

Šafariková, M., and Šafarik, I. (1999). Magnetic solid-phase extraction. *J. Magn. Magn. Mater*. 194, 108–112. doi: 10.1016/S0304-8853(98)00566-6

Sarp, G., and Yilmaz, E. (2019). A flower-like hybrid material composed of Fe 3 O 4, graphene oxide and CdSe nanodots for magnetic solid phase extraction of ibuprofen prior to its quantification by HPLC detection. *Microchim. Acta* 186:744. doi: 10.1007/s00604-019-3875-x

Sereshti, H., Toloutehrani, A., and Rashidi, H. (2020). Determination of cholecalciferol (vitamin D 3) in bovine milk by dispersive micro-solid phase extraction based on the magnetic three-dimensional graphene-sporopollenin sorbent. *J. Chromatogr. B* 1136:121907. doi: 10.1016/j.jchromb.2019.121907

Serrano, M., Chatzimitakos, T., Gallego, M., and Stalikas, C. D. (2016). 1-Butyl-3-aminopropyl imidazolium-functionalized graphene oxide as a nanoadsorbent for the simultaneous extraction of steroids and β-blockers via dispersive solid-phase microextraction. *J. Chromatogr. A* 1436, 9–18. doi: 10.1016/j.chroma.2016.01.052

Shah, J., and Jan, M. R. (2020). Eco-friendly alginate encapsulated magnetic graphene oxide beads for solid phase microextraction of endocrine disrupting compounds from water samples. *Ecotoxicol. Environ. Saf*. 190:110099. doi: 10.1016/j.ecoenv.2019.110099

Shamaila, S., Sajjad, A. K. L., and Iqbal, A. (2016). Modifications in development of graphene oxide synthetic routes. *Chem. Eng. J*. 294, 458–477. doi: 10.1016/j.cej.2016.02.109

Shi, X., Qiao, L., and Xu, G. (2015). Recent development of ionic liquid stationary phases for liquid chromatography. *J. Chromatogr. A* 1420, 1–15. doi: 10.1016/j.chroma.2015.09.090

Singh, R. K., Kumar, R., and Singh, D. P. (2016). Graphene oxide: strategies for synthesis, reduction and frontier applications. *RSC Adv*. 6, 64993–65011. doi: 10.1039/C6RA07626B

Sitko, R., Zawisza, B., and Malicka, E. (2013). Graphene as a new sorbent in analytical chemistry. *TrAC* 51, 33–43. doi: 10.1016/j.trac.2013.05.011

Smith, A. T., LaChance, A. M., Zeng, S., Liu, B., and Sun, L. (2019). Synthesis, properties, and applications of graphene oxide/reduced graphene oxide and their nanocomposites. *Nano Mater. Sci*. 1, 31–47. doi: 10.1016/j.nanoms.2019.02.004

Solís-Fernández, P., Bissett, M., and Ago, H. (2017). Synthesis, structure and applications of graphene-based 2D heterostructures. *Chem. Soc. Rev*. 46, 4572–4613. doi: 10.1039/C7CS00160F

Sotolongo, A. C., Martinis, E. M., and Wuilloud, R. G. (2018). An easily prepared graphene oxide-ionic liquid hybrid nanomaterial for micro-solid phase extraction and preconcentration of Hg in water samples. *Anal. Methods* 10, 338–346. doi: 10.1039/C7AY02201H

Speltini, A., Sturini, M., Maraschi, F., Consoli, L., Zeffiro, A., and Profumo, A. (2015). Graphene-derivatized silica as an efficient solid-phase extraction sorbent for pre-concentration of fluoroquinolones from water followed by liquid-chromatography fluorescence detection. *J. Chromatogr. A* 1379, 9–15. doi: 10.1016/j.chroma.2014.12.047

Sun, L. (2019). Structure and synthesis of graphene oxide. *Chinese J. Chem. Eng*. 27, 2251–2260. doi: 10.1016/j.cjche.2019.05.003

Sun, M., Bu, Y., Feng, J., and Luo, C. (2016). Graphene oxide reinforced polymeric ionic liquid monolith solid-phase microextraction sorbent for high-performance liquid chromatography analysis of phenolic compounds in aqueous environmental samples. *J. Sep. Sci*. 39, 375–382. doi: 10.1002/jssc.201500904

Sun, M., Cui, P., Ji, S., Tang, R., Wu, Q., Wang, C., et al. (2014). Octadecyl-modified graphene as an adsorbent for hollow fiber liquid phase microextraction of chlorophenols from honey. *Bull. Korean Chem. Soc*. 35, 1011–1015. doi: 10.5012/bkcs.2014.35.4.1011

Szejtli, J. (2004). Past, present, and future of cyclodextrin research. *Pure Appl. Chem*. 76, 1825–1845. doi: 10.1351/pac200476101825

Tahmasebi, E. (2018). Adsorption efficiency enhancing of electrospun polycaprolactone nanofibers towards acidic polar drugs through the incorporation of a composite of graphene oxide nanosheets and Al 30 polyoxocations: a comparative study. *Analyst* 143, 4684–4698. doi: 10.1039/C8AN01066H

Tan, P., and Hu, Y. (2017). Improved synthesis of graphene/β-cyclodextrin composite for highly efficient dye adsorption and removal. *J. Mol. Liq*. 242, 181–189. doi: 10.1016/j.molliq.2017.07.010

Tang, H., Wang, Y., Li, S., Wu, J., Li, J., Zhou, H., et al. (2019). Graphene oxide composites for magnetic solid-phase extraction of twelve quinolones in water samples followed by MALDI-TOF MS. *Anal. Bioanal. Chem*. 411, 7039–7049. doi: 10.1007/s00216-019-02081-w

Tang, S., Liu, S., Guo, Y., Liu, X., and Jiang, S. (2014). Recent advances of ionic liquids and polymeric ionic liquids in capillary electrophoresis and capillary electrochromatography. *J. Chromatogr. A* 1357, 147–157. doi: 10.1016/j.chroma.2014.04.037

Tang, S., Sun, J., Xia, D., Zang, B., Gao, Y., Chen, C., et al. (2019). In-syringe extraction using compressible and self-recoverable, amphiphilic graphene aerogel as sorbent for determination of phenols. *Talanta* 195, 165–172. doi: 10.1016/j.talanta.2018.11.038

Tang, T., Cao, S., Xi, C., Li, X., Zhang, L., Wang, G., et al. (2020). Green synthesis of magnetic carbon nanodot graphene oxide hybrid.pdfOptimization by response surface methodology of a dispersive magnetic solid.pdfChitosa. *Int. J. Biol. Macromol*. 146, 343–352. doi: 10.1016/j.ijbiomac.2019.12.259

Tashakkori, P., Erdem, P., Merdivan, M., and Bozkurt, S. S. (2019). Determination of phthalate esters in water and coffee by solid-phase microextraction using

vinyl terminated imidazolium based ionic liquid grafted on graphene oxide coatings. *ChemistrySelect* 4, 2307–2313. doi: 10.1002/slct.201803214

Toffoli, A. L., de, Lanças, F. M., de Toffoli, A. L., Maciel, E. V. S., Fumes, B. H., and Lanças, F. M. (2018). The role of graphene-based sorbents in modern sample preparation techniques. *J. Sep. Sci.* 41, 288–302. doi: 10.1002/jssc.201700870

Tu, C., Guo, Y., Dai, Y., Wei, W., and Wang, W. (2019). Determination of chloramphenicol in honey and milk by HPLC coupled with aptamer-functionalized Fe_3O_4/graphene oxide magnetic solid-phase extraction. *J. Food Sci.* 84, 3624–3633. doi: 10.1111/1750-3841.14955

Ungku Abdullah, U. A. A., Mohamad Hanapi, N. S., Ibrahim, W. N. W., Saiful, A., Ishak, N. S., and Hamid, R. D. (2019). Rapid magnetic solid-phase extraction based on graphene oxide/magnetite nanoparticles for the determination of non-steroidal anti-inflammatory drugs and bisphenol-A in tap water U.A.A. *Asian J. Chem.* 31, 1294–1300. doi: 10.14233/ajchem.2019.21899

Vadivel, D., Speltini, A., Zeffiro, A., Bellani, V., Pezzini, S., Buttafava, A., et al. (2017). Reactive carbons from Kraft lignin pyrolysis: stabilization of peroxyl radicals at carbon/silica interface. *J. Anal. Appl. Pyrolysis* 128, 346–352. doi: 10.1016/j.jaap.2017.09.016

Vasconcelos Soares Maciel, E., Henrique Fumes, B., Lúcia de Toffoli, A., and Mauro Lanças, F. (2018). Graphene particles supported on silica as sorbent for residue analysis of tetracyclines in milk employing microextraction by packed sorbent. *Electrophoresis* 39, 2047–2055. doi: 10.1002/elps.201800051

Walden, P. (2003). Academician pavel ivanovich walden (on 140th anniversary of his birthday). *Russ. J. Appl. Chem.* 76, 1186–1190. doi: 10.1023/A:1026399420965

Wang, C., Li, T., Liu, Z., Guo, Y., Li, C., Dong, C., et al. (2016). An ultra-sensitive sensor based on β -cyclodextrin modi fi ed magnetic graphene oxide for detection of tryptophan. *J. Electroanal. Chem.* 781, 363–370. doi: 10.1016/j.jelechem.2016.07.028

Wang, N., Yu, H., and Shao, S. (2016). Preparation of a graphene oxide/silica composite modified with nitro-substituted tris(indolyl)methane as a solid-phase extraction sorbent for the extraction of organic acids. *J. Sep. Sci.* 39, 1700–1708. doi: 10.1002/jssc.201501362

Wang, X., Li, G., and Row, K. H. (2017a). Graphene and graphene oxide modified by deep eutectic solvents and ionic liquids supported on silica as adsorbents for solid-phase extraction. *Bull. Korean Chem. Soc.* 38, 251–257. doi: 10.1002/bkcs.11074

Wang, X., Li, G., and Row, K. H. (2017b). Magnetic graphene oxide modified by imidazole-based ionic liquids for the magnetic-based solid-phase extraction of polysaccharides from brown alga. *J. Sep. Sci.* 40, 3301–3310. doi: 10.1002/jssc.201700393

Wang, X., Liu, B., Lu, Q., and Qu, Q. (2014). Graphene-based materials: fabrication and application for adsorption in analytical chemistry. *J. Chromatogr. A* 1362, 1–15. doi: 10.1016/j.chroma.2014.08.023

Wang, Y., Ou, Y., Xie, S., Chen, D., Wang, X., Pan, Y., et al. (2019). Magnetic graphene solid-phase extraction for the determination of 47 kinds of non-steroidal anti-inflammatory drug residues in animal food with liquid chromatography tandem mass *spectrometry. Food Anal. Methods* 12, 1346–1368. doi: 10.1007/s12161-019-01440-8

Welton, T. (2018). Ionic liquids: a brief history. *Biophys. Rev.* 10, 691–706. doi: 10.1007/s12551-018-0419-2

Wu, J., Zhao, H., Xiao, D., Chuong, P. H., He, J., and He, H. (2016). Mixed hemimicelles solid-phase extraction of cephalosporins in biological samples with ionic liquid-coated magnetic graphene oxide nanoparticles coupled with high-performance liquid chromatographic analysis. *J. Chromatogr. A* 1454, 1–8. doi: 10.1016/j.chroma.2016.05.071

Wu, Q., Feng, C., Wang, C., and Wang, Z. (2013). A facile one-pot solvothermal method to produce superparamagnetic graphene—Fe_3O_4 nanocomposite and its application in the removal of dye from aqueous solution. *Colloids Surf. B Biointerf.* 101, 210–214. doi: 10.1016/j.colsurfb.2012.05.036

Xian, Y., Liang, M., Wu, Y., Wang, B., Hou, X., Dong, H., et al. (2020). Fluorine and nitrogen functionalized magnetic graphene as a novel adsorbent for extraction of perfluoroalkyl and polyfluoroalkyl substances from water and functional beverages followed by HPLC-Orbitrap HRMS determination. *Sci. Total Environ.* 723:138103. doi: 10.1016/j.scitotenv.2020.138103

Xiao, R., Wang, S., Hassan, M., Idris, H., Shan, D., Chen, J., et al. (2019). Three-dimensional hierarchical frameworks based on molybdenum disulfide-graphene oxide-supported magnetic nanoparticles for enrichment fluoroquinolone antibiotics in water. *J. Chromatogr. A* 1593, 1–8. doi: 10.1016/j.chroma.2019.02.005

Xie, X., Ma, X., Guo, L., Fan, Y., Zeng, G., Zhang, M., et al. (2019). Novel magnetic multi-templates molecularly imprinted polymer for selective and rapid removal and detection of alkylphenols in water. *Chem. Eng. J.* 357, 56–65. doi: 10.1016/j.cej.2018.09.080

Xiong, Y., Lu, Z., Wang, D., Yang, M. O., and Guo, H. (2019). Application of polydopamine functionalized magnetic graphene in triazole fungicides residue analysis. *J. Chromatogr. A* 1614:460725. doi: 10.1016/j.chroma.2019.460725

Xu, L., Feng, J., Liang, X., Li, J., and Jiang, S. (2012). C18 functionalized graphene oxide as a novel coating for solid-phase microextraction. *J. Sep. Sci.* 35, 1531–1537. doi: 10.1002/jssc.201200124

Yang, X., Hu, Y., Li, G., and Zhang, Z. (2015). Acrylamide-functionalized graphene micro-solid-phase extraction coupled to high-performance liquid chromatography for the online analysis of trace monoamine acidic metabolites in biological samples. *J. Sep. Sci.* 38, 1380–1387. doi: 10.1002/jssc.201401432

Yang, X., Zhang, X., Ma, Y., Huang, Y., and Chen, Y. (2009). Superparamagnetic graphene oxide—Fe 3 O 4 nanoparticles hybrid for controlled targeted drug *carriers. J. Mater. Chem.* 86, 2710–2714. doi: 10.1039/b821416f

Yavuz, E., Tokalioglu, S., and Patat, S. (2018). Dispersive solid-phase extraction with tannic acid functionalized graphene adsorbent for the preconcentration of trace beryllium from water and street dust samples. *Talanta* 190, 397–402. doi: 10.1016/j.talanta.2018.08.001

Ye, N., and Shi, P. (2015). Applications of graphene-based materials in solid-phase extraction and solid-phase microextraction. *Sep. Purif. Rev.* 44, 183–198. doi: 10.1080/15422119.2014.912664

Yilmaz, E., and Sarp, G. (2020). Graphene-like MoS 2 -modified magnetic C-dot nanoflowers: an efficient magnetic solid-phase extraction adsorbent for monitoring of trace amounts of ibuprofen. *Anal. Methods* 12, 1570–1578. doi: 10.1039/C9AY02752A

Yu, H., Merib, J., and Anderson, J. L. (2016). Faster dispersive liquid-liquid microextraction methods using magnetic ionic liquids as solvents. *J. Chromatogr. A* 1463, 11–19. doi: 10.1016/j.chroma.2016.08.007

Yu, L., Ma, F., Zhang, L., and Li, P. (2019). Determination of aflatoxin B1 and B2 in vegetable oils using Fe3O4/rGO magnetic solid phase extraction coupled with high-performance liquid chromatography fluorescence with post-column photochemical derivatization. *Toxins*. 11:621. doi: 10.3390/toxins11110621

Yu, M., Wang, L., Hu, L., Li, Y., Luo, D., and Mei, S. (2019). Recent applications of magnetic composites as extraction adsorbents for determination of environmental pollutants. *Trends Anal. Chem.* 119:115611. doi: 10.1016/j.trac.2019.07.022

Yuvali, D., Narin, I., Soylak, M., and Yilmaz, E. (2020). Green synthesis of magnetic carbon nanodot/graphene oxide hybrid material (Fe3O4 @ C-nanodot @ GO) for magnetic solid phase extraction of ibuprofen in human blood samples prior to HPLC-DAD determination. *J. Pharm. Biomed. Anal.* 179:113001. doi: 10.1016/j.jpba.2019.113001

Zhang, H., and Lee, H. K. (2011). Plunger-in-needle solid-phase microextraction with graphene-based sol–gel coating as sorbent for determination of polybrominated diphenyl ethers. *J. Chromatogr. A* 1218, 4509–4516. doi: 10.1016/j.chroma.2011.05.016

Zhang, H., Wu, X., Yuan, Y., Han, D., Qiao, F., and Yan, H. (2018). An ionic liquid functionalized graphene adsorbent with multiple adsorption mechanisms for pipette-tip solid-phase extraction of auxins in soybean sprouts. *Food Chem.* 265, 290–297. doi: 10.1016/j.foodchem.2018.05.090

Zhang, Q., Zhou, D., Li, F., Wang, Y., and Yang, F. (2019). *Extraction of* nucleobases, nucleosides and nucleotides by employing a magnetized graphene oxide functionalized with hydrophilic phytic acid and titanium (IV) ions. *Mikrochim. Acta* 186:187. doi: 10.1007/s00604-019-3308-x

Zhang, R., Su, P., and Yang, Y. (2014). Microwave-assisted preparation of magnetic nanoparticles modified with graphene oxide for the extraction and analysis of phenolic. *J. Sep. Sci.* 37, 3339–3346. doi: 10.1002/jssc.201400767

Zhang, S., Yao, W., Zhou, C., Wang, J., and Zhao, H. (2019). Fabrication of magnetic zeolitic imidazolate framework-7 supported graphene oxide for the extraction of fungicides from environmental water and soil samples. *Int. J. Environ. Anal. Chem.* 1–10. doi: 10.1080/03067319.2019.1670821

Zhang, Y., Zhao, Y.-G. G., Chen, W.-S. S., Cheng, H.-L. L., Zeng, X.-Q. Q., and Zhu, Y. (2018). Three-dimensional ionic liquid-ferrite functionalized graphene oxide nanocomposite for pipette-tip solid phase extraction of 16 polycyclic aromatic hydrocarbons in human blood sample. *J. Chromatogr. A* 1552, 1–9. doi: 10.1016/j.chroma.2018.03.039

Zhao, P., Wang, Z., Gao, X., Guo, X., and Zhao, L. (2019). Simultaneous enantioselective determination of 22 chiral pesticides in fruits and vegetables using chiral liquid chromatography coupled with tandem mass spectrometry. *Food Chem*. 277, 298–306. doi: 10.1016/j.foodchem.2018.10.128

Zheng, S., Tu, Q., Li, S., and Mi, B. (2017). Swelling of graphene oxide membranes in aqueous solution : characterization of interlayer spacing and insight into water. *ACS Nano* 6, 6440–6450. doi: 10.1021/acsnano.7b02999

Zhou, J., Wang, R., and Chen, Z. (2019). Stir bar sorptive extraction with a graphene oxide framework-functionalized stainless-steel wire for the determination of Sudan dyes in water samples. *Anal. Methods* 11, 2050–2056. doi: 10.1039/C9AY00321E

Zhou, T., Ding, L., Che, G., Jiang, W., and Sang, L. (2019). Recent advances and trends of molecularly imprinted polymers for specific recognition in aqueous matrix: preparation and application in sample pretreatment. *TrAC* 114, 11–28. doi: 10.1016/j.trac.2019.02.028

Zhou, X., Zhang, Y., Huang, Z., Lu, D., Zhu, A., and Shi, G. (2016). Ionic liquids modified graphene oxide composites: a high efficient adsorbent for phthalates from aqueous solution. *Sci. Rep*. 6:38417. doi: 10.1038/srep38417

Zhou, Y., Zhao, M., and Meng, Z. (2019). *A magnetic graphene-like MoS 2 nanocomposite for simultaneous preconcentration of multi-residue herbicides prior to UHPLC with ion trap mass spectrometric* detection. *Mikrochim. Acta* 186:486. doi: 10.1007/s00604-019-3536-0

Zhu, Q.-Y., Zhang, Q.-Y., Cao, J., Cao, W., Xu, J.-J., and Peng, L.-Q. (2016). Cyclodextrin-assisted liquid-solid extraction for determination of the composition of jujube fruit using ultrahigh performance liquid chromatography with electrochemical detection and quadrupole time-of-flight tandem mass spectrometry. *Food Chem*. 213, 485–493. doi: 10.1016/j.foodchem.2016.06.115

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CAPÍTULO 6

CONCLUSÃO GERAL E PERSPECTIVAS FUTURAS

6.1 Conclusão geral

O desenvolvimento de novos materiais sorventes proporcionou eficiência de extração e seletividade para os analitos de interesse, com resultados adequados quando aplicados para a determinação de isoflavonas em sucos à base de soja utilizando a técnica MEPS e determinação de isoflavonas em urina humana utilizando a técnica *automated needle-sleeve* SPME.

O material sintetizado Si@GO@β-CD se mostrou seletivo para extração dos analitos estudados nesta tese. O desenvolvimento de novos nanomateriais, em especial materiais derivados de grafeno, para uso como sorventes, é atualmente uma tendência, juntamente com técnicas de preparação de amostras miniaturizadas. A fase de extração composta por óxido de grafeno e β-ciclodextrina, mantém as características de alta absorção de materiais à base de grafeno, melhorando a seletividade de extração para os analitos alvo, resultando em um bom *clean-up* da amostra e ausência de efeitos da matriz, o que é especialmente relevante ao trabalhar-se com fontes de ionização ESI-MS.

A utilização da microtécnica de extração *automated needle-sleeve* SPME como preparo de amostra totalmente automatizado acoplada *on-line* com HPLC-UV apresentou resultados satisfatórios para determinação de isoflavonas em urina, mostrando ser uma técnica alternativa aos métodos *on-line* de SPME, tal como a *in-tube* SPME. Além disso, a mesma apresentou várias vantagens sobre métodos *off-line* como praticidade, rapidez, quantidades de solvente reduzidas e reutilização da fase extratora. Quando comparado com métodos *on-line*, a abordagem proposta apresentou como vantagem a possibilidade de sincronização total e ininterrupta do processo.

6.2. Perspectivas futuras

Na etapa original do projeto de doutorado, havia o planejamento de desenvolver uma fase extratora e empregá-la em uma técnica *on-line* de preparo de amostra, a *in-tube* SPME.

A *in-tube* SPME é uma técnica de preparo de amostra derivada da SPME. Em seu formato original era empregado um pedaço de coluna analítica capilar de cromatografia gasosa, ou seja, um capilar de sílica com uma fase estacionária comercial. A abordagem *on-line* favorece a automatização do processo, reduz tempo de análise, melhora a exatidão, precisão e sensibilidade. Entretanto as fases comerciais disponíveis apresentam baixa capacidade e eficiência de extração para espécies polares e iônicas, são frágeis, instáveis a determinados solventes e apresentam pouca sensibilidade (1,2). Apesar desses pontos negativos apresentados, a *in-tube* SPME supera a maioria dos problemas de fragilidade e baixa capacidade de sorção da SPME convencional, e ainda elimina o uso das interfaces típicas SPME-HPLC (3).

Muitos trabalhos foram publicados pelo grupo de cromatografia do IQSC-USP (CROMA) nesta área de pesquisa. Por exemplo, em 2006 o grupo publicou os resultados de uma abordagem em *microcolumn-switching* para análise de fluoxetina em plasma humano (4). Em 2008 essa abordagem *on-line* foi aprimorada e reportada na forma *in-tube* SPME para análise de antidepressivos em plasma humano demonstrando alta sensibilidade, precisão e reprodutibilidade (5). Em 2017 foi reportado o uso de uma coluna capilar empacotada com partículas de sílica funcionalizada com C-18 e 10 µm de diâmetro para determinação de ocratoxinas em vinho (6).

O uso de grafeno suportado em aminopropil sílica em *in-tube* SPME demonstrou bom fator de enriquecimento, atribuídos aos elétrons π delocalizados e à grande afinidade por compostos aromáticos, em análises de triazinas em água (7). Há ainda a possibilidade da funcionalização do grafeno e derivados com outros materiais. Mejía-Carmona e Lanças reportaram o uso de aminopropil-sílica recoberta com grafeno e funcionalizada com C-18, apresentando aumento na capacidade de extração (8).

Nesta linha de desenvolvimento seria interessante o uso do material sintetizado e reportado neste trabalho (Si@GO@β-CD) como fase extratora em *in-tube* SPME e, assim, a avaliação de desempenho de um material seletivo empregado nesta técnica de preparo de amostra, tal como o desenvolvimento de novos materiais com outras ciclodextrinas como a α-ciclodextrina e a γ-ciclodextrina.

No entanto, a abordagem desenvolvida e apresentada no capítulo 4, foi proposta como uma modificação e uma alternativa à abordagem até então estudada em

nosso grupo de pesquisa. A *automated needle-sleeve* SPME apresentou praticidade, reutilização superior da fase de extração, com confiabilidade e sensibilidade competitivas. Nesta abordagem, diferentemente da *in-tube* SPME, enquanto é feito a análise de uma amostra é possível realizar o preparo da amostra seguinte, o que resulta em ganho de tempo. Outra vantagem a ser observada é a ausência de entupimento do sistema em todo o estudo realizado, o que é possível ocorrer na abordagem *in-tube* SPME com colunas extratoras empacotadas com materiais à base de grafeno.

O acoplamento com detectores de alta sensibilidade como espectrômetros de massa, também é uma possibilidade para a diminuição dos limites de detecção e quantificação do método apresentado.

Um ponto que pode ser desenvolvido em parceria com outros grupos de pesquisas que atuam em áreas correlacionadas à robótica, informática e afins, a partir do presente estudo, seria o desenvolvimento de um protótipo de equipamento, para um futuro amostrador automático de nível comercial. O desenvolvimento de agulhas com fase extratora quimicamente ligadas é outro ponto que pode ser explorado e que pode produzir bons resultados. A produção padronizada desses dispositivos, fazendo com que a técnica possa ser aplicada utilizando o amostrador automático do próprio cromatógrafo, seria um avanço importante. Isto implicaria em algumas modificações no hardware - para promover agitação - e no software para contagem do tempo nas etapas de extração, clean-up e dessorção.

Em suma, esta área de investigação é ainda recente, porém altamente promissora, trazendo muitos novos e motivadores desafios a serem enfrentados pelos pesquisadores, nos anos vindouros.

Referências bibliográficas

1 ASIABI, H.; YAMINI, Y.; SEIDI, S.; SHAMSAYEI, M.; SAFARI, M.; REZAEI, F. On-line electrochemically controlled in-tube solid phase microextraction of inorganic selenium followed by hydride generation atomic absorption spectrometry. **Analytica Chimica Acta**, v. 922, p. 37–47, 2016a.

2 MEJÍA-CARMONA, K.; JORDAN-SINISTERRA, M.; LANÇAS, F. Current Trends in Fully Automated On-Line Analytical Techniques for Beverage Analysis. **Beverages**, v. 5, n. 1, p. 13, 2019b.

3 FUMES, B. H.; ANDRADE, M. A.; FRANCO, M. S.; LANÇAS, F. M. On-line approaches for the determination of residues and contaminants in complex samples. **Journal of Separation Science**, v. 40, n. 1, p. 183–202, 2017c.

4 SANTOS NETO, A. J.; RODRIGUES, J. C.; FERNANDES, C.; TITATO, G. M.; ALVES, C.; LANÇAS, F. M. Automated microcolumn-switching system for drug analysis by direct injection of human plasma. **Journal of Chromatography A**, v. 1105, n. 1- 2 SPEC. ISS., p. 71–76, 2006d.

5 SILVA, B. J. G.; LANÇAS, F. M.; QUEIROZ, M. E. C. In-tube solid-phase microextraction coupled to liquid chromatography (in-tube SPME/LC) analysis of nontricyclic antidepressants in human plasma. **Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences**, v. 862, n. 1–2, p. 181–188, 2008e.

6 ANDRADE, M. A.; LANÇAS, F. M. Determination of Ochratoxin A in wine by packed in-tube solid phase microextraction followed by high performance liquid chromatography coupled to tandem mass spectrometry. **Journal of Chromatography A**, v. 1493, p. 41–48, 2017f.

7 DE TOFFOLI, A. L.; FUMES, B. H.; LANÇAS, F. M. Packed in-tube solid phase microextraction with graphene oxide supported on aminopropyl silica: Determination of target triazines in water samples. **Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes**, v. 53, n. 7, p. 434–440, 2018g.

8 MEJÍA-CARMONA, K.; LANÇAS, F. M. Modified graphene-silica as a sorbent for in-tube solid-phase microextraction coupled to liquid chromatography-tandem mass spectrometry. Determination of xanthines in coffee beverages. **Journal of Chromatography A**, v. 1621, 2020h.