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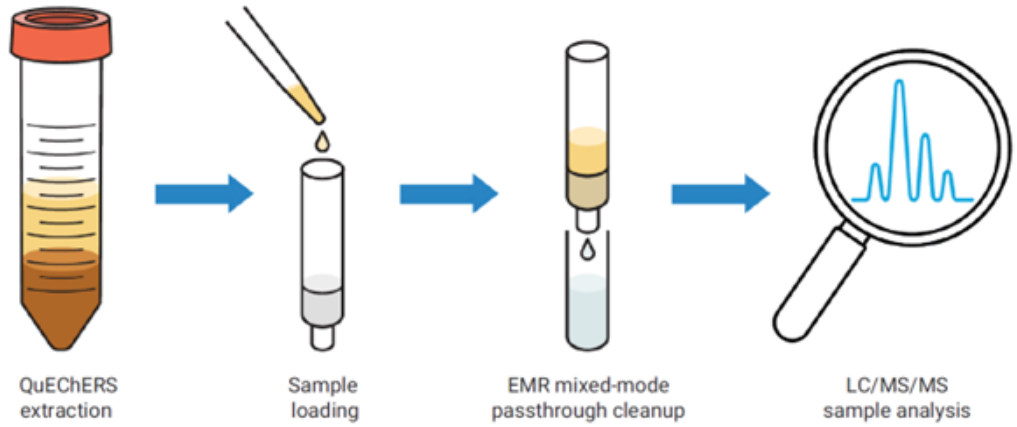
Enhanced Matrix Removal (EMR) Mixed-Mode Passthrough Cleanup Methodology

A reference guide using Captiva EMR cartridges for food safety analysis workflows



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Preface

1.1 Multiclass multiresidue food analysis challenges

The Enhanced Matrix Removal (EMR) mixed-mode passthrough cleanup approach using Captiva EMR cartridges was first introduced in 2017, providing a streamlined solution for effective matrix removal with minimal impact on analyte recoveries. Since its introduction, this matrix-focused sample preparation strategy has been widely applied in multiclass, multiresidue food safety workflows, demonstrating high efficiency and selectivity for matrix interference removal while maintaining robust analyte recovery.

This comprehensive reference guide describes the EMR passthrough cleanup methodology and its underlying mechanisms, the Captiva EMR product portfolio and usage recommendations, method development considerations, performance comparisons, and practical tips for implementation. Application-specific discussions are presented across five primary food safety workflows, including pesticides, veterinary drugs, PFAS, mycotoxins, polycyclic aromatic hydrocarbons (PAHs), and other contaminant classes.

A separate Captiva EMR application compendium summarizes published application notes and technical briefs utilizing Captiva EMR cartridges across major food safety analysis workflows.[1] For each application note, a concise application highlight is provided to enable rapid understanding of the analytical approach and key results. Each highlight also includes a direct link to the corresponding application note for convenient access to the full document. This compendium serves as a practical and user-friendly resource for identifying relevant application notes based on target analytes and food matrices.

In multiclass multiresidue food analysis, laboratories encounter significant analytical challenges. The need to monitor large panels of chemically diverse targets across highly variable food matrices introduces complexity at every stage of the workflow. Sample preparation remains a critical bottleneck, often involving labor-intensive, time-consuming procedures that increase variability and risk of error.

From an analytical perspective, analysts seek streamlined workflows that minimize manual steps and reduce method development time without compromising data integrity. Key requirements include effective removal of matrix interferences, high recoveries across a broad spectrum of analytes, and reproducibility under rugged conditions. Achieving these goals ensures reliable quantitation and compliance with regulatory standards while maintaining operational efficiency.

Analyte recovery and matrix cleanup are critical objectives in sample preparation. Ideally, analysts strive for maximum analyte recovery and complete matrix elimination; however, achieving both simultaneously is challenging due to the diverse physicochemical properties of hundreds or even thousands of analytes. As a result, compromises are often necessary, typically reducing matrix removal to maintain acceptable recoveries. Excessive compromise, however, can introduce significant matrix effects, which stress instruments, increase downtime, and drive up maintenance costs. These issues may also degrade data quality or even lead to analytical failure. Therefore, it is essential to apply a balanced approach that maximizes analyte recovery while minimizing matrix interference to ensure robust, reliable results and instrument performance.

1.2 EMR passthrough cleanup methodology

Enhanced matrix removal (EMR) methodology was developed with a deep understanding of analysts' pain points in modern food safety analysis. Its purpose is to tackle these challenges by combining simplified sample preparation with optimized cleanup strategies. Unlike traditional approaches that focus on extracting target analytes, the EMR methodology emphasizes removing unwanted matrix interferences while allowing the target analytes to flow through. This shift in strategy ensures cleaner extracts and more reliable results without compromising analyte recovery.

EMR methodology leverages matrix-based chemical filtration to effectively remove co-extracted matrix interferences without compromising analyte recovery. It utilizes blended sorbents engineered through proprietary Agilent sorbents and optimized formulation. As a result, EMR enables mixed-mode interaction mechanisms, including combinatory size exclusion and hydrophobic interactions for highly efficient and selective lipids removal; ionic and hydrophilic interactions for organic acids, fatty acids, and other carbohydrates removal; planar interaction for pigments removal; and typical hydrophobic interaction for other hydrophobic interferences removal.

This mixed-mode cleanup mechanism enables the comprehensive and targeted removal of major matrix components such as lipids, fat, organic acids, pigments, and a wide range of hydrophilic and hydrophobic interferences. This design ensures broad applicability across diverse and complex food matrices. The cartridge-based format facilitates seamless integration with both manual and automated workflows, reducing sample handling and improving reproducibility. Furthermore, the method is fully compatible with common extraction protocols, including QuEChERS, direct solvent extraction, and protein precipitation, making implementation straightforward within existing laboratory workflows. Figure 1-1 illustrates the general workflow for applying the EMR mixed-mode passthrough methodology for matrix removal following sample extraction.

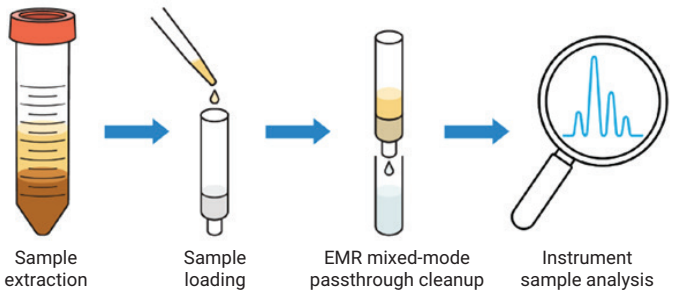


Figure 1-1. General workflow for applying EMR mixed-mode passthrough cleanup following sample extraction.

The EMR passthrough cleanup methodology is designed to achieve the optimal balance between matrix removal and analyte recovery. This "sweet spot" is achieved using highly selective sorbents and optimized formulations. These formulations were developed through extensive evaluations across hundreds of analytes and multiple food matrices. It also leverages the matrix masking effect, where low-abundance analytes are protected because sorbent active sites are occupied by high-abundance co-extractives, solvents, and additives. This mechanism plays a critical role in preventing analyte loss and is frequently leveraged during method optimization to maintain the desired balance. Several critical factors must be considered when applying EMR methodology for food analysis to ensure acceptable performance and reproducibility.

- Front-end sample extraction is required before loading samples onto EMR cartridges.
- Acetonitrile (ACN) is the preferred extraction solvent. Other acceptable solvents include isopropanol (IPA), ethanol (EtOH), partial ethyl acetate (EtOAc), or mixtures of these solvents. Methanol (MeOH) is generally not recommended as the sole solvent for EMR cartridges that include EMR–Lipid sorbent because it may compromise lipid retention during sample loading.
- For analysis with diverse properties of analytes, always load extract in a high percentage of organic solvent, preferably > 80% ACN.
- Do not use EMR methodology for neat standards.
- Avoid direct loading of aqueous or high-water-content liquid samples unless analytes are highly hydrophilic or ionic.
- Matrix masking effect can be optimized by adjusting sample size during extraction and crude extract loading volume on EMR cartridges.
- Water and acids can influence matrix masking effects. However, water may reduce solubility of hydrophobic analytes in the crude extract mixture, potentially causing recovery loss.

1.3 Captiva EMR portfolio overview

Captiva EMR passthrough cleanup methodology was first introduced in 2017 with the Captiva EMR–Lipid products. These products employ proprietary Agilent EMR–Lipid sorbent blended with C18, providing highly selective and efficient removal of lipids and fats from sample matrices. Since many foods contain significant fatty components, Captiva EMR–Lipid products have demonstrated broad applicability in food analysis, especially for veterinary drugs analysis in all animal-origin food matrices.

In 2022, a series of Captiva EMR with Carbon S products were introduced, featuring blended sorbents with optimized formulations for multiclass, multiresidue pesticides analysis in fresh and dry plant-based food and feed matrices. Five Captiva EMR cartridges were developed for various complex plant-based sample types:

- Captiva EMR–GPD for general pigmented dry matrices
- Captiva EMR–LPD for low pigmented dry matrices
- Captiva EMR–GPF for general pigmented fresh matrices
- Captiva EMR–HCF 1 and 2 for high-chlorophyll fresh matrices

In 2024, the Captiva EMR portfolio expanded further with the launch of Captiva EMR PFAS Food I and II cartridges, Captiva EMR Mycotoxins, and Captiva EMR–Lipid HF cartridges. Captiva EMR PFAS Food I and II cartridges were designed for PFAS analysis in complex sample matrices, providing enhanced mixed-mode matrix removal and excellent PFAS recovery. Captiva EMR Mycotoxins cartridges employed special formulation to ensure ultrasensitive mycotoxins recovery. Captiva EMR–Lipid HF incorporated an engineering improvement to enhance cartridge usability and deliver more consistent gravity-based sample elution.

Figure 1-2 illustrates the evolution of the Captiva EMR portfolio, which continues to grow with new EMR products to meet emerging analytical challenges.

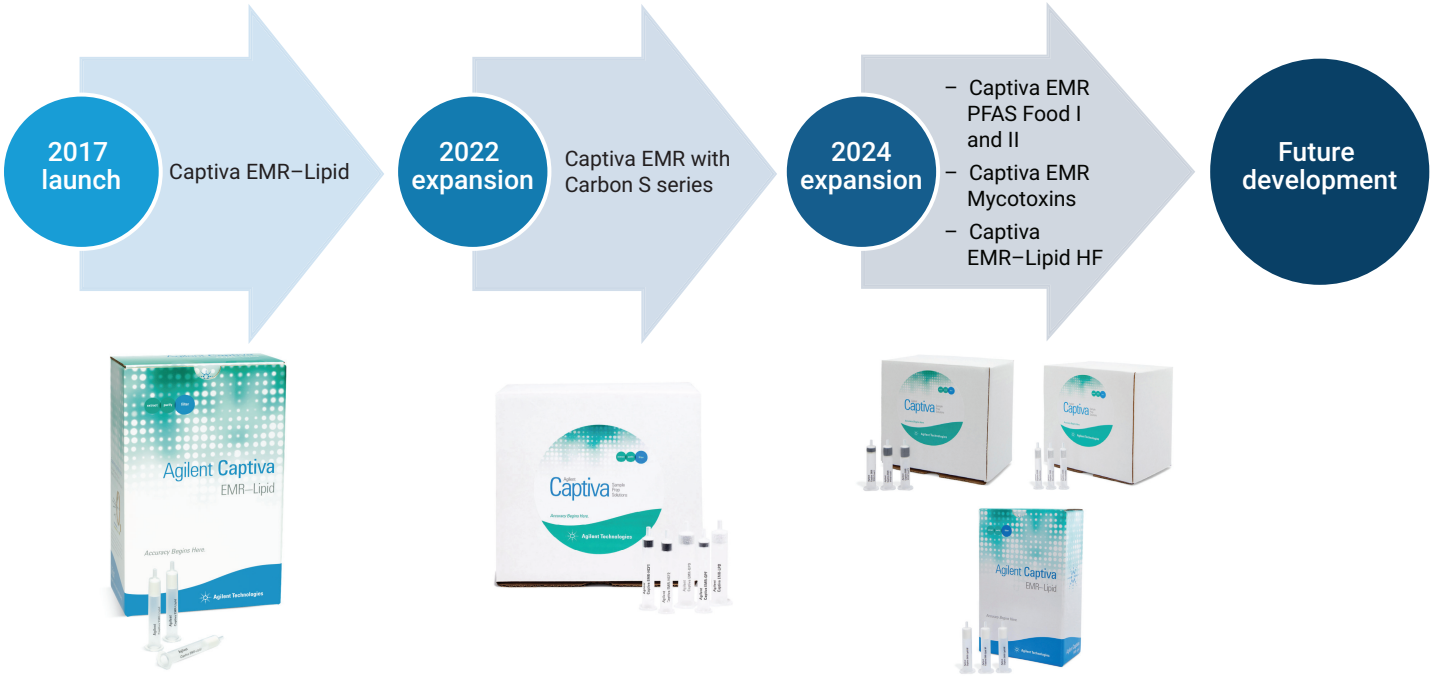


Figure 1-2. Captiva EMR portfolio development.

1.4 Sorbents in Captiva EMR cartridges

EMR–Lipid sorbent

EMR–Lipid sorbent is a patented Agilent material that provides highly efficient and selective interaction with molecules containing long aliphatic chains—typically lipids—through a combination of size exclusion and hydrophobic mechanisms. This sorbent is incorporated into many Captiva EMR cartridges designed for animal-origin matrices, dry or oily plant-origin matrices, and other complex food and feed matrices. To achieve optimal lipid removal efficiency with EMR–Lipid sorbent, the sample crude extract should be mixed with 10 to 20% water prior to loading.

Carbon S sorbent

Carbon S sorbent is also an Agilent proprietary material designed as an advanced hybrid carbon with optimized carbon content and pore structure. Its pigment removal capability is based on planar and π-π interactions, effectively retaining common pigment components such as chlorophyll, lutein, anthocyanidins, carotenoids, and xanthophylls. Compared to traditional graphitized carbon black (GCB) sorbent, Carbon S provides equivalent or superior pigment removal while significantly improving the recovery of sensitive analytes, particularly for compounds with a planar structural feature. This optimized design delivers a better balance between analyte recovery and matrix pigment removal efficiency than conventional GCB sorbent.

PSA sorbent

PSA is a sorbent that incorporates a primary-secondary amine structure, which has been widely used in sample preparation. This sorbent is primarily employed to remove fatty acids and other organic acids present in sample matrices, and it can also partially contribute to the removal of other polar interferences such as sugars. The removal of fatty acids by PSA serves as an excellent complement to EMR–Lipid sorbent for achieving complete lipid cleanup in a sample. However, because its interaction mechanism is not highly selective, the matrix masking effect becomes critical in EMR methodology to prevent the loss of analytes containing acidic functional groups. This effect is achieved when acidic matrix co-extractives, along with acidified solvents and residue water, compete for active sites on the PSA sorbent, thereby protecting sensitive targets from unwanted interactions.

EC-C18 sorbent

End-capped C18 (EC-C18) sorbent is incorporated into the cartridges to provide complementary removal of hydrophobic matrix interferences such as sterols or waxes. However, because the hydrophobic interaction mechanism of EC-C18 is not highly selective, excessive amounts of this sorbent can lead to loss of hydrophobic target analytes. To prevent this, the level of EC-C18 sorbent in EMR formulations is carefully controlled to maintain effective matrix cleanup without compromising analyte recovery.

The blended sorbents used in Captiva EMR cartridges incorporate some or all the aforementioned sorbents through formulation optimization, developed with careful consideration of both analyte recovery and matrix removal.

Reference

1. Multiclass Multiresidue Analysis for Food Safety Application Workflows: An application compendium using Agilent Captiva EMR cartridges. *Agilent Technologies application compendium*, publication number 5997-8859EN, **2026**.

2.1 Introduction of application

A pesticide is defined by the Food and Agriculture Organization (FAO) of the United Nations as “any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies”.[1] Pesticide residues are commonly found in or on plant-origin foods such as vegetables, fruits, grains, cereals, spices, tea, coffee, edible oils, and herbs as well as in many animal-derived foods such as milk, fish, eggs, and meat. These residues can pose potential health risks and raise environmental concerns.

Based on their intended function, pesticides can be classified into various categories, including algicides, avicides, bactericides, fungicides, herbicides, insecticides, miticides/acaricides, molluscicides, nematicides, rodenticides, or virucides, respectively. However, pesticides residues found on food commodities are primarily associated with three major classes—insecticides, fungicides, and herbicides. These classes encompass thousands of compounds from diverse chemical classes, such as organochlorine, organophosphorus, carbamate, benzoylurea, pyrethroid, anilinopyrimidine, imidazole, dithiocarbamate, strobilurin, triazole, chroroacetamide, imidazolinone, phenylurea, sulfonylurea, etc.[2]

Modern pesticide detection is commonly performed using LC/MS/MS, GC/MS/MS, or both, due to their high sensitivity, selectivity, and broad suitability for multiclass multiresidue pesticides. Depending on their chemical properties and suitability to different detection, pesticides can be classified as GC-amenable, LC-amenable, or suitable for both detection methods. LC-amenable pesticides are typically more polar to moderately polar and contain various functional polar groups. In contrast, GC-amenable pesticides tend to be nonpolar to moderately polar with less polar groups. Approximately 60 to 70% of pesticides are LC-amenable, while 30 to 40% are GC-amenable. About one-third of pesticides can be detected using both techniques.

Pesticides present many analytical challenges when tested in food matrices. Pesticides with planar structures are particularly sensitive to sorbent such as GCB used for pigment removal, often requiring compromises to achieve acceptable recovery. Organochlorine and pyrethroid pesticides, due to their high lipophilicity, are difficult to extract from edible oils. Labile pesticides are prone to degradation or loss along the GC flow path, necessitating the use of inert GC flow path consumables such as GC columns and liners. Pesticides containing acidic or basic functional groups may be affected by sorbent such as PSA or by acidic extraction solvents. Highly polar pesticides—such as glyphosates—pose significant challenges in both sample preparation and liquid chromatography.

The QuEChERS sample preparation approach, introduced in 2003, stands for quick, easy, cheap, effective, rugged, and safe.[3] It was originally developed for the multiresidue analysis of pesticides in fruits and vegetables and was quickly extended to a wide variety of food matrices. Designed to complement modern GC/MS and LC/MS instrumentation, the method leverages their high selectivity, sensitivity, and broad analytical scope. This enables a highly streamlined workflow with just good enough cleanup, small solvent volumes, no concentration steps, and compatibility with both GC- and LC-amenable pesticides using a single extraction. QuEChERS has since been widely adopted by food testing laboratories around the world and has become one of the most popular methods for food safety testing, particularly for multiclass multiresidue pesticides analysis. Key features of the method include:

- Effective matrix cleanup to remove major matrix interferences
- Minimal impact on target analytes, supporting broad-spectrum extraction
- Wide applicability across diverse food matrices with minimal modification
- Compatibility with both LC and GC analysis workflows
- Simple, fast, and reliable sample preparation for high-throughput food testing

The traditional QuEChERS approach consists of two main steps: salting-out extraction and dispersive SPE (dSPE) cleanup. The extraction is performed using acetonitrile, followed by a salting-out induced phase separation to partition the ACN and aqueous layers. This is achieved using either buffered or nonbuffered salts. Compared to the original method, which used nonbuffered salts (NaCl and $MgSO_4$), buffered salts—either the acetate salts used in AOAC method 2007.01 or the citrate salts in EN method 15662—offer advantages for extracting labile pesticides.[4,5] These buffered salts help maintain a more controlled pH condition during extraction, improving the stability and recovery of pH-sensitive compounds. The phase separation step also significantly reduces polar co-extractives from sample matrix, contributing to cleaner extracts.

The dSPE cleanup has been recommended for post-extraction matrix removal since the method was developed. Depending on the specific kit and format, the appropriate volume of crude sample extract is transferred into a dSPE tube. The tube is capped and vortexed for 3 to 5 minutes, followed by centrifuging for 5 minutes. The supernatant is then ready for analysis. This approach is relatively simple, fast, and user friendly, offering good enough matrix cleanup while maintaining acceptable pesticide recovery.

However, matrix cleanup efficiency by dSPE cleanup is limited when dealing with moderately to highly complex food matrices—such as high pigment components, high fat/lipids levels, or dry herbal compositions. These matrices often lead to unacceptable matrix effects due to significant co-extracted interferences, compromising the reliability and consistency of pesticides analysis. The final samples without efficient cleanup can also negatively impact instrument detection reliability and robustness. Additionally, the use of GCB and PSA may result in the loss of certain sensitive pesticides, particularly planar and acidic compounds. Endcapped C18 (EC-C18) sorbents are also insufficient for effectively removing fatty matrix components. While the QuEChERS method is theoretically compatible with both LC/MS/MS and GC/MS/MS analyses, it can be challenging to apply effectively for complex matrices such as herbal supplements, spices, tea, and essential oils, using one standard protocol due to the difference in instrument tolerance and selectivity for matrix interferences. In such cases, more sophisticated and tailored sample preparation methods are required to achieve acceptable analytical results.

The large variety of available dSPE kits often leads to confusion and complicates the selection process, making method alignment for dSPE cleanup challenging. Additionally, the dSPE cleanup protocol remains time consuming and labor intensive, involving multiple manual steps such as repeated transfers and frequent uncapping and capping of tubes. Another limitation is the relatively low sample volume recovery—typically around 50%. This low volume yield complicates the transfer, as residue salt can easily be drawn into pipette tips. It also restricts certain post-treatment steps, such as drying and reconstitution for sample concentration, further impacting overall method efficiency and reliability.

2.2 Captiva EMR product recommendations

The application of EMR passthrough cleanup methodology to multiclass multiresidue pesticide analysis is one of the primary applications for Captiva EMR cartridges. Initially introduced with Captiva EMR–Lipid products, the technology was later advanced with the development of Captiva EMR with Carbon S cartridges, which provide high selectivity and efficiency in comprehensive matrix removal, offering a convenient, rapid, and effective matrix cleanup solution that is fully compatible with QuEChERS extraction workflows. Given the significance of multiclass multiresidue pesticides—covering over a thousand compounds across diverse food matrices—multiple EMR cartridges are applied based on the specific matrix type. For plant-origin food matrices (excluding edible oils), Captiva EMR with Carbon S cartridges are recommended. The selection is guided by key matrix characteristics, such as whether the sample is fresh or dry, or whether it contains high, moderate, or low pigment level. For animal-origin foods and edible oils, Captiva EMR–Lipid and EMR–Lipid HF cartridges are the most suitable options to start with.

Table 2-1 provides the ordering information for Captiva EMR cartridges used in pesticides analysis. Figure 2-1 illustrates the overall recommended EMR passthrough cleanup for pesticides analysis in foods. Figure 2-2 presents detailed guidance on selecting Captiva EMR with Carbon S for various plant-origin food matrices.

Table 2-1. Ordering information for Agilent Captiva EMR cartridges used in pesticides analysis.

Product Name	Cartridge Format	Sorbent Bed Mass	Part Number
Captiva EMR–Lipid	3 mL	300 mg	5190-1003
Captiva EMR–Lipid	6 mL	600 mg	5190-1004
Captiva EMR–Lipid HF	3 mL	300 mg	5610-2235
Captiva EMR–Lipid HF	6 mL	600 mg	5610-2236
Captiva EMR–GPF	3 mL	135 mg	5610-2090
Captiva EMR–HCF1	3 mL	180 mg	5610-2088
Captiva EMR–HCF2	3 mL	180 mg	5610-2089
Captiva EMR–GPD	6 mL	595 mg	5610-2091
Captiva EMR–LPD	6 mL	570 mg	5610-2092

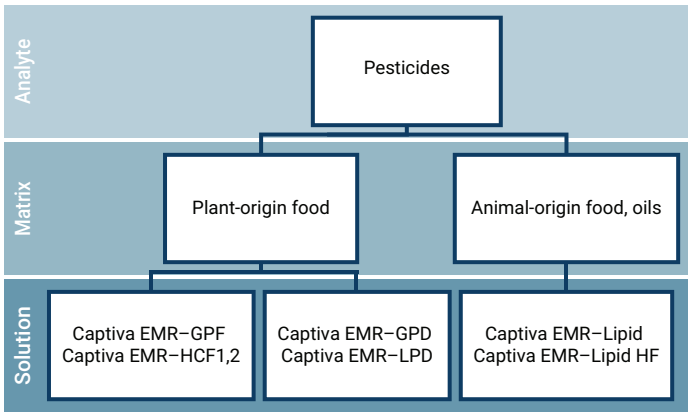


Figure 2-1. EMR passthrough cleanup for multiclass multiresidue pesticides analysis in food matrices.

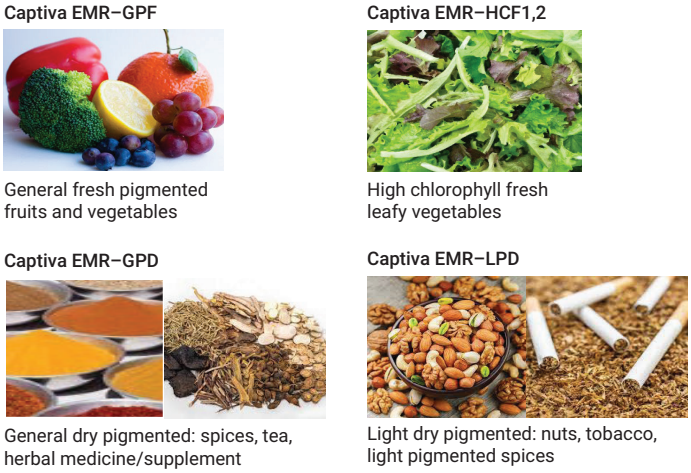


Figure 2-2. Recommendations of Agilent Captiva EMR with Carbon S cartridges for plant-origin food matrices.

Captiva EMR passthrough cleanup is a simple and easy procedure. The crude sample extract from a previous sample extraction is transferred onto appropriate Captiva EMR cartridges, either through direct transfer or with 10% premixed water. Sample elution usually uses gravity or low-level external forces, such as positive pressure or vacuum. The eluent subjects for direct injection or further post-treatment as needed. Figure 2-3 presents the simplified passthrough cleanup workflow using Captiva EMR with Carbon S cartridge for various food matrices.

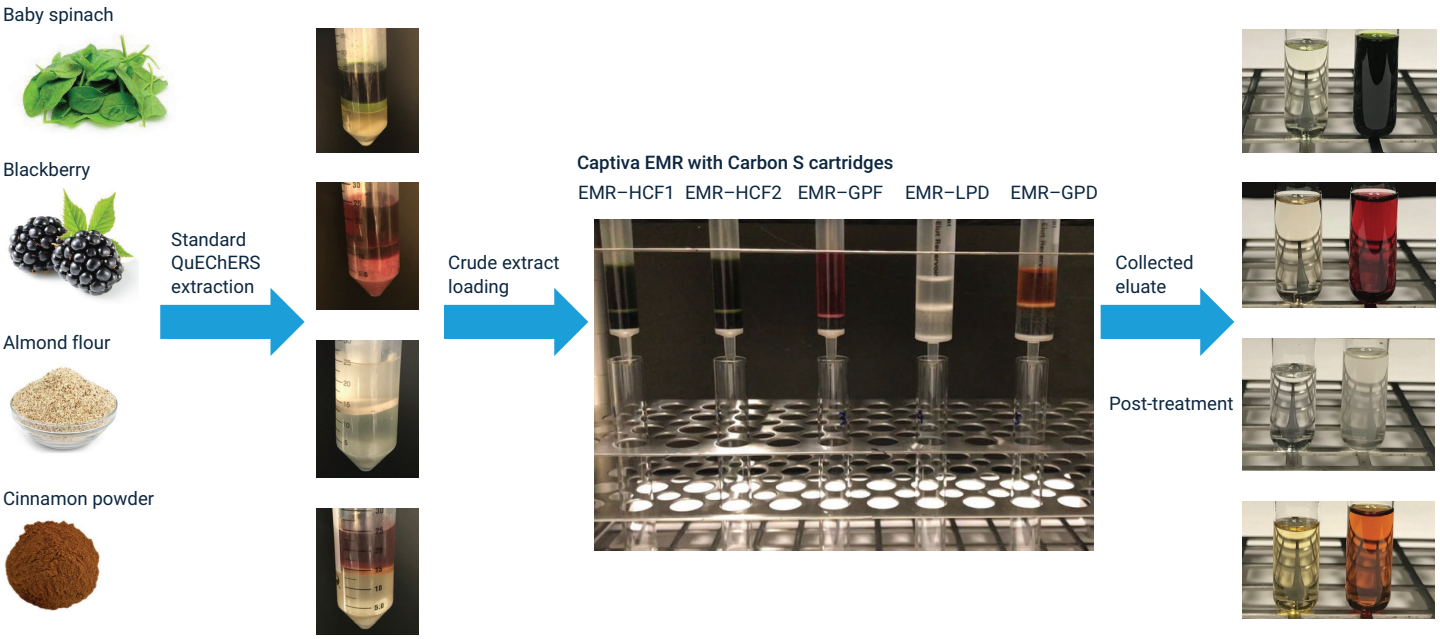


Figure 2-3. EMR passthrough cleanup procedure using Agilent Captiva EMR with Carbon S procedure for plant-origin food matrices.

2.3 Method development and comparison

2.3.1 Sample extraction

As illustrated previously, the QuEChERS salting-out extraction method is commonly used as the initial step in pesticides residue analysis in plant-origin matrices except edible oils. This approach can also be applied to many animal-origin foods, such as milk, eggs, meat, and fish. The procedure typically begins with 10 or 15 g of fresh produce sample, or 1 to 5 g of dry sample, followed by the addition of water for sample hydration of dry matrix. Depending on the specific protocol, 10 or 15 mL of extraction solvent—either ACN or ACN with 1% acetic acid—is then added. Next, QuEChERS extraction salts are added, which may be either buffered or nonbuffered. Nonbuffered salts (part number 5982-5550 or 5982-5550CH) are used in the original method, while buffered salts are employed in the AOAC method (part number 5982-5755 or 5982-5755CH) and EN method (part number 5982-5650 or 5982-5650CH). Buffered salts are widely preferred in pesticides analysis due to their capability to stabilize labile pesticides during extraction. Overall, both AOAC and EN buffered extraction salts perform comparably, offering high extraction efficiency across a broad range of pesticide compounds. The selection of extraction kit typically depends on the regulatory method that the testing laboratory follows or the preferred sample size.

The use of ceramic homogenizers (CHs, p/n 5982-9313) is highly recommended during QuEChERS salting-out extractions. CHs enhance the consistency of sample extraction by breaking up salt agglomerates, facilitating thorough homogenization, and thereby improving pesticide recovery from sample matrices. Figure 2-4 visually compares food samples after (A) vertical shaking and after (B) centrifugation. In each step, two sample tubes—(A) one with CHs and (B) one without CHs—are shown side by side to highlight differences in sample homogeneity. The comparison clearly demonstrates that samples processed with CHs result in a much more uniform sample/salt mixture with significantly fewer visible salt clumps.

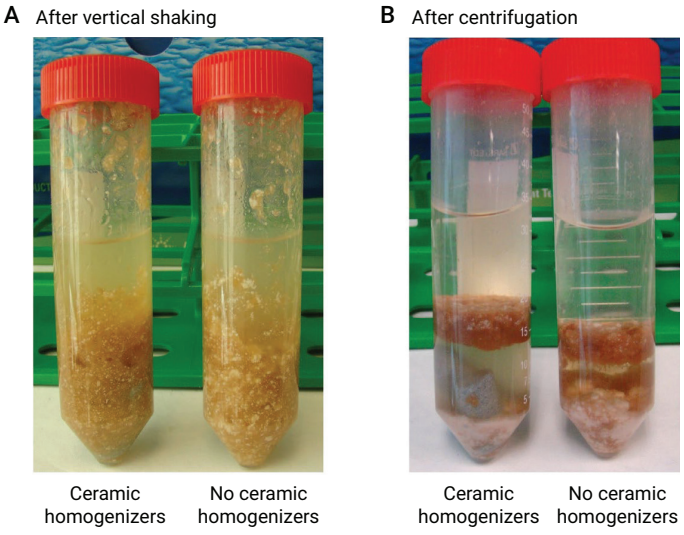


Figure 2-4. A comparison study of the use of ceramic homogenizers for QuEChERS extraction.

Pesticide extraction from edible oils is particularly challenging due to the high hydrophobicity of oil matrices. In such cases, salting-out extraction using ACN may lead to inefficient extraction efficiency, especially lipophilic compounds such as organochlorine pesticides. To overcome this limitation, direct solvent extraction with a stronger solvent mixture—such as 80:20 ACN:ethyl acetate—is commonly applied. To further enhance extraction efficiency, repeated extraction steps with multiple smaller aliquots of extraction solvent and extended extraction times are beneficial, ensuring more complete recovery of target analytes from oil matrix.

2.3.2 Sample size and dry sample hydration

For fresh produces, sample size is mostly standardized as either 10 or 15 g, depending on which extraction method is followed. This sample size is also applied for milk and eggs extraction.

For sample matrices with low moisture content—such as dry plant-origin food, edible oils, meat, and fish—a reduced sample size of 0.5 to 5 g is commonly used due to the concentrated matrix components in these matrices. For fresh meat and fish, a typical sample size is 5 g. For dry plant matrices and edible oils, a smaller amount—ranging from 0.5 to 2 g—is recommended, depending on the complexity of the matrix. In the cases of dry, plant-seeded matrices, it is strongly advised to conduct a prescreening study using a 1 g sample. This helps determine the appropriate sample size and prevents potential contamination of analytical instruments from unexpectedly heavy matrix loads.

Adding water to dry matrices to rehydrate these samples prior to QuEChERS extraction is essential for efficient extraction, particularly for relatively polar analytes. However, excessive water during rehydration has been observed to negatively impact analytical results. First, higher water volumes tend to increase the extraction of matrix polar co-extractives. Second, using less water during rehydration has shown to improve recoveries by approximately 10 to 15% for many intermediate to more hydrophobic pesticides. As a result, it is recommended to use a small volume of water or aqueous buffer—typically 3 to 5 mL—for rehydrating dry samples. The approach balances effective extraction with minimal matrix interference.

2.3.3 EMR mixed-mode passthrough cleanup

For fresh produce matrices, lipids/fats are usually insignificant. Sample crude extract after extraction is directly loaded on the Captiva EMR–GPF or EMR–HCF1 or 2 cartridges for passthrough cleanup.

Other more complex food matrices often contain higher levels of lipids and fats, making the removal of lipid co-extractives from the crude extract a critical step. In such cases, it is essential to premix the crude extract with 10 to 20% of water before loading it onto EMR cartridges, as water addition facilitates effective lipid removal on EMR–Lipid sorbent. Specifically, when using Captiva EMR–Lipid cartridges for fatty food matrices, a 10 to 20% water premix is typically recommended. For EMR mixed-mode passthrough cleanup using EMR–GPD and EMR–LPD, a lower water content—approximately 10%—is preferred. This approach helps balance the influence of water in the sample mixture on various sorbents and preserves analyte integrity by minimizing unwanted interactions with the mixed-mode blended sorbents.

Sample loading volume on Captiva EMR–Lipid or Captiva EMR–Lipid HF cartridges depends on the cartridge format. Typically, 2.5 to 3 mL loading volume is applied for 3 mL cartridges, while 5 to 6 mL is used for 6 mL cartridges. However, for Captiva EMR with Carbon S cartridges, including EMR–GPF, EMR–HCF 1 and 2, EMR–GPD, and EMR–LPD, sample volume is relatively consistent regardless of the cartridge tube format—3 or 6 mL. This is because the sorbent bed mass in these cartridges is tailored to approximately 3 mL of sample extract. Captiva EMR–GPD and EMR–LPD are designed for highly complex dry matrices, thus containing more blended sorbent bed mass. To accommodate higher bed mass, 6 mL tubes are therefore used.

The loading volume is an important parameter for managing matrix masking effects and their influence on analyte recoveries. The matrix masking effect refers to the phenomenon where matrix co-extractives interact with sorbents during EMR passthrough cleanup, thereby shielding target analytes from unwanted interactions with the sorbents. When the sample matrix is relatively simple and lacks sufficient co-extractives to provide this masking effect, analyte loss may occur—particularly for pesticides that are sensitive to certain sorbents. In such cases, increasing the sample loading volume can enhance the matrix masking effect and help preserve analyte recovery. However, excessive sample loading may exceed the capacity of the EMR cartridge, leading to reduced matrix removal efficiency. Therefore, optimizing the loading volume is a useful strategy to balance analyte recovery and effective matrix cleanup. Figure 2-5 shows the sample loading volume impact on sensitive pesticides recovery from celery extract when using Captiva EMR–GPF cleanup.

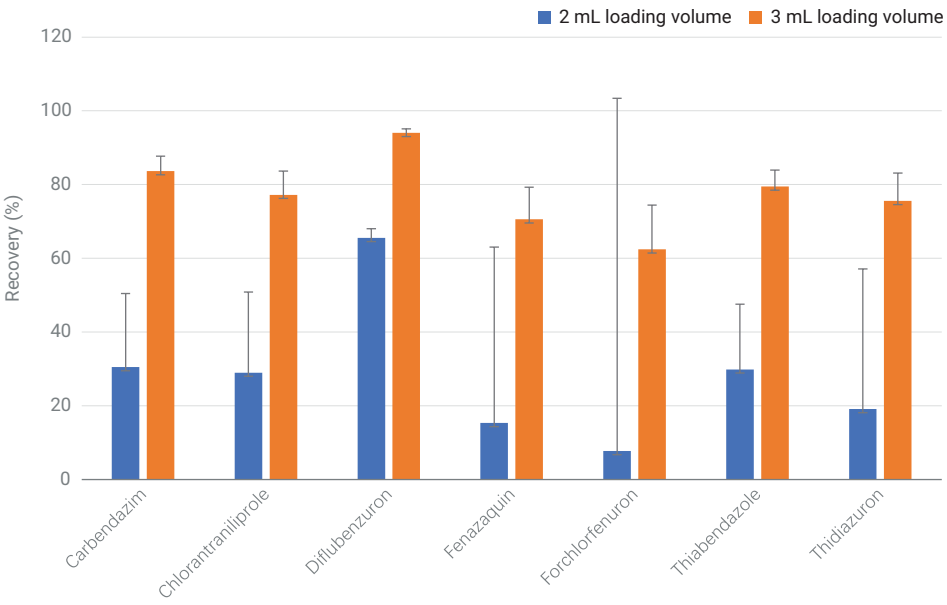


Figure 2-5. Sample loading volume effect on planar pesticides recovery from celery extract using Agilent Captiva EMR–GPF passthrough cleanup.

2.3.4 Post-treatment after EMR cleanup

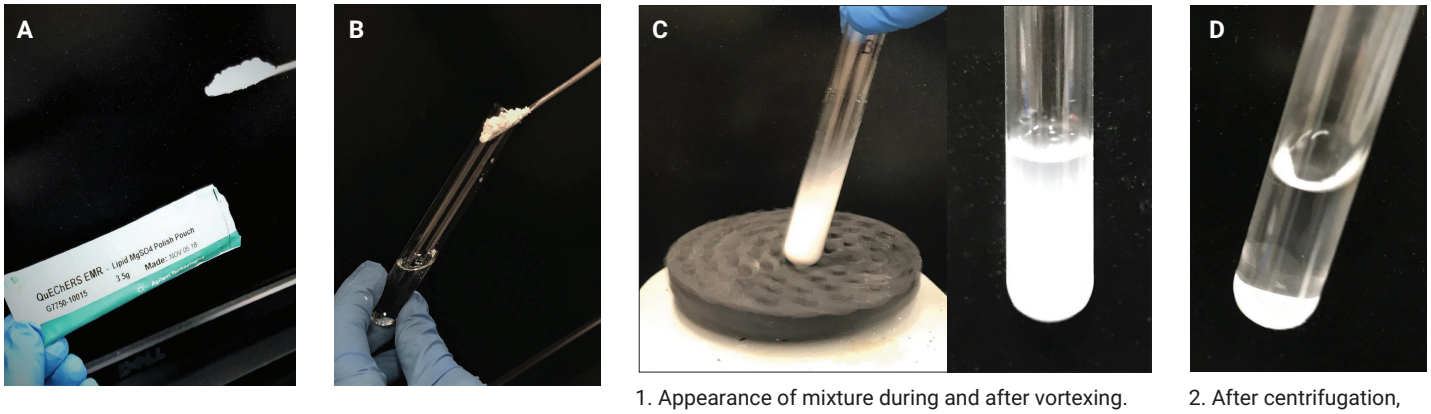
After EMR cleanup, the sample eluate—typically in 100% ACN or a 90:10 ACN:water mixture—is subjected for instrumental analysis. However, appropriate post-treatment steps are necessary before injecting on the instrument for analysis to ensure acceptable chromatographic performance and detection integrity.

For LC/MS/MS detection, injecting samples in a high-organic solvent can lead to solvent effects, which negatively impact the peak shapes of early-eluting analytes. These effects include peak fronting, splitting, shouldering, and distortion, primarily caused by the mismatch between the high organic content of the sample and the aqueous starting conditions of the chromatographic method. To mitigate solvent effects, the sample eluate should be diluted with water to reduce the organic solvent content to below 20%. However, when method sensitivity does not allow for such dilution—due to the need to meet strict limits of quantitation (LOQs)—a drying and reconstitution step is required to perform a solvent switch.

An alternative strategy to reduce solvent effects without compromising detection limits or adding extra preparation time is to use an appropriate injection program. This can be achieved through a sandwiched injection program on a standard multisampler or the feed injection mode available on a hybrid multisampler.

For GC/MS/MS detection, the sample eluate must be completely dried to eliminate residual water. Notably, even eluates that are nominally in 100% ACN—such as those obtained from EMR–GPF cleanup—still contain trace amounts of water. For multiclass multiresidue pesticides analysis, residual water can be removed using either an anhydrous $MgSO_4$ drying step or a drying and reconstitution step. The $MgSO_4$ drying approach is quicker and simpler, but the final sample remains in ACN, which is not always considered ideal for GC analysis due to its solvent properties. The drying and reconstitution approach takes more time but allows the sample to be redissolved in a GC-friendly solvent such as acetone or toluene, thus improving compatibility with GC analysis. It also provides an opportunity to further concentrate sample extract by dissolving the dried residue in a smaller volume of reconstitution solution.

For the drying step using anhydrous $MgSO_4$, a simple addition of approximately 200 to 300 mg per 1 mL of EMR eluate—roughly a small spatula scoop—of anhydrous $MgSO_4$ powder from a Bond Elut QuEChERS EMR–Lipid polish pouch (part number 5982-0102) is sufficient for sample eluate after EMR passthrough cleanup. Precise measurement is not required for effective drying, and complete removal of residual water can be confirmed by two visual indications: (1) milky white homogeneity of the sample mixture during vortex, indicating proper dispersion, and (2) powder-like settling of salts at the bottom of the tube after standing, rather than forming coagulated chunks. Figure 2-6 illustrates the step-by-step process of sample drying following EMR passthrough cleanup and prior to GC/MS/MS analysis.



1. Appearance of mixture during and after vortexing.

2. After centrifugation, the salt settles to the bottom, and has no large grains.

Figure 2-6. Sample drying after EMR passthrough cleanup for GC/MS/MS analysis using anhydrous $MgSO_4$ powder.

2.4 Performance review

2.4.1 Enhanced matrix cleanup efficiency

Captiva EMR sorbents and products are designed to enhance matrix cleanup efficiency, particularly for complex food matrices that traditional dSPE cleanup methods cannot effectively address. This improved performance is evidenced across various challenging matrices through multiple evaluations. Table 2-2 shows the reduction in dried sample residue for edible oils, highlighting the effectiveness of Captiva EMR–Lipid cleanup. Figure 2-7 presents a GC/MS full scan chromatographic profile and dried residue study of cayenne pepper extract, illustrating reduced matrix interferences provided by Captiva EMR–GPD cleanup. Figure 2-8 displays an LC/MS total ion chromatogram (TIC) for black pepper extract processed by different cleanup methods, further confirming the improved cleanup capability delivered by hyphenated Captiva EMR–GPD and EMR–GPF cleanup.

Table 2-2. Edible oil matrix co-extractive residues and removal using LLE followed by either Agilent Captiva EMR–Lipid cleanup or C18 dSPE cleanup.[6]

	Olive Oil	Corn Oil	Soybean Oil	Canola Oil
Dried Residue Weight per 1 mL Oil Extract (mg), n = 2				
Sample with No Cleanup	7.64	16.11	14.49	8.44
Sample with Captiva EMR–Lipid Cleanup	1.14	0.34	0.16	0.08
Sample with C18 dSPE Cleanup	3.47	6.05	9.68	4.36
Matrix Removal (%) by Dried Residue Weight				
Captiva EMR–Lipid Cleanup	85	98	99	99
dSPE C18 Cleanup	55	62	33	48

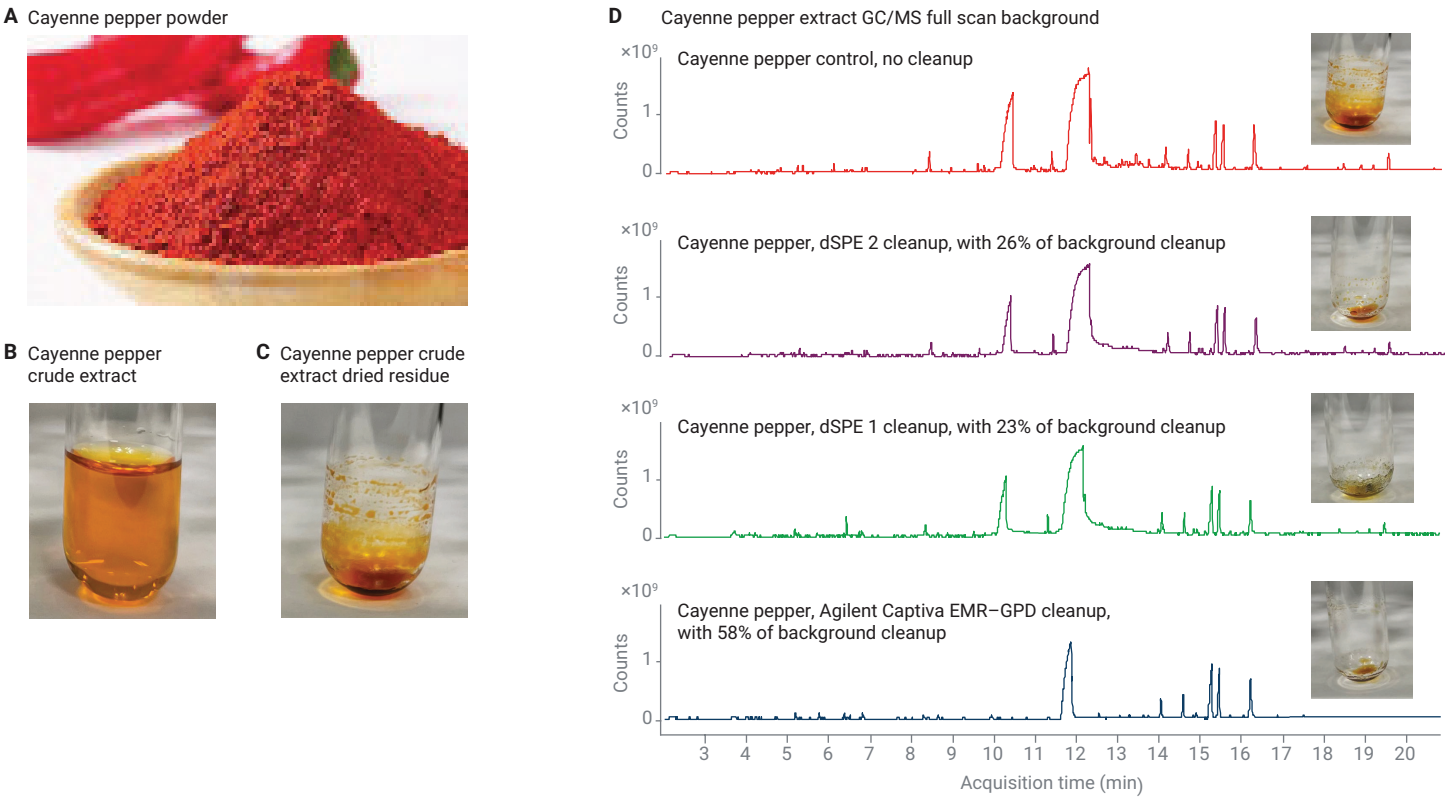


Figure 2-7. Cayenne pepper matrix removal study. (A) Typical cayenne pepper powder, (B) crude extract after QuEChERS extraction, (C) dried residue of crude extract, (D) GC/MS background profile full scan chromatograms and dried residue of final samples using different cleanup methods.

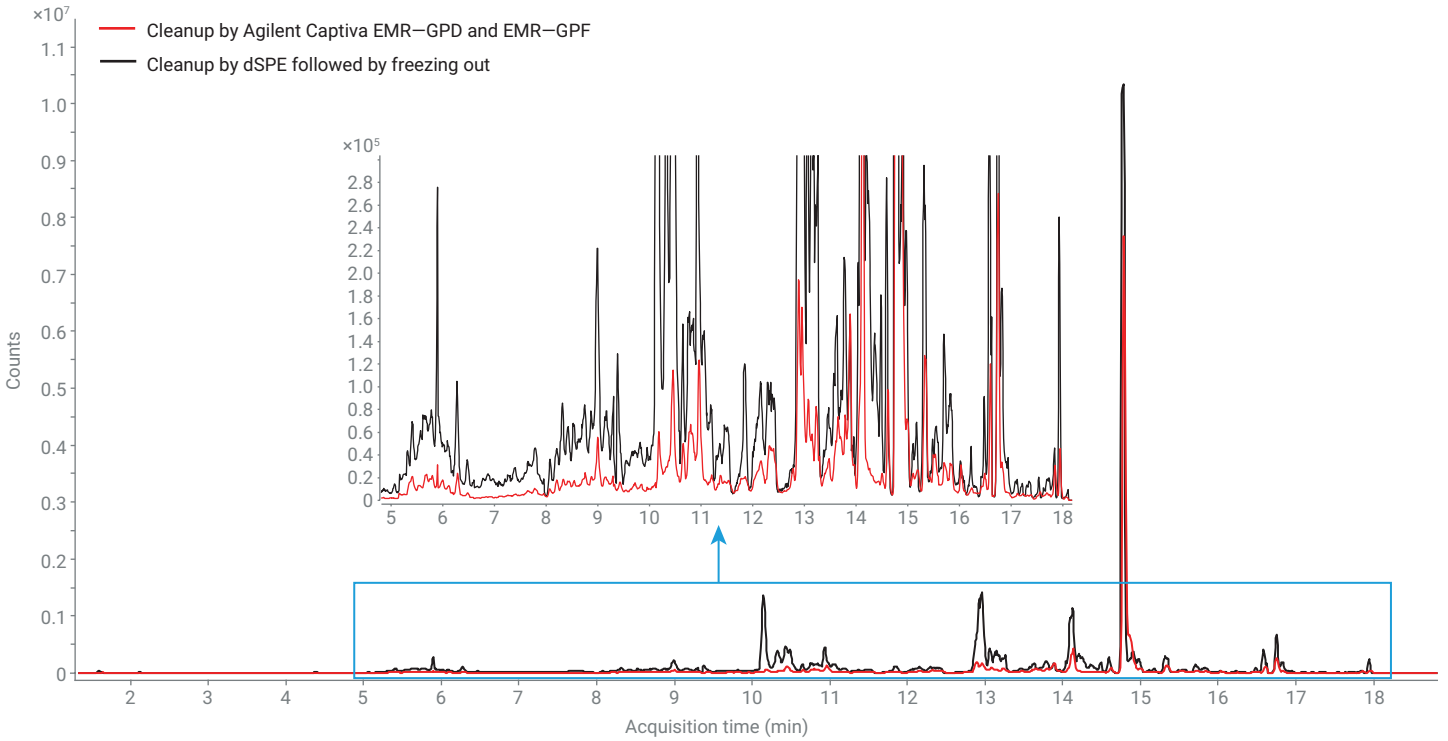


Figure 2-8. TIC chromatograms of black pepper by LC/TQ with the sequential EMR mixed-mode passthrough cleanup using Agilent Captiva EMR–GPD and EMR–GPF cartridges, compared to dSPE followed by freezing-out cleanup.

2.4.2 Influence of cleaning samples on pesticides analysis

Cleaner samples for pesticides analysis significantly improve analytical performance across multiple dimensions. Compared to samples prepared using traditional QuEChERS methods, those processed with EMR passthrough cleanup yield a much cleaner chromatographic background. This improvement enhances method selectivity and contributes to more consistent and accurate quantitation. Figure 2-9 displays GC/MS/MS MRM chromatograms of bell pepper extracts prepared using different matrix cleanup methods. The expanded images within the ovals highlight the MRM chromatograms for the compound molinate collected within a 1.5-minute acquisition window. The comparison clearly demonstrates that Captiva EMR–GPF cleanup provides a cleaner analyte background, resulting in improved analyte integration accuracy.

Matrix ion suppression on LC/MS/MS is another indicator for the sample matrix cleanliness evaluation, where the cleaner sample resulted in less suppression and higher responses. Figure 2-10 shows peak response for spirodiclofen and fenproximate in cayenne pepper extract, and temephos and retenone in cumin extract, when using EMR passthrough cleanup versus dSPE cleanup. Compared to dSPE cleanup, EMR passthrough cleanup leads to reduced ion suppressions and higher peak responses.

Continuous injections of cleaner samples into the instrument help reduce matrix accumulation along the flow path and at the MS source. This enhances overall detection robustness and decreases instrument downtime. Figure 2-11 presents the LC/MS/MS responses of tralkoxydim in cayenne pepper extracts, illustrating improved detection consistency across multiple injections.

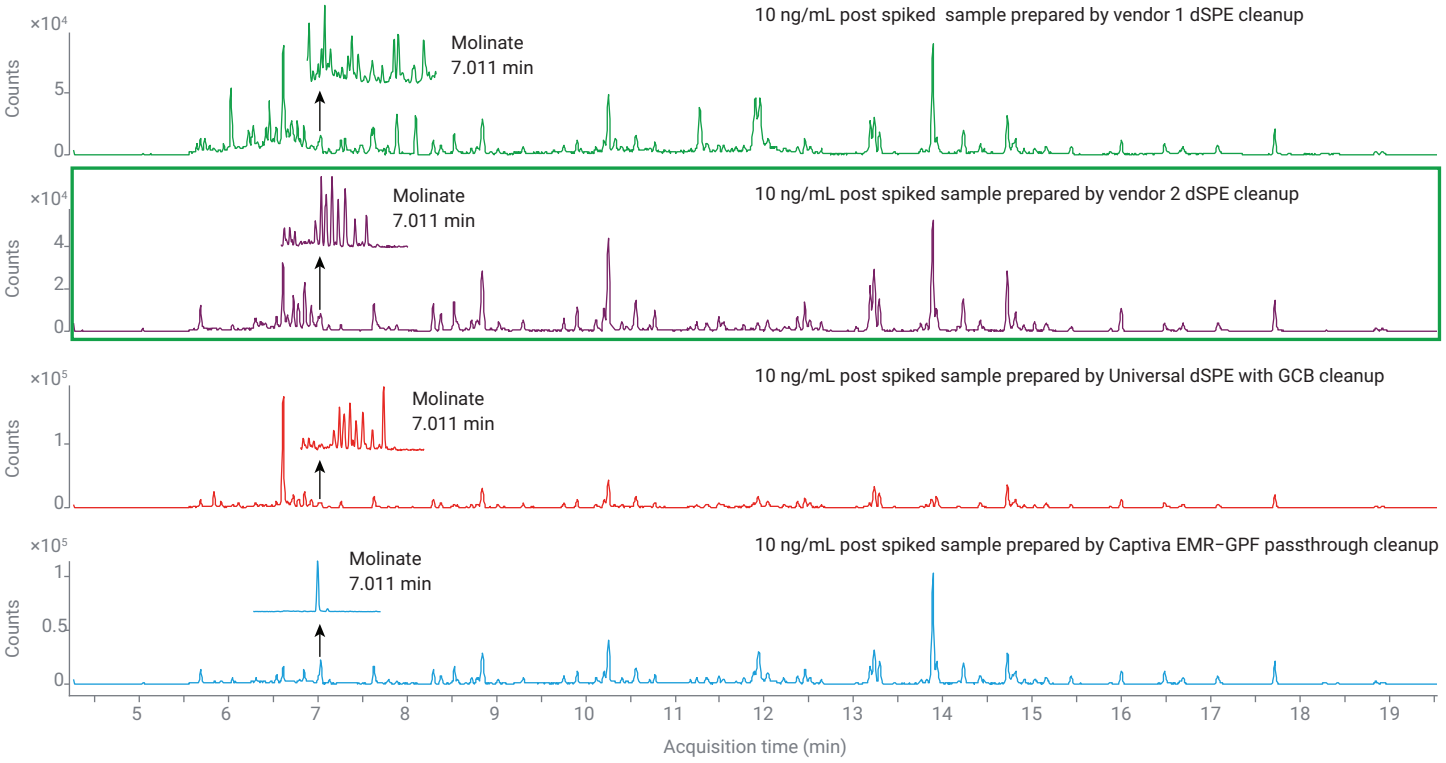


Figure 2-9. GC/MS/MS MRM chromatograms for bell pepper extracts prepared by different cleanup methods.

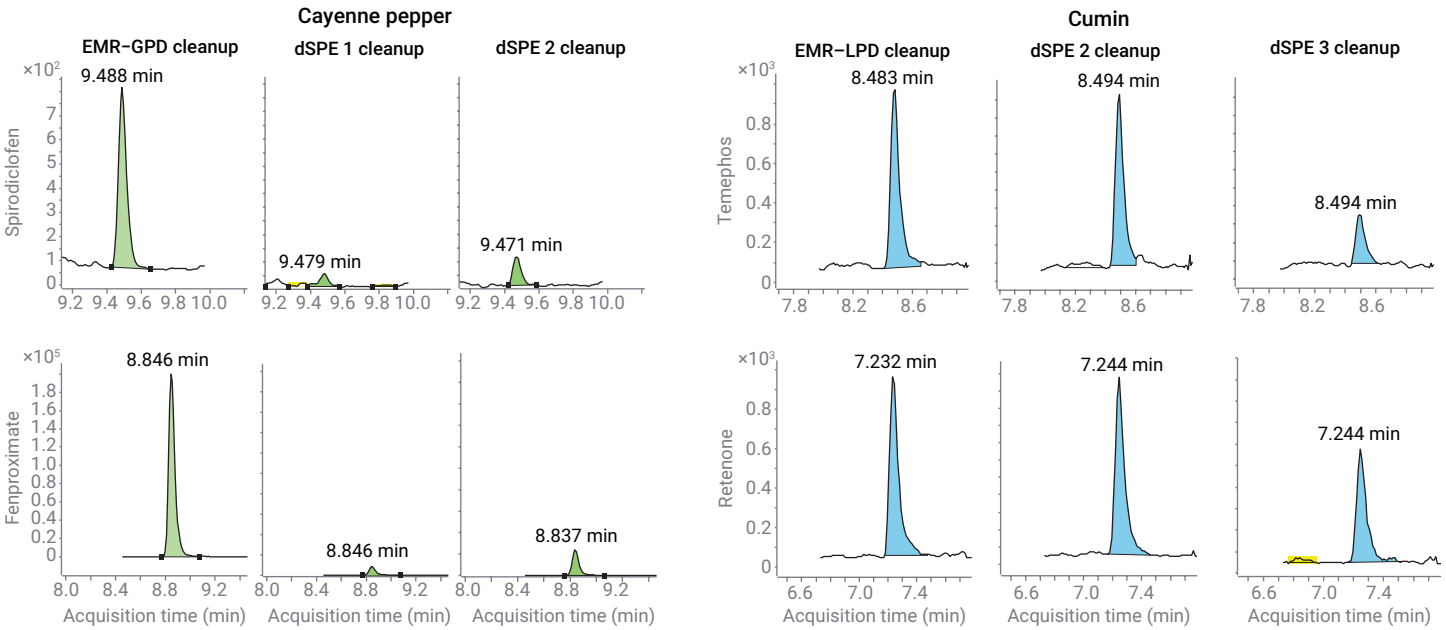


Figure 2-10. Representative pesticides responses on LC/MS/MS detection in cayenne pepper extract and cumin extract using EMR passthrough cleanup versus dSPE cleanup.

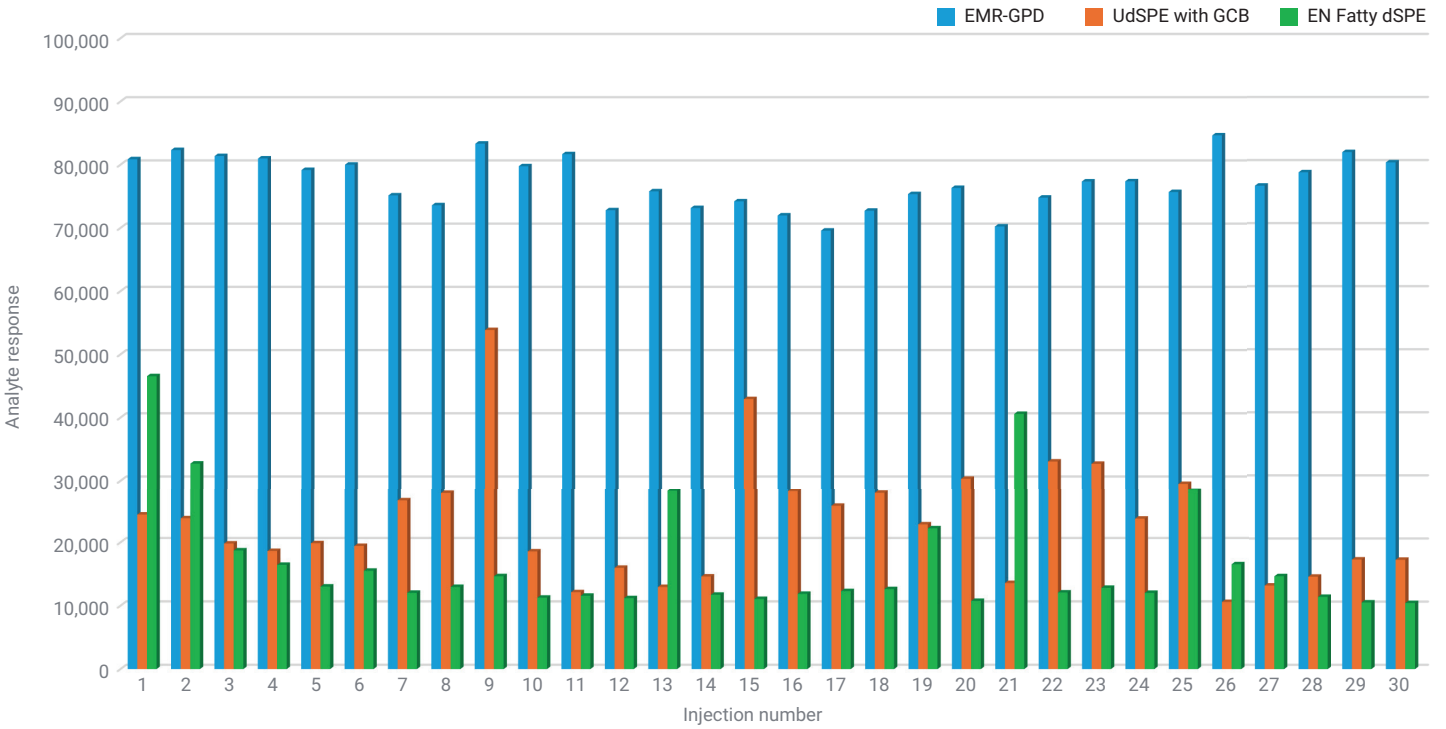


Figure 2-11. Tralkoxydim responses on LC/MS/MS for 30 injections of cayenne pepper extract by Agilent Captiva EMR–GPD cleanup versus traditional dSPE cleanup.

2.4.3 Improved quantitation performance and overall pass rate

The use of highly selective sorbents in Captiva EMR cartridges—such as EMR–Lipid and Carbon S sorbents—combined with the optimized formulation that balances pesticide recovery and matrix removal, results in significantly higher recoveries of many pesticides that are often lost during traditional dSPE cleanup. Figure 2-12 illustrates the improved sensitive pesticide recovery from green leafy vegetables when using Captiva EMR–HCF passthrough cleanup.

For large-scale pesticides analysis panels involving the analysis of several hundred compounds, the improved quantitation performance for sensitive pesticides translates to a lower failure rate, or conversely, a higher pass rate. This improvement is demonstrated by the statistical summary of quantitation results for over 400 pesticides in botanical tea materials using both LC/MS/MS and GC/MS/MS detections, as shown in Figure 2-13.

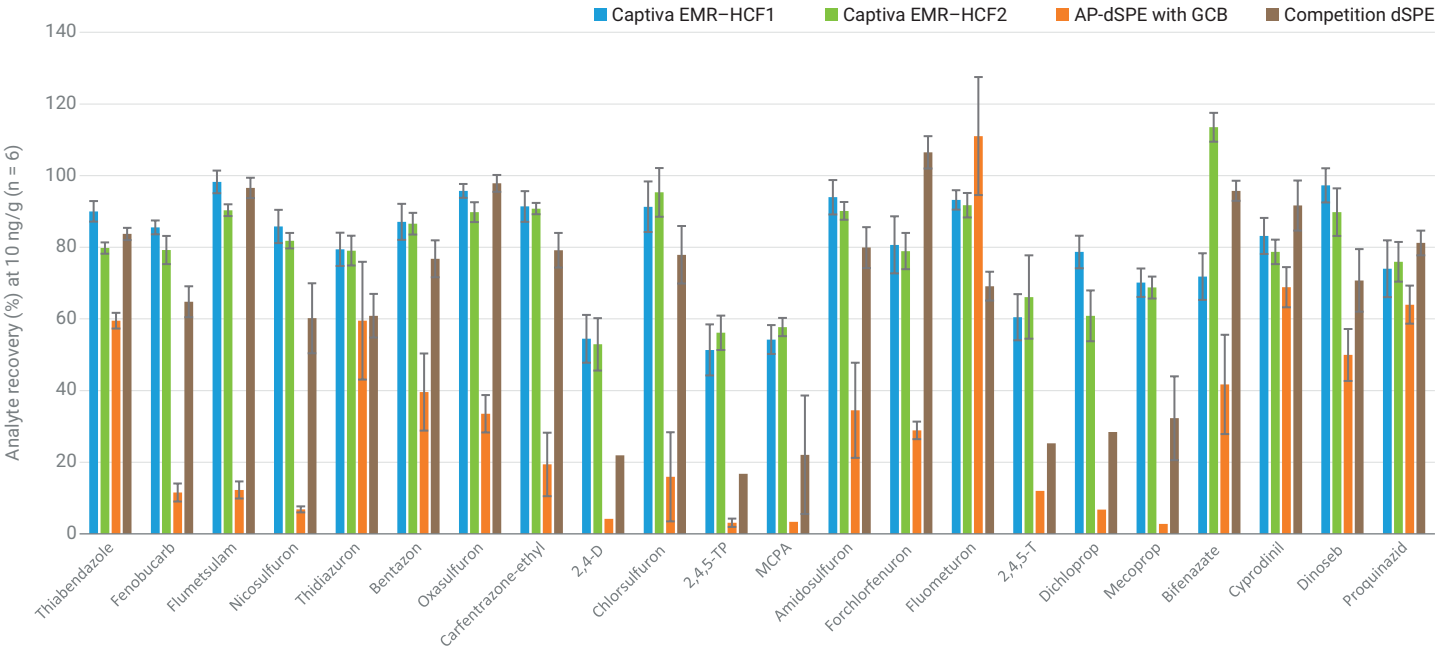


Figure 2-12. Sensitive pesticides recovery comparison for samples prepared using different cleanup methods.

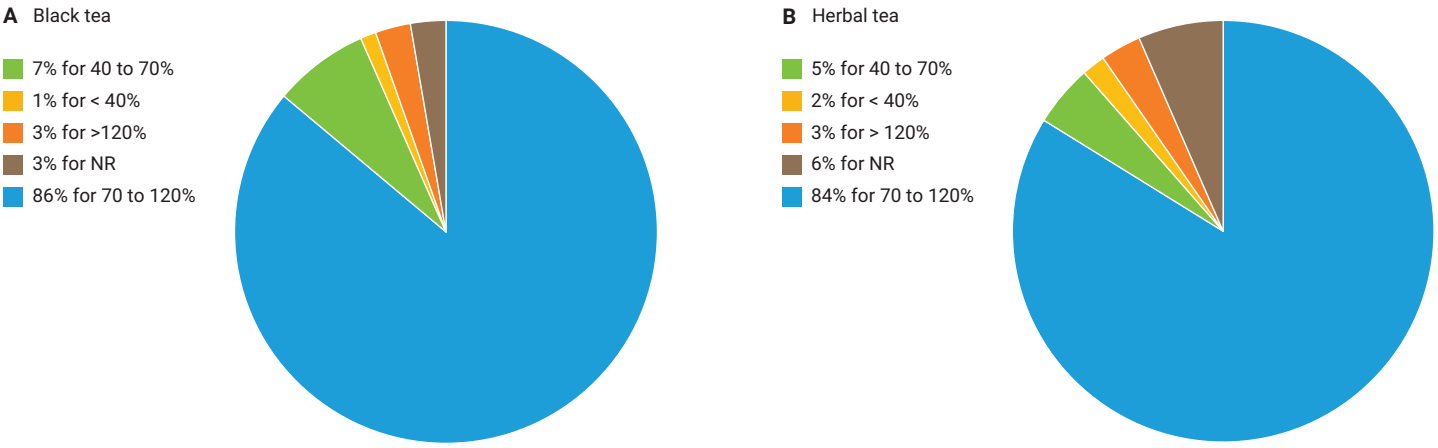


Figure 2-13. Statistical summary for over 400 pesticides analysis failure rate in botanical teas.

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Multilcass Multiresidue Veterinary Drugs Analysis in Food

3.1 Introduction of application

Veterinary drugs are widely used in animal-origin food production to prevent animal diseases or as a growth promoter. Despite obvious benefits, extensive use of these drugs can lead to residues in animal food products such as meat, milk, and eggs, causing adverse effects to consumers. The impacts and concerns make the presence of veterinary drug residues and other contaminants in edible tissues one of the key issues for food safety, initiating great public concern. With the increase and wider attention on food safety, regulation of drugs used in animal food production has been imposed in nearly every country, and global regulations define maximal residue limits (MRLs) for veterinary drugs in food of animal origin to protect public health.[1–3]

Commonly used vet drugs include multiple classes of compounds based on either chemical classes or main functional use. The main classes include antibiotics such as beta-lactams, tetracyclines, macrolides, quinolones, sulfonamides, aminoglycosides, and amphenicols. Anthelmintics include benzimidazoles, avermectins, and nitrolmidazoles. Other classes include NSAIDs (nonsteroidal anti-inflammatory drugs), tranquilizers, hormones, antimicrobials like furans, and growth promoters such as corticosteroids, beta-agonists, and anabolic steroids. Additionally, there are insecticides like organophosphates, fungicides, dyes, coccidiostats, antivirals, antipyretic analgesics, glucocorticoids, and sedatives.

Many vet drugs are labile and sensitive to aggressive sample preparation procedures or chemicals being used. Certain compounds present unique challenges. Tetracyclines tend to chelate, or bind, with metal ions from salts or container surfaces. Beta-lactams are very labile, meaning they are prone to degradation. Hormones and steroids are hydrophobic and have planar structural features, making them difficult to work with. Quinolones are sensitive to acid scavenger sorbents like PSA, and aminoglycosides are quite polar, which makes them difficult to extract. All these challenges make multiclass multiresidue vet drugs analysis difficult since targets with different physical features can add restrictions to the analytical method, especially sample preparation techniques.

Among the various detection techniques developed for vet drugs analysis, LC/MS/MS has become increasingly popular in recent years due to its high sensitivity and selectivity for simultaneously detecting multiclass multiresidue vet drugs. However, matrix effect using MS detection can be significant due to the limited sample preparation that can be used. Foods from animal origin such as muscle, liver, and eggs are complex matrices, which may contain 2 to 47% fat and 10 to 30% protein. Therefore, it is critical to use appropriate sample preparation methods to achieve efficient protein, fat, and lipid removal to mitigate matrix effects for vet drugs analysis by LC/MS/MS. However, highly selective and efficient matrix cleanup is challenging given the need to achieve the desired recovery of multiple veterinary drug compounds.

Generally, the sample preparation methods used in multiclass multiresidue veterinary drug analysis include two major categories, methods focused on removing unwanted food matrices (category 1), and methods focusing on capturing target analytes (category 2). In category 1, sample preparation methods tyically involve the use of more general and less selective sample extractions such as solid-liquid extraction or QuEChERS extraction.[4–6] Solid-liquid extraction using a mixture of aqueous buffer or water and organic solvent such ACN or MeOH can extract multiple veterinary drugs, but can also extract many matrix co-extractives simultaneously, especially when using MeOH as the extraction solvent. QuEChERS extraction involves the use of inorganic salt for phase separation after solvent extraction, which can cause loss of sensitive or polar analytes due to degradation, metal chelation, or limited solubility of polar compounds in ACN. Therefore, QuEChERS extraction is usually not recommended for multiclass multiresidue vet drugs analysis, except for single or few classes of vet drugs that are not sensitive to the use of salts. Both extraction techniques require further matrix cleanup methods after extraction. Traditionally these techniques include dispersive solid phase extraction (dSPE) cleanup with various sorbents, freezing-out, and hexane defatting. However, these matrix cleanup techniques can either lack selectivity due to analyte loss, require substantial time, provide inefficient lipid removal especially for lipids and fats, or require method development work.

In category 2, SPE is often used to extract targets from complex matrices, where the unwanted matrix co-extractives are washed away before eluting target analytes.[7,8] This presents challenges when developing multiclass multiresidue methods as multiple analyte classes have different binding affinity to the sorbent. Mixed-mode sorbents can have advantages over single-mode SPE, where analytes are trapped using the combined reversed-phase and ion-exchange interactions. As a result, the methods for multiclass multiresidue veterinary drug analysis in category 2 are usually applicable to a limited number of compounds and classes relative to category 1 techniques. For these reasons, a matrix removal-based sample preparation method (category 1) is preferable for multiclass multiresidue analysis. However, there is a need to improve the matrix removal efficiency.

3.2 Captiva EMR product recommendations

Given the sensitive classes of vet drugs like quinolones, beta-lactams, tetracyclines, steroids, etc., the multiclass multiresidue analysis for vet drugs can be highly sensitive to carbon material and PSA sorbents. Plus, the typical animal-origin food matrices contain a high percentage of fats and lipids. The front-end sample extraction typically removes proteins, therefore, the matrix removal after sample extract mainly focuses on lipid and fat removal, and Captiva EMR–Lipid cartridges fit the best for this purpose.[9] Captiva EMR–Lipid HF (high flow) was introduced later to improve the sample elution flow under gravity. Given the demonstrated equivalence of this product with Captiva EMR–Lipid, Captiva EMR–Lipid HF can be advantageous for vet drugs analysis in fatty food matrices and provides the benefit of easy elution flow under gravity.

Table 3-1 shows the ordering information for Captiva EMR–Lipid cartridges and EMR–Lipid HF cartridges. Figure 3-1 shows the recommendation of EMR passthrough cleanup for vet drugs analysis in animal-origin food.

Table 3-1. Captiva EMR–Lipid and EMR–Lipid HF cartridges for order.

Product Name	Cartridge Format	Sorbent Bed Mass	Part Number
Captiva EMR–Lipid	3 mL	300 mg	5190-1003
Captiva EMR–Lipid	6 mL	600 mg	5190-1004
Captiva EMR–Lipid HF	3 mL	300 mg	5610-2235
Captiva EMR–Lipid HF	6 mL	600 mg	5610-2236

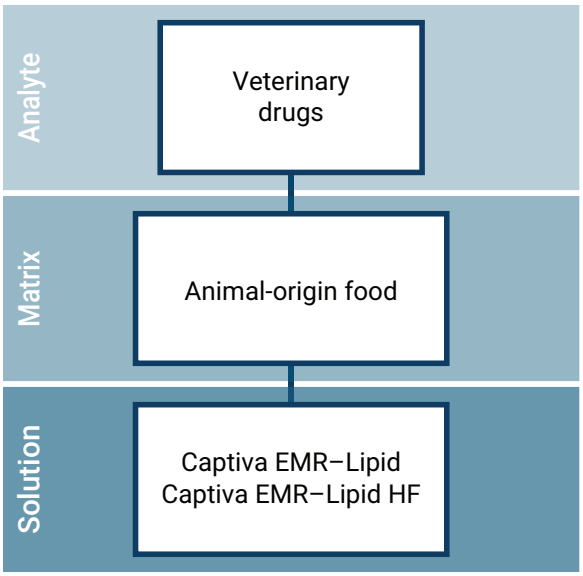

Figure 3-1. EMR passthrough cleanup for multiclass multiresidue vet drugs analysis in animal-origin food matrices.

3.3 Method development and comparison

As previously discussed, solvent extraction for protein removal is typically recommended for multiclass multiresidue vet drugs analysis. To preserve some labile vet drugs, the sample was first extracted with a small aliquot of water or aqueous buffer. The use of aqueous buffer with EDTA is highly recommended to prevent tetracyclines loss during extraction. Acidified ACN was then added to further extract the rest of the vet drugs and remove proteins from the matrix. The acid addition in the extraction solvent improved protein precipitation efficiency during sample extraction. It also increased the recoveries for tetracyclines, quinolones, macrolides, and a few other classes of vet drugs. The disadvantage of using acidified solvent was the decreased beta-lactams response. Figure 3-2 shows the investigation of the recoveries of representative vet drugs from multiple classes using various formic acid concentrations in ACN. As a compromise, ACN with 2% formic acid extraction solvent was used to achieve acceptable results for most classes of vet drugs and efficient protein removal through sample extraction.

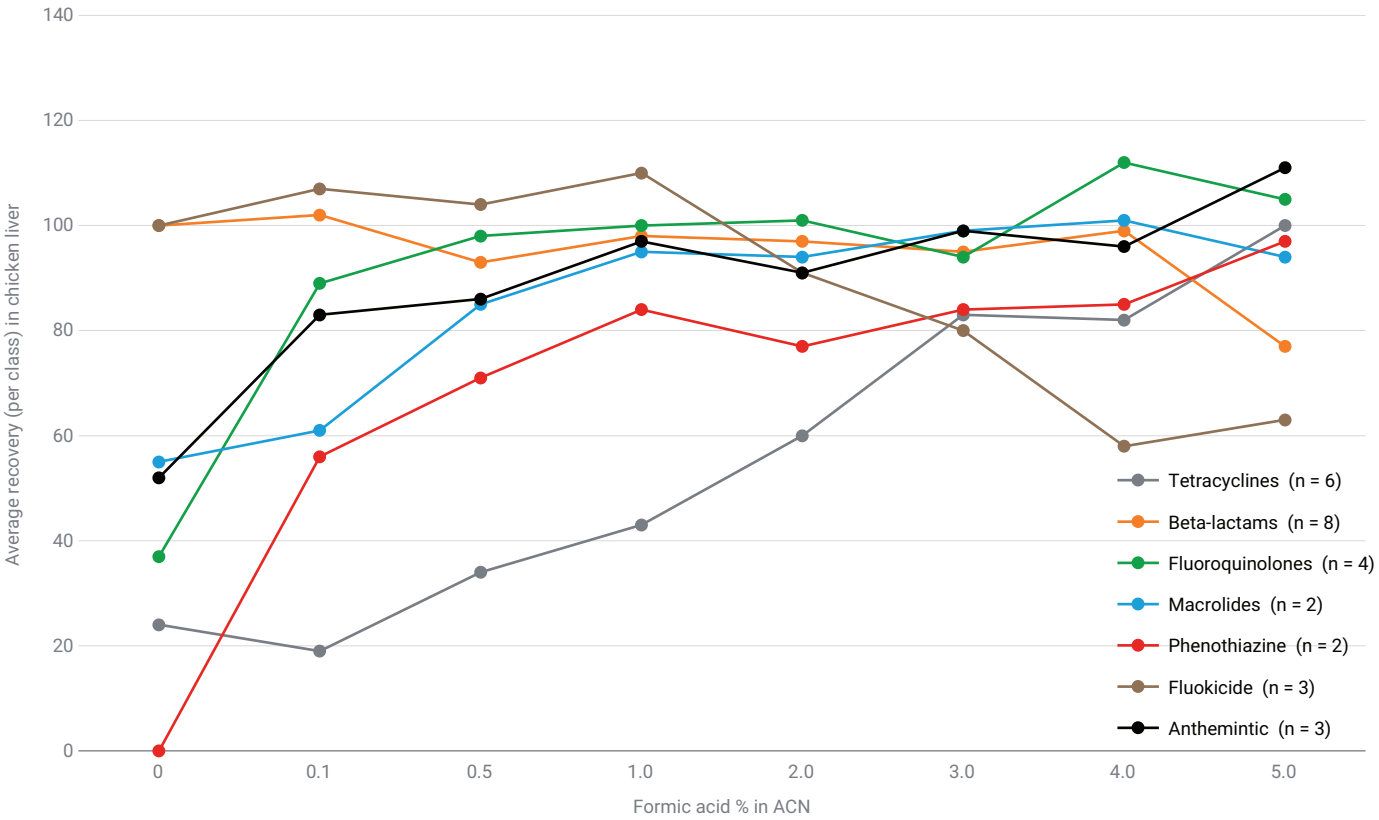

Figure 3-2. Acid addition in ACN for food sample extraction and its impact on different veterinary drugs recoveries. The number for each veterinary drug class indicates the number of veterinary drug targets used for evaluation.

After solvent extraction, the crude extract containing 15 to 20% aqueous can be directly loaded onto Captiva EMR–Lipid or EMR–Lipid HF cartridges for passthrough cleanup. For 3 mL cartridges, the loading volume is approximately 2.5 to 3 mL, while for 6 mL cartridges, it is about 5 to 6 mL. The elution can be either driven by gravity or low-level external force. For most fatty food matrices, gravity elution within a reasonable time (< 30 minutes) is achievable on Captiva EMR–Lipid HF, which requires less attention during sample elution and improves elution consistency. Table 3-2 shows the comparison of typical fatty food sample extracts elution under gravity between Captiva EMR–Lipid HF cartridges, Captiva EMR–Lipid cartridges, and cartridges from another vendor. Captiva EMR–Lipid HF cartridges demonstrate equivalent analyte recovery and fatty matrix removal efficiency, as shown in Figure 3-3.

Table 3-2. Complex food matrix extract gravity elution time comparison using different cartridges for matrix passthrough cleanup.

Food Matrix Extract	Sample Extract Gravity Elution Time (min)		
	Agilent Captiva EMR–Lipid HF	Captiva EMR–Lipid Cartridge	Competition Cartridge
Beef	18 to 22	45 to 47	38 to 46
Pork	22 to 24	41 to 45	32 to 47
Bovine Kidney	22 to 26	48 to 51	31 to 54
Salmon	15 to 20	36 to 40	19 to 26
Eggs	11 to 15	23 to 25	34 to 37
Infant Formula	12 to 14	15 to 17	10 to 12
Chocolate	12 to 14	30 to 37	20 to 74
Peanut Oil	13 to 17	19 to 22	74 to 76
Pumpkin Seed Oil	20 to 25	23 to 25	> 90

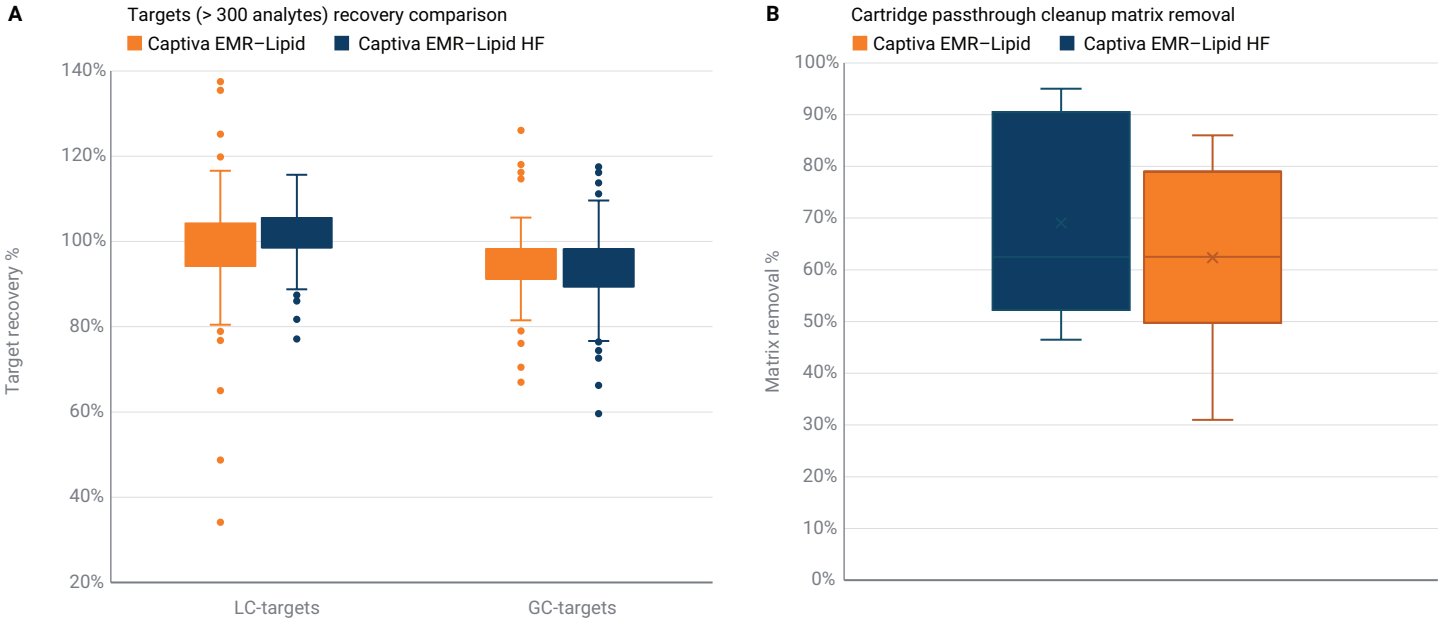

Figure 3-3. (A) Analyte recovery comparison between Agilent Captiva EMR–Lipid and Captiva EMR–Lipid HF cartridges. (B) Matrix cleanup efficiency for eight typical fatty food matrices including pork, beef, bovine kidney, salmon, egg, infant formula, peanut oil, and pumpkin seed oil.

For vet drugs analysis, it is recommended to use an additional elution with an aliquot of 80:20 ACN:water solution, which improves the recoveries of some critical vet drugs like tetracyclines during matrix cleanup on EMR–Lipid cartridges. Figure 3-4 shows the comparison of representative vet drugs recoveries with versus without the additional elution step. Results showed that the additional elution step improved recoveries 10 to 30% for these sensitive vet drugs.

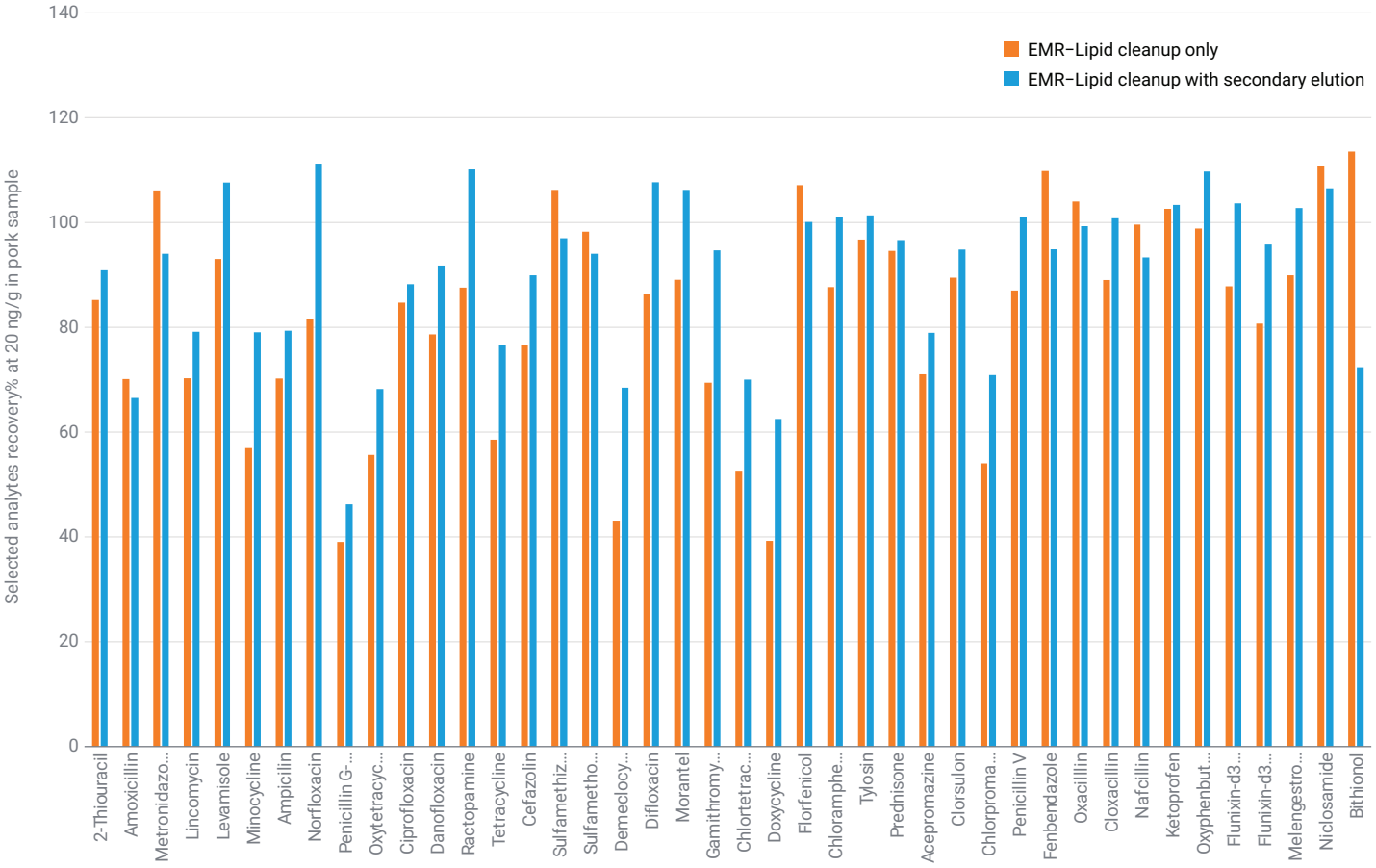

Figure 3-4. The improvement of using an additional elution step on Agilent Captiva EMR–Lipid cartridges for sensitive vet drugs.

3.4 Performance review

The method provided acceptable recoveries and reproducibility for multiclass multiresidue vet drugs (~ 200 targets) in fatty food matrices using Captiva EMR–Lipid cleanup, including muscle meats, edible offal, milk, and eggs. The average recoveries of each vet drugs class were within 60 to 120% recovery range, with RSD < 20% for over 95% of targets, in various fatty food matrices. Due to the challenges with tetracyclines, the average recoveries for this class of drugs were slightly below 60% but still with acceptable RSD < 20%.

For large panel vet drugs analysis in animal-origin matrices, the method delivered a high pass rate in multiple fatty food matrices. Considering the acceptance window for average recovery is between 60 and 130% with RSD < 20%, a greater than 95% pass rate was achieved in muscle meat, 93% pass rate in milk, 94% pass rate in eggs, and 90% pass rate in edible offal. Figure 3-5 shows the average recovery range for various classes of vet drugs in meat. Figure 3-6 shows the average recovery range for 193 vet drugs in typical meat matrices.

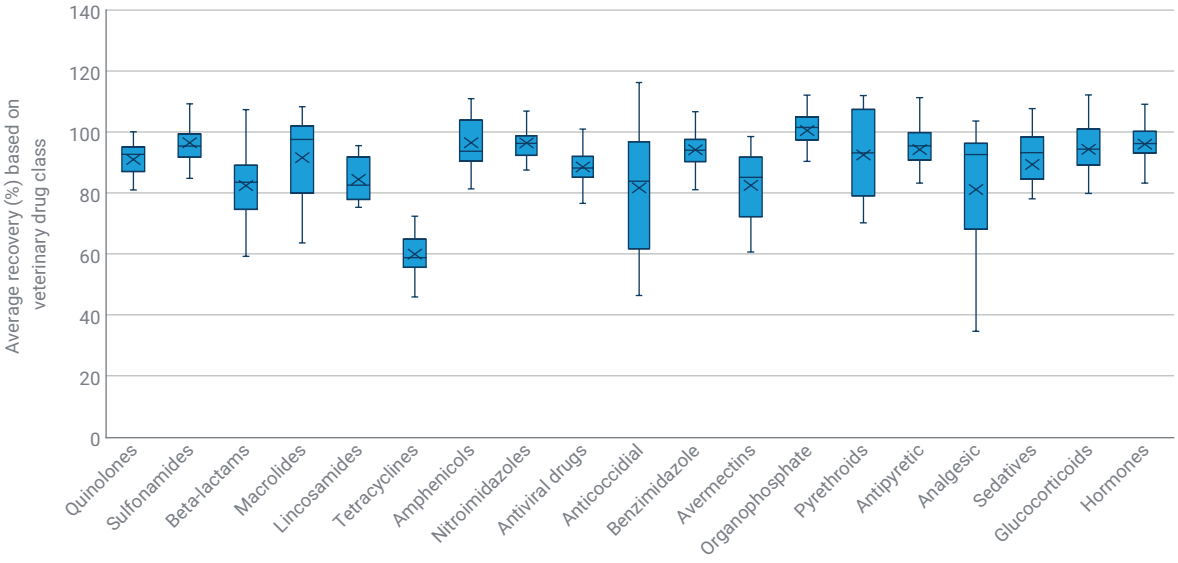

Figure 3-5. Average recovery ranges for each veterinary drug class from meat matrix.

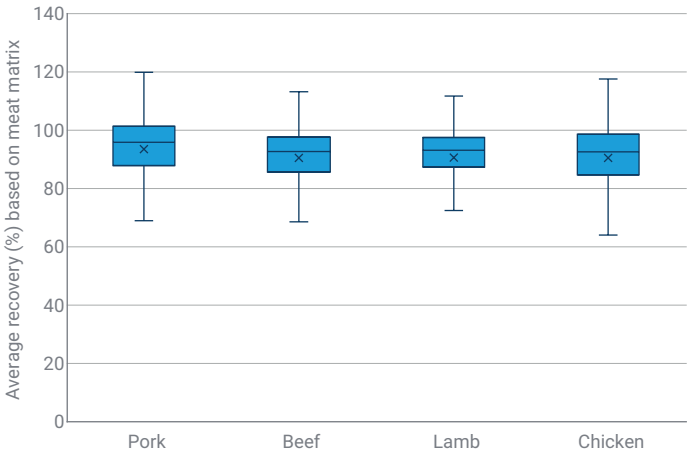

Figure 3-6. Recovery ranges for 193 veterinary drugs in four types of muscle meat.

Compared to other matrix cleanup methods after QuEChERS extraction, which apply either a typical commercial SPE cartridge or a special dSPE adjusted for vet drugs analysis, the EMR–Lipid passthrough cleanup demonstrated improved targets recoveries. Figure 3-7 shows the comparison of representative vet drugs recoveries using Captiva EMR–Lipid cleanup versus a competitor's SPE product that targeted for vet drug analysis. Figure 3-8 shows the comparison of 58 glucocorticoids recoveries using Captiva EMR–Lipid cleanup versus a special adjusted dSPE kit. The improvement of vet drug target recoveries using Captiva EMR–Lipid cleanup is attributed to the high selectivity of the sorbent interaction mechanism, resulting in efficient fatty matrix cleanup without compromising vet drug recovery.

EMR–Lipid passthrough cleanup provides significantly improved matrix cleanup efficiency and reduces the introduction of matrix co-extractives to the detection flow path. As a result, it increases overall method robustness for long-term use and reduces instrument downtime for maintenance and troubleshooting. Figure 3-9 shows a study that demonstrates excellent method robustness over 400 continuous injections of food samples prepared by Captiva EMR–Lipid passthrough cleanup.

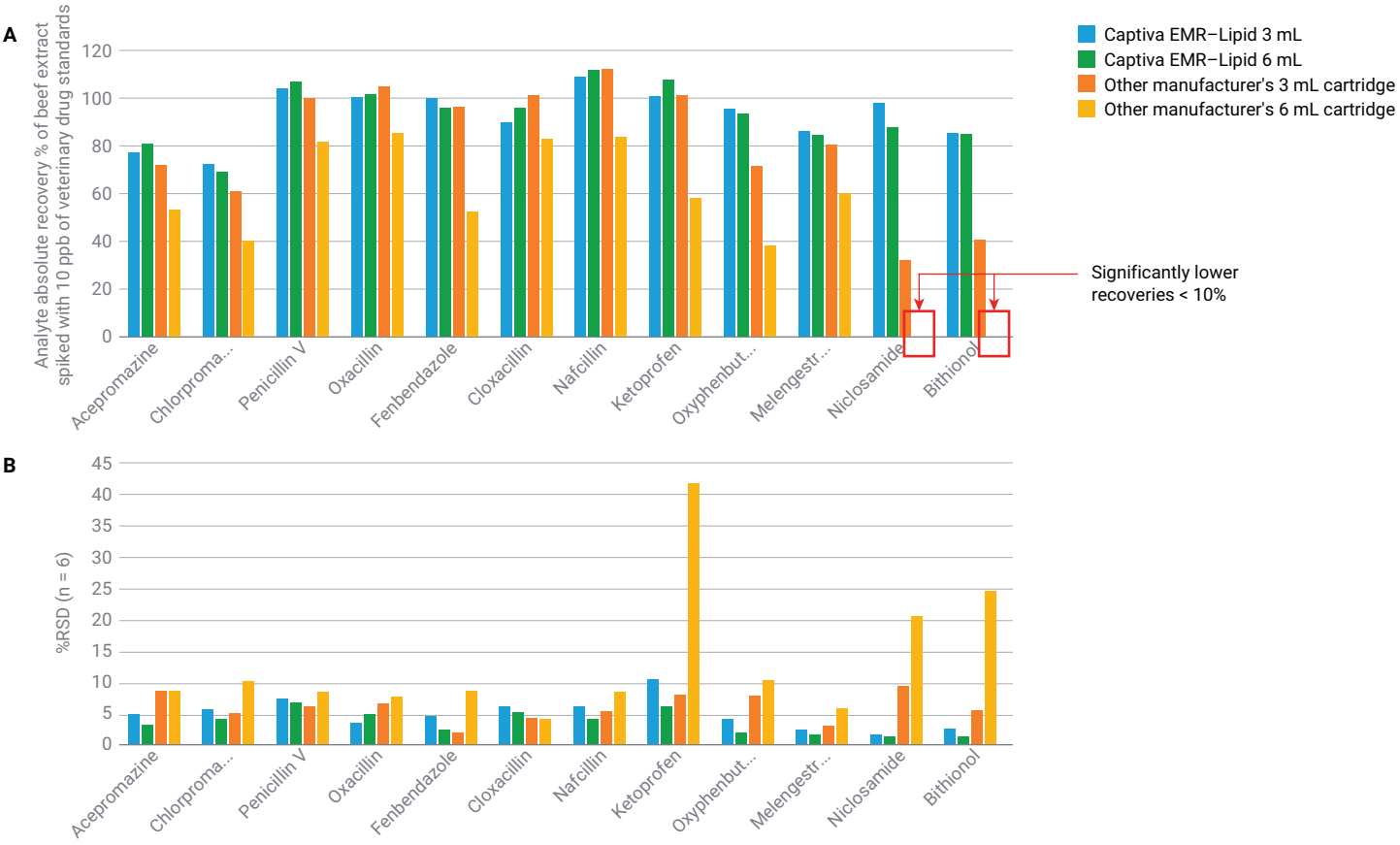

Figure 3-7. Comparison of hydrophobic veterinary drugs (A) recovery and (B) reproducibility using Agilent Captiva EMR–Lipid passthrough cleanup versus a competitive SPE cartridge cleanup.

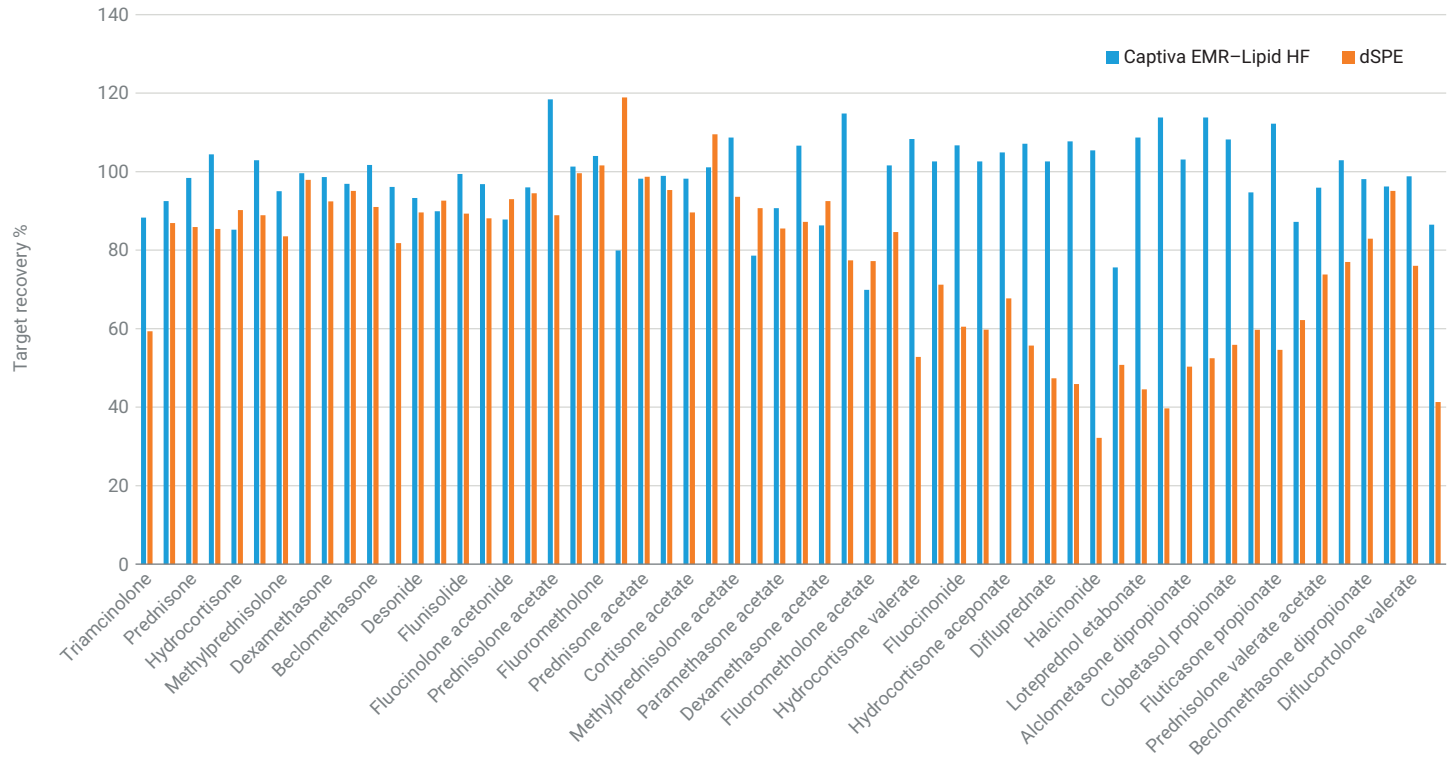

Figure 3-8. Comparison of vet drugs (58 glucocorticoids) recoveries using Agilent Captiva EMR–Lipid HF passthrough cleanup versus traditional dSPE cleanup.

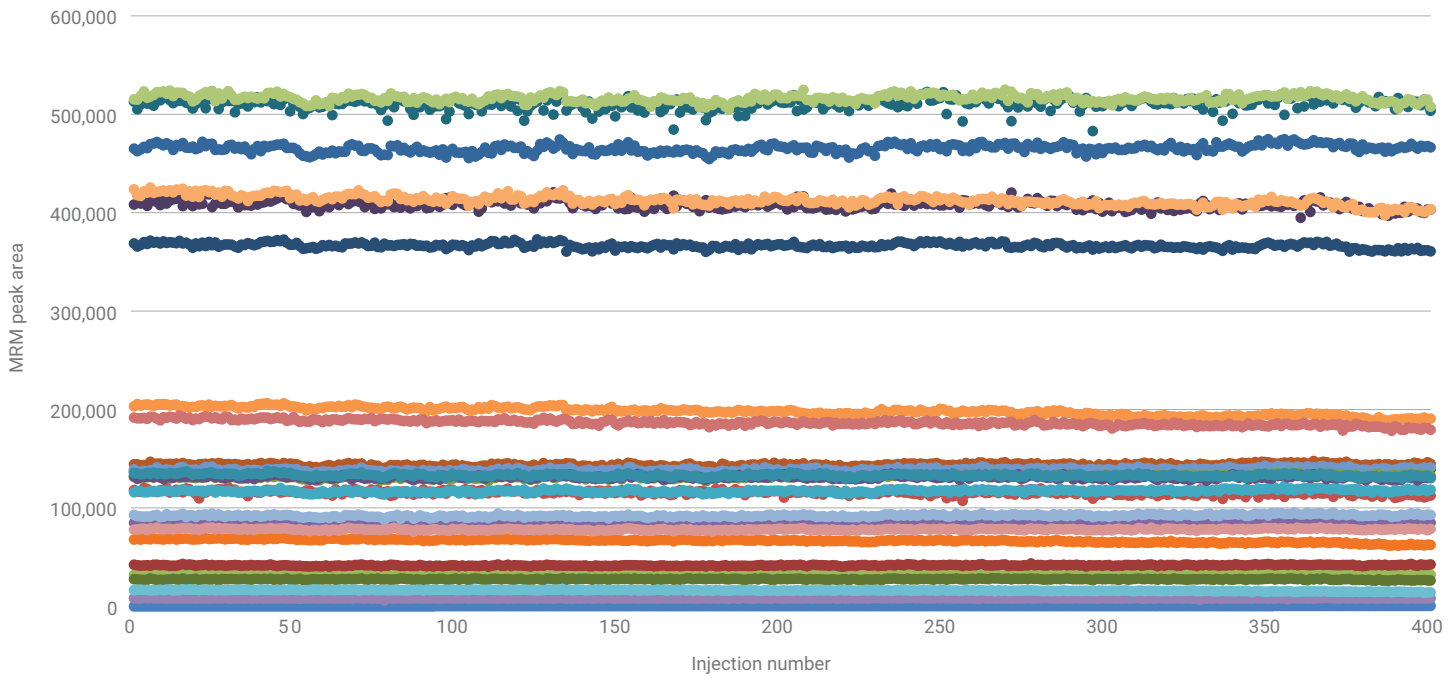

Figure 3-9. Method robustness for 25 representative vet drugs responses over 400 injections of milk samples prepared by Agilent Captiva EMR–Lipid passthrough cleanup after solvent extraction.

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Multiclass Multiresidue PFAS Analysis in Food, Feed, and Other Complex Matrices

4.1 Introduction of application

Determination of PFAS residues in food has become an issue of growing concern, attracting increasing attention in recent years. In April 2023, the European Commission implemented regulations targeting four specific PFAS compounds—PFOS, PFOA, PFNA, and PFHxS—in food categories such as eggs, fish, seafood, meat, and offal.[1] Later, in November 2023, AOAC International released the SMPR 2023.003, establishing performance requirements for the analysis of 30 PFAS compounds across a broad range of food matrices, including produce, beverages, dairy products, eggs, seafood, meat products, and animal feed.[2]

LC/MS/MS-based methods for PFAS analysis have been widely applied in the analysis of environmental water and soil.[3,4] The acidic functional groups presented in PFAS compounds allow for efficient ionization in negative ion mode, offering significant advantages in terms of method sensitivity and selectivity.

In food analysis, sample preparation plays a critical role in ensuring efficient PFAS extraction and effective removal of matrix co-extractives. The wide variety and high complexity of food matrices pose significant challenges—not only in terms of extraction and matrix cleanup efficiency, but also in achieving overall method simplicity, processing efficiency, and adaptability across different sample types. Weak anion exchange (WAX) sorbent-based solid phase extraction (SPE) methods have been widely used for PFAS analysis in environmental samples such as aqueous, solid, tissue, and biosolid. However, applying SPE to complex solid matrices is more challenging, as these samples must first be extracted before being loaded onto the SPE cartridge. Additionally, the typical SPE workflow—which includes conditioning, equilibration, loading, washing, and elution—is particularly time consuming and solvent consumption intensive. For complex solid matrices such as biological tissue and biosolid, method performance can be compromised due to inefficient matrix cleanup.

QuEChERS extraction followed by conventional dispersive SPE (dSPE) cleanup has been reported for PFAS sample preparation in food.[5,6] However, dSPE alone often fails to provide sufficient matrix removal for many food types, making it difficult to meet the lower limits of quantitation (LOQs) required in food analysis. As a result, an additional cleanup step using WAX SPE is typically needed following dSPE.[5] This added step increases the time and labor required, significantly reducing sample processing throughput. Moreover, such multistep cleanup procedures can lead to the high risk of PFAS loss and contamination introduction, compromising method qualification performance.

4.2 Captiva EMR PFAS Food I and II cartridges

Agilent Captiva EMR PFAS Food cartridges were specifically developed and optimized for PFAS analysis in food, offering a comprehensive mixed-mode passthrough cleanup. Two cartridge types (I and II) were designed to accommodate the wide diversity of food matrices. The EMR mixed-mode passthrough cleanup provides a simple and efficient approach for removing matrix interferences such as organic acids, pigments, fats, lipids, and other hydrophobic and hydrophilic co-extractives.

Captiva EMR PFAS Food I cartridges contain a simplified sorbent formulation and are recommended for fresh and processed fresh plant-based foods, including fruits, vegetables, baby food, juices, and beverages. These cartridges have also demonstrated excellent cleanup efficiency for relatively simple environmental solid matrices, such as soil and sediment. They are available in two formats—packed with 340 and 680 mg of blended sorbents—to support varying sample loading capacities and matrix complexities.

The 340 mg cartridges are suitable for cleanup of up to 5 mL of produce crude extract, while the 680 mg cartridges can accommodate up to 10 mL. The higher-capacity 680 mg cartridges are also recommended for the cleanup of complex environmental samples such as soil and sediment.

Captiva EMR PFAS Food II cartridges contain higher amounts of blended sorbents with a more complex formulation. These cartridges are recommended for fresh and processed foods of animal origin—such as milk, eggs, meat, fish, and infant formula—as well as certain plant-based foods like dry seeds, animal foods, and oils. These cartridges also demonstrate high cleanup efficiency for complex environmental solid matrices, including biosolids and biological tissues. The proprietary Agilent EMR–Lipid sorbent is incorporated into this cartridge, providing highly efficient and selective removal of lipids and fat from these challenging matrices. Table 4-1 summarizes the specifications of the Captiva EMR PFAS Food cartridges.

Table 4-1. Captiva EMR PFAS Food cartridges for order.

Product Name	Cartridge Tube Size	Sorbent Bed Mass	Part Number
Captiva EMR PFAS Food I	6 mL	340 mg	5610-2230
Captiva EMR PFAS Food I	6 mL	680 mg	5610-2231
Captiva EMR PFAS Food II	6 mL	750 mg	5610-2232

The selection of Captiva EMR PFAS Food cartridges is guided by the food matrix complexity and the sample loading volume, which is influenced by regulatory limits and the sensitivity of the analytical instrumentation. In addition to food applications, these cartridges have been successfully extended to environmental solid matrices such as soil, sediment, biosolid, and biological tissues. Figure 4-1 provides a selection guide for choosing the appropriate Captiva EMR PFAS Food cartridge based on matrix type and application needs.

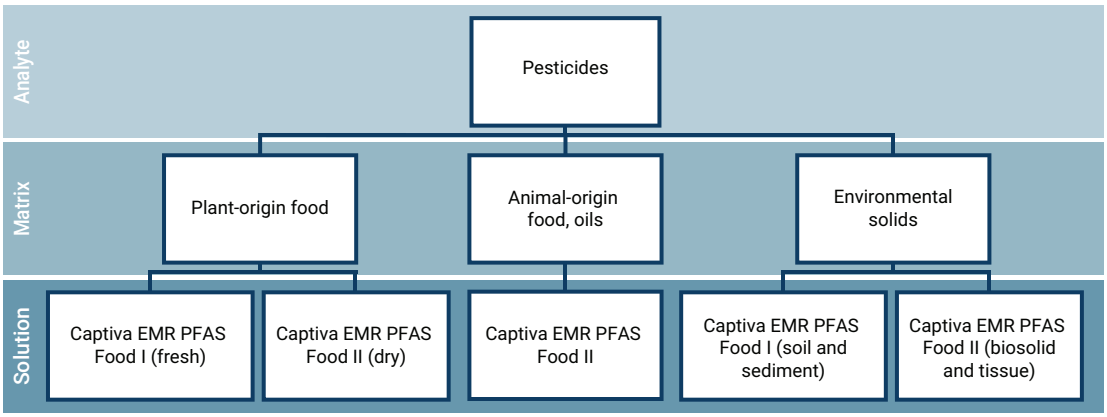

Figure 4-1. Captiva EMR PFAS Food cartridges selection guide.

4.3 Method performance evaluation and comparison

The use of EMR mixed-mode passthrough cleanup simplifies the overall sample preparation workflow by reducing the number of steps, thereby saving time, effort, and consumables. The newly developed method consists of two main steps: QuEChERS extraction followed by EMR passthrough cleanup. In contrast, the traditional method for PFAS analysis in food involves three major steps: QuEChERS extraction, dSPE cleanup, and WAX SPE extraction.[5] When processing the same number of samples, the new method reduces preparation time by 30% or more, representing a significant improvement in laboratory productivity. Figure 4-2 illustrates a comparison of the sample preparation workflows between the new and traditional methods for PFAS analysis in food.

The QuEChERS-EMR approach has also been extended to environmental solid samples such as soil, sediment, biosolids, and biological tissues—matrices for which EPA Method 1633 is currently the most widely used protocol for PFAS analysis. Compared to the conventional EPA method, the QuEChERS-EMR workflow offers more than 50% savings in preparation time and over 50% reduction in reagent and consumable usage. These efficiencies contribute to a substantial improvement in overall laboratory productivity. Table 4-2 provides a side-by-side comparison of procedure time, reagent consumption, and consumables required for the traditional EPA Method 1633 versus the QuEChERS-EMR method.

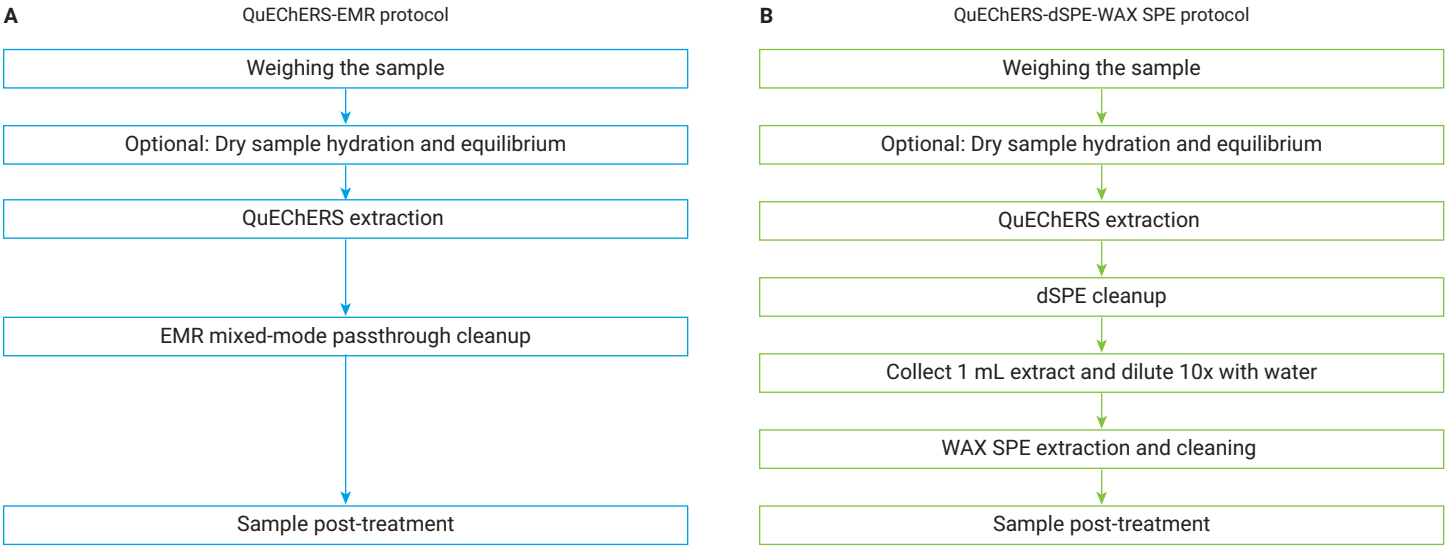

Figure 4-2. Sample preparation methods procedure comparison for PFAS in food analysis using (A) the new method versus (B) the traditional method.

Table 4-2. Comparing time, organic solvent, and consumables needed to prepare one sample across three QuEChERS-EMR methods versus standard EPA Method 1633 protocols for biological tissue sample preparation for PFAS analysis.

	QuEChERS$_{ext}$-EMR Method				Solvent$_{ext}$-Carbon/WAX SPE Method				Solvent$_{ext}$-Carbon dSPE-WAX SPE Method			
Preparation Main Phases	Main Steps	Time (hr)	Solvent Used (mL)	Consumables	Main Steps	Time (hr)	Solvent Used (mL)	Consumables	Main Steps	Time (hr)	Solvent Used (mL)	Consumables
Prework	Make two reagents	0.25			Make six reagents, pack glass wool in SPE cartridge	1		Glass wool	Make six reagents, pack glass wool in SPE cartridge	1		Glass wool
Sample Extraction	One-step QuEChERS extraction	0.75	10	50 mL Tube (1), Extraction salt (1), CHs (2)	Three-step solvent extraction and digestion	17.5	25	15 mL Tube (1), 50 mL Tube (1)	Three-step solvent extraction and digestion, carbon dSPE cleanup	18	25	15 mL Tube (1), 50 mL Tube (2), Carbon material (10 mg)
Transition Step	Dilution with 10% water	0.25		5 mL Tube (1)	Drying and redissolving, pH check and adjustment	1.25		pH paper (1-2)	Drying and redissolving, pH check and adjustment	1.25		pH paper (1–2)
Sample Further Extraction or Cleanup	EMR passthrough cleanup	0.25		EMR cartridge (1), 15 mL Tube (1)	Carbon/WAX SPE extraction and cleanup	2	25	Carbon/WAX SPE cartridge (1), Loading 50 mL tube (1), Connection fitting (1), 15 mL tube (1)	WAX SPE extraction and cleanup	1.25	25	WAX SPE cartridge (1), Loading 50 mL tube (1), Connection fitting (1), 15 mL tube (1)
Sample Post-Treatment	NIS post-spike	0.25		2 mL PP vial (1)	Neutralization sample, NIS post-spike, sample filtering	0.5		2 mL PP vial (1), 5 mL Syringe (1), Syringe filter (1)	Neutralization sample, NIS post spike, sample filtering	0.5		2 mL PP vial (1), 5 mL Syringe (1), Syringe filter (1)
Total (Per Sample)		1.75	10			22.25	50			22	50	

The passthrough cleanup using Captiva EMR PFAS Food cartridges is simple and efficient. The crude extract obtained after QuEChERS extraction can be used directly for cleanup, with no need for a solvent exchange to accommodate cartridge loading. For Captiva EMR PFAS Food I cartridges, the upper ACN layer from the extraction is loaded directly onto the cartridge and allowed to elute by gravity. For Captiva EMR PFAS Food II cartridges, the crude ACN extract must first be premixed with 10% water.

The EMR mixed-mode passthrough cleanup using Captiva EMR PFAS Food cartridges significantly enhances matrix cleanup efficiency, as evidenced by a cleaner chromatographic background compared to traditional cleanup approaches. Figure 4-3 presents a comparison of chromatographic background profiles for plant-based baby food, showing over 50% matrix reduction when using Captiva EMR PFAS Food I for cleanup. Figure 4-4 further highlights the superior matrix removal performance for complex food matrices such as infant formula and eggs. The use of Captiva EMR PFAS Food II achieved over 90% matrix cleanliness compared to conventional methods. Additionally, Figure 4-5 demonstrates environmental complex sample matrices background reduction using GC/MS full scan monitoring. More than 85% matrix background reduction was observed for both biosolid and soil samples, enabling reliable quantitative performance for PFAS analysis in these complex environmental matrices.

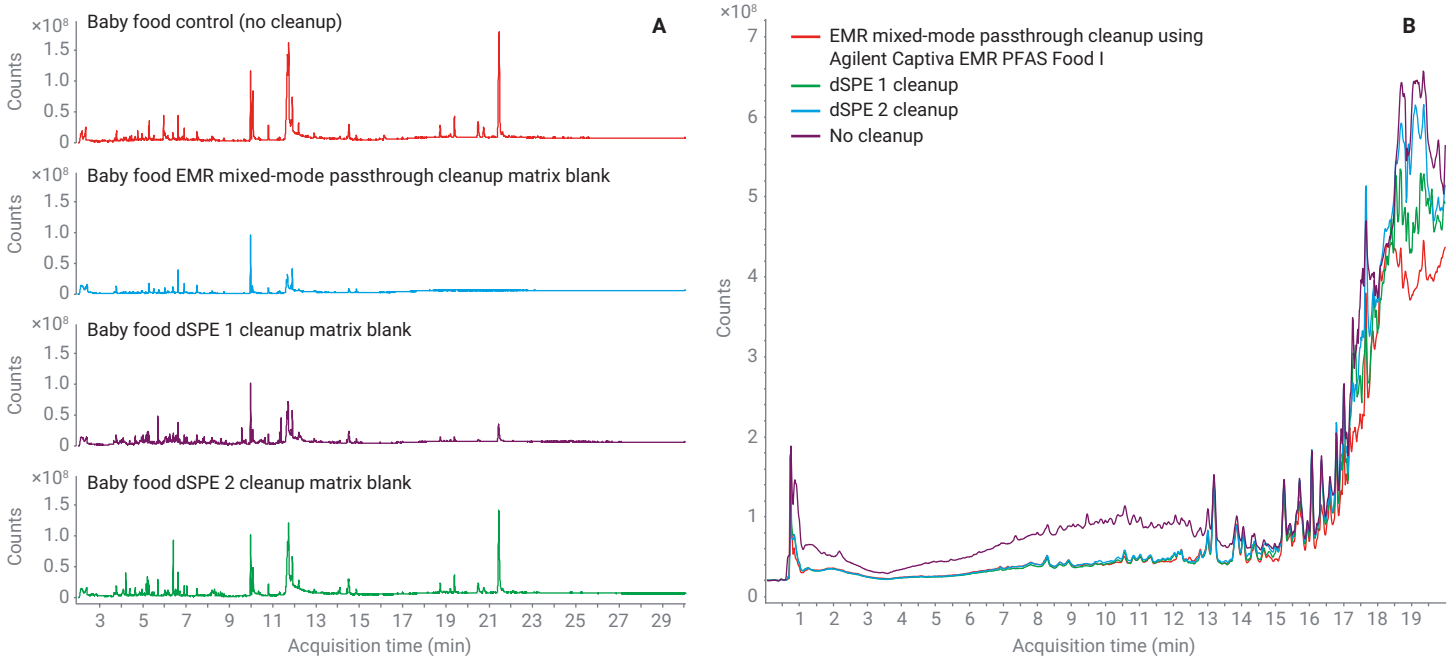

Figure 4-3. Baby food matrix removal comparison using (A) GC/MS full scan and (B) LC/Q-TOF full scan (+).

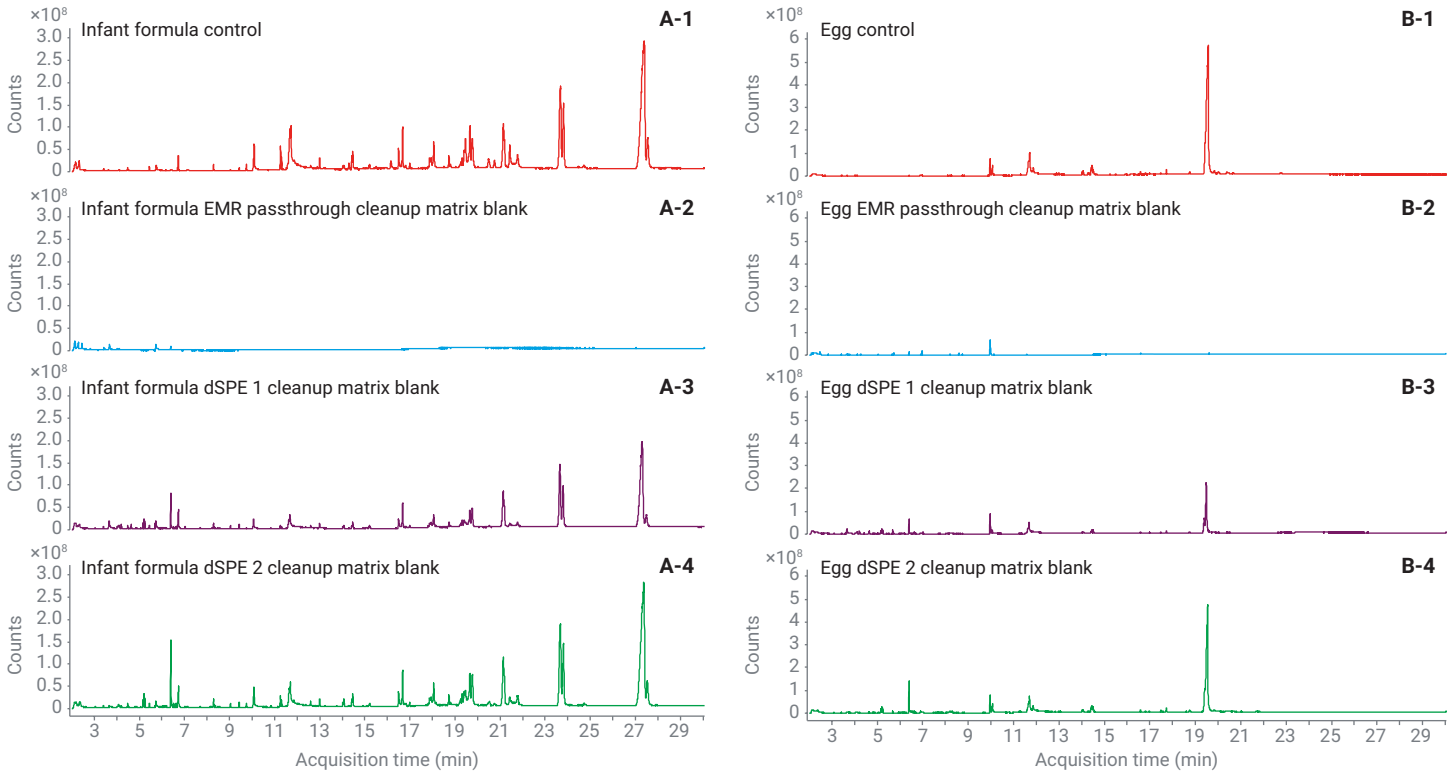

Figure 4-4. (A) Infant formula and (B) eggs matrix blank sample comparisons on GC/MS full scan chromatography background.

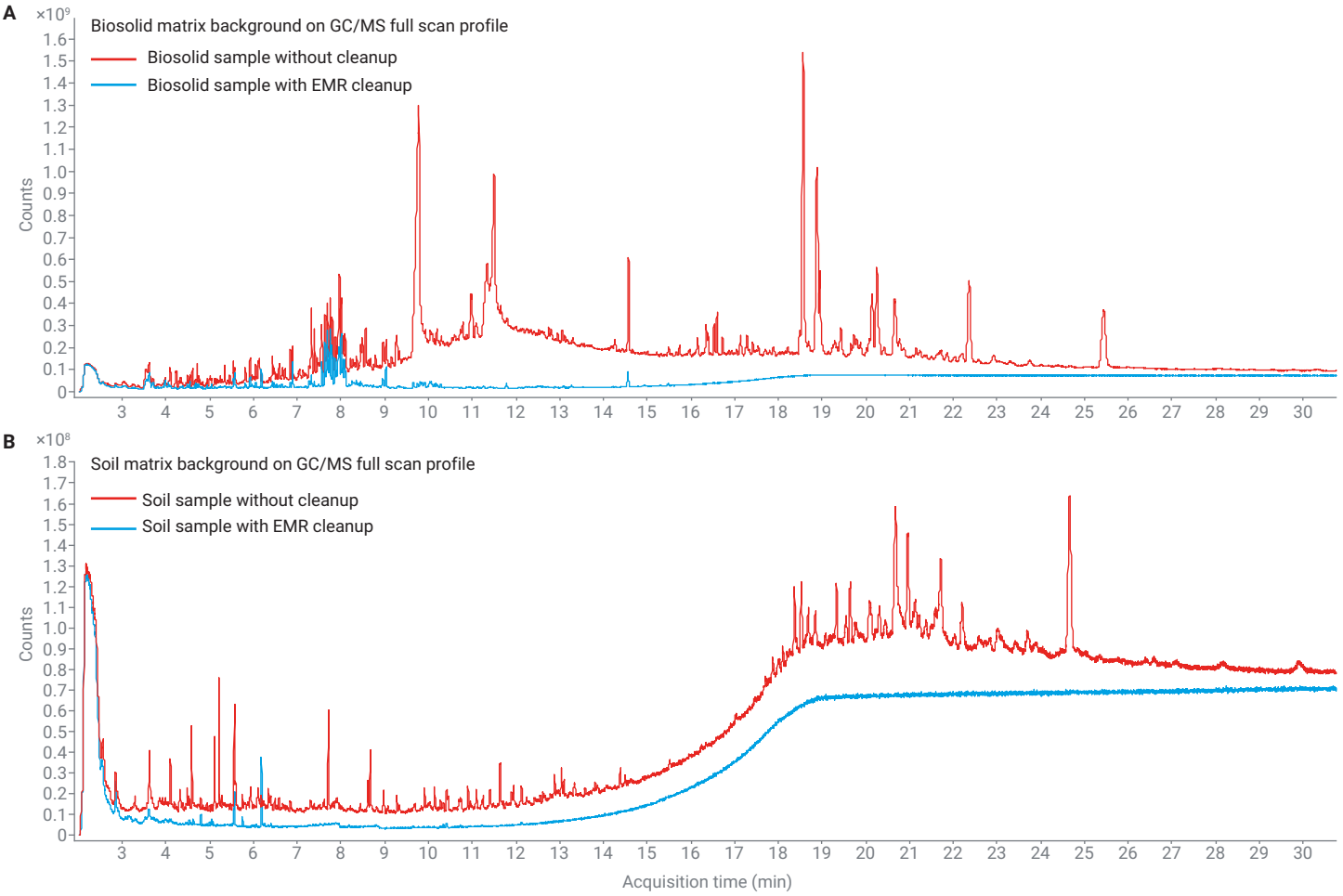

Figure 4-5. Environmental (A) biosolid and (B) soil matrix reduction using EMR passthrough cleanup, demonstrated by GC/MS full scan chromatographic background comparison on sample with (blue) EMR cleanup versus (red) without cleanup.

The recommended sample loading volume typically ranges from 3 to 5 mL, depending on the complexity of the crude extract and whether a post-concentration step is needed to achieve the desired method LOQ. In the early stages of method development, a dry-down and reconstitution step was commonly used to achieve a 5- to 10-fold concentration factor. However, with advancements in instrumentation, this time-consuming drying step has been largely eliminated—reducing sample preparation time by up to 50%.

These improvements include the use of the highly sensitive Agilent 6495D LC/TQ system, as well as injection strategies that mitigate solvent effects, such as feed injection with the Agilent 1260 II or 1290 III hybrid multisampler or a sandwiched injection program using a standard multisampler. These approaches enable direct injection of the sample eluent after EMR cleanup without compromising chromatographic performance or sensitivity. Figure 4-6 illustrates a chromatographic comparison of PFAS neat standards in 90:10 ACN/water using various injection programs.

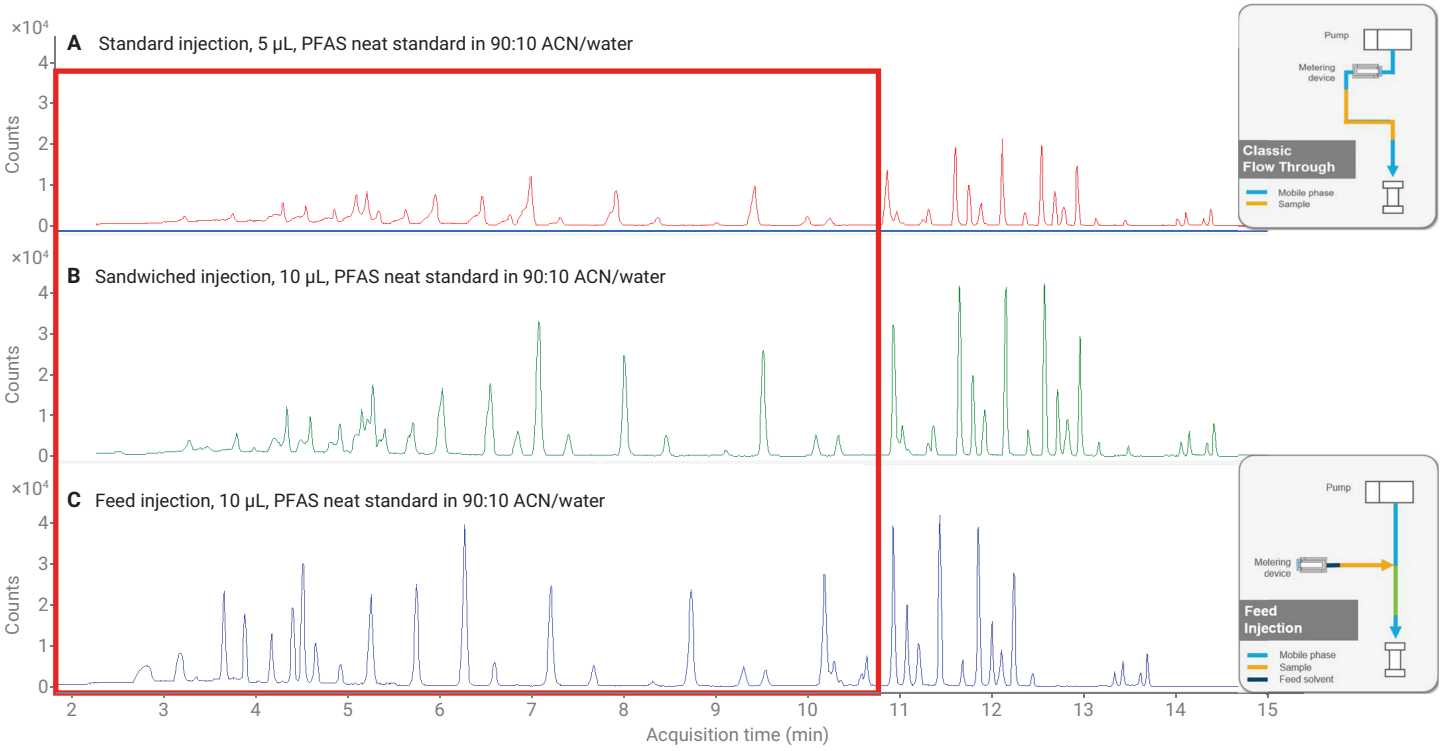

Figure 4-6. MRM chromatograms of PFAS neat standard dissolved in 90:10 ACN/water using different injection programs: (A) classic injection using a standard multisampler with 5 µL injection; (B) sandwiched injection program using a standard multisampler with 10 µL injection; (C) feed injection program using a hybrid multisampler with 10 µL injection.

It has also been observed that slightly reducing the sample loading volume (for example, from 4.5–5 mL to 3–3.5 mL) can help prevent overloading the EMR cartridge, thereby enhancing matrix removal efficiency. For highly complex matrices such as biosolids, the loading volume can be further reduced to as low as 2 mL.

The new matrix cleanup method not only significantly enhances matrix removal—particularly for complex food matrices—but also improves PFAS recovery and method reproducibility, resulting in more accurate and precise quantitation. PFAS recovery using the EMR passthrough cleanup was evaluated across representative food samples and compared with conventional dSPE cleanup. Captiva EMR PFAS Food I cartridges were used for baby food and grape extracts, while Captiva EMR PFAS Food II cartridges were applied to soybean, infant formula, tuna, and egg extracts. Figure 4-7 presents comparison results based on the average recovery of each PFAS target in each food matrix, demonstrating a marked improvement in recovery using the EMR mixed-mode passthrough cleanup compared to dSPE.

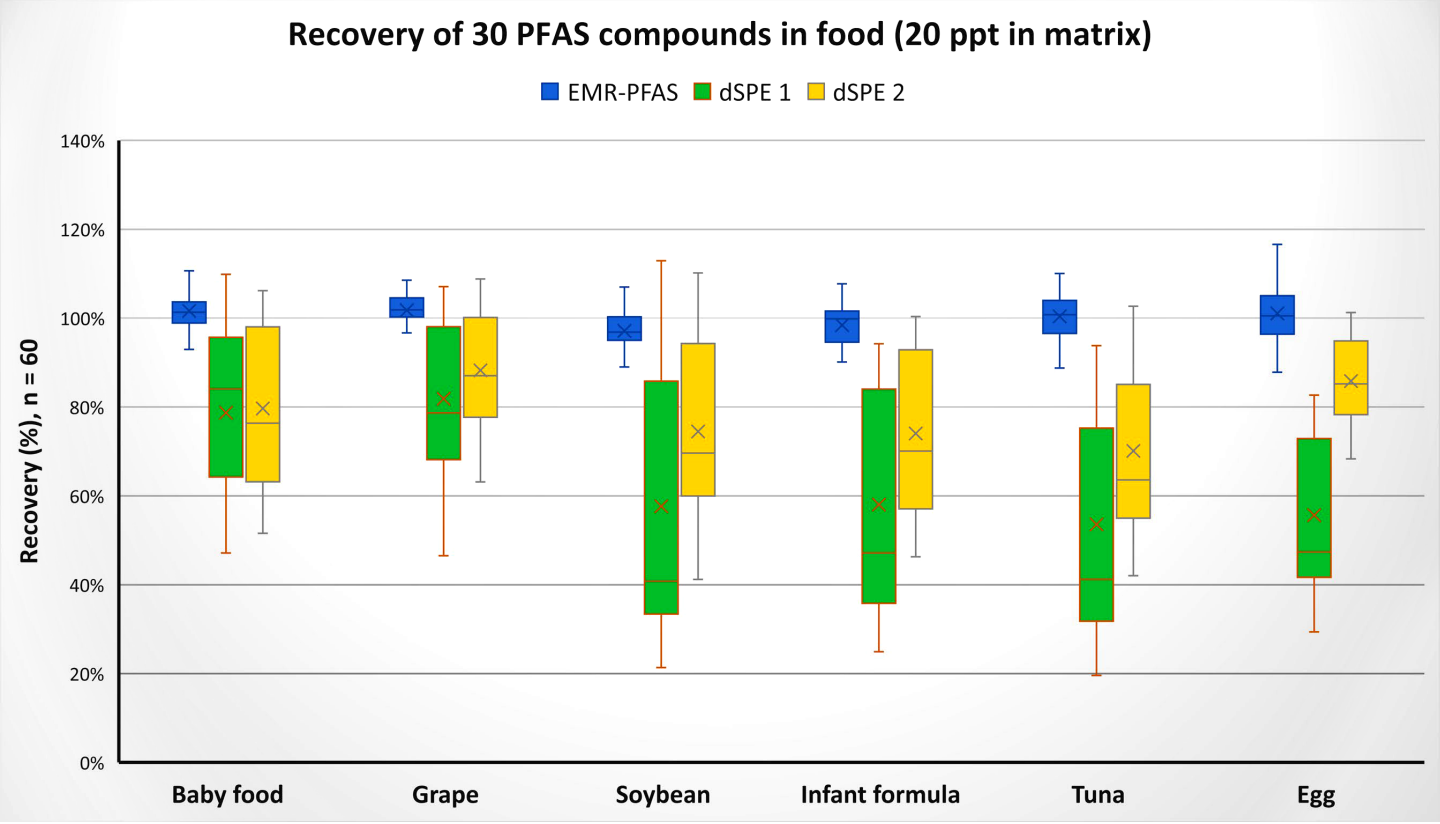

Figure 4-7. PFAS recovery comparison in food extract using multiple cleanup methods: EMR mixed-mode passthrough cleanup versus traditional dSPE cleanup.

The QuEChERS-EMR method also demonstrated improved quantitative performance—delivering better accuracy and precision—compared to the conventional EPA Method 1633, which employs WAX-based SPE cleanup. Figure 4-8 presents a comparison of recoveries for extraction internal standards (EIS) and nonextraction internal standards (NIS) between the new QuEChERS-EMR method and the traditional EPA Method 1633. The enhanced quantitation performance is attributed to high matrix removal efficiency and improved apparent recovery of analytes achieved with the QuEChERS-EMR workflow.

Captiva EMR PFAS Food cartridges can also be integrated into the conventional EPA Method 1633 workflow to enhance matrix reduction efficiency for complex solid matrices such as biosolids.[6] This provides an alternative approach for improving matrix cleanup while maintaining the core steps of EPA Method 1633, which may be required for regulatory compliance.

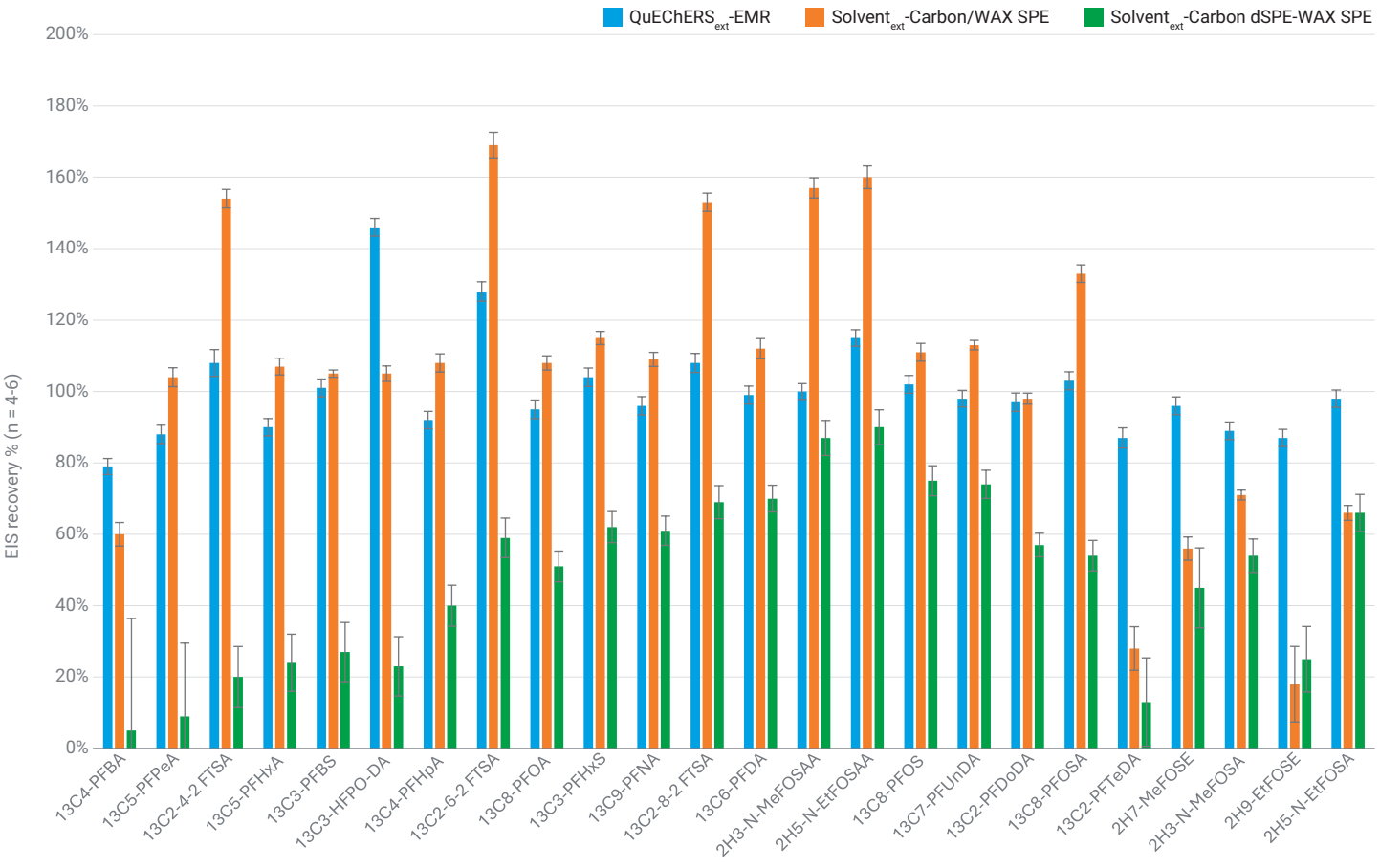

Figure 4-8. Sample preparation method comparison for EIS compounds recovery in tilapia, using QuEChERS-EMR method versus traditional EPA Method 1633 protocols.

4.4 Method validation

The new method was validated across 16 representative food matrices spanning 11 food categories outlined in the AOAC SMPR. The validation process strictly followed AOAC SMPR guidelines, including assessments of method suitability and selectivity, limit of quantitation (LOQ), quantitation accuracy (recovery), and precision (RSD). European Union regulations were also considered, particularly with respect to LOQ requirements. In addition to food matrices, the method was validated for environmental solid matrices in accordance with the quality control criteria specified in EPA Method 1633. Table 4-3 summarizes the range of applications for which the EMR method was validated. A recently published application note in Korea demonstrated the applicability of the expanded QuEChERS-EMR approach for PFAS analysis in cosmetics, where Captiva EMR PFAS Food II cartridges were applied.

Attributed to improved PFAS target recovery and enhanced matrix cleanup efficiency, the method demonstrates ultralow LOQs that meet the requirements of both the AOAC SMPR guidelines and EU regulations—except in cases where LOQ determination was impacted by positive detections in food matrix blanks. Figure 4-9 shows matrix blanks for baby food and milk, along with the validated LOQs for four critical PFAS targets. Samples were prepared using the validated method with Captiva EMR PFAS Food I cartridges (for baby food) and EMR PFAS Food II cartridges (for milk). The validated LOQs for 30 PFAS analytes across 16 food matrices met the acceptance criteria for their respective food categories, with a failure rate of less than 1%, primarily due to matrix-related positive detections or interferences.

The validated method demonstrated acceptable quantitation accuracy (65 to 135% recovery) and precision (≤ 20% RSD) across all 16 food matrices, with a failure rate of less than 1%. These results confirm the excellent quantitation performance of the entire workflow for PFAS determination in complex food matrices.

Table 4-3. Summary of validated method for PFAS analysis in food and environmental matrices.

Sample Matrix Category	Quantitation Guideline	Represented Matrices for Method Validation	Application Notes
Produce	AOAC SMPR 2023.003	Grape, mushroom, carrot, tomato, lettuce, orange juice	5994-7369EN
Foods for Infants and Young Children		Baby food	5994-7367EN
Milk (Liquid)		Whole milk	5994-7366EN
Eggs		Eggs	
Fish Meat and Meat of Terrestrial Animals		Canned tuna, beef	5994-7368EN
Seafood (Crustaceans And Mollusks)		Shrimp	
Edible Offal of Terrestrial Animals		Bovine kidney	5994-7370EN
Pet Food and Animal Feed		Dry soybean	5994-7371EN
Coffee		Coffee powder	5994-8610EN
Fish Oil		Fish oil	
Dairy Powders and Plant-Based Protein Powders		Protein powder	
Alcoholic Drink		Beers and wines	5994-8813EN
Environmental Solid	EPA Method 1633 quality control	Soil	5994-8778EN
Biological Tissue		Tilapia	5994-8232EN
Environmental Biosolid		Biosolid sludge	5994-8777EN

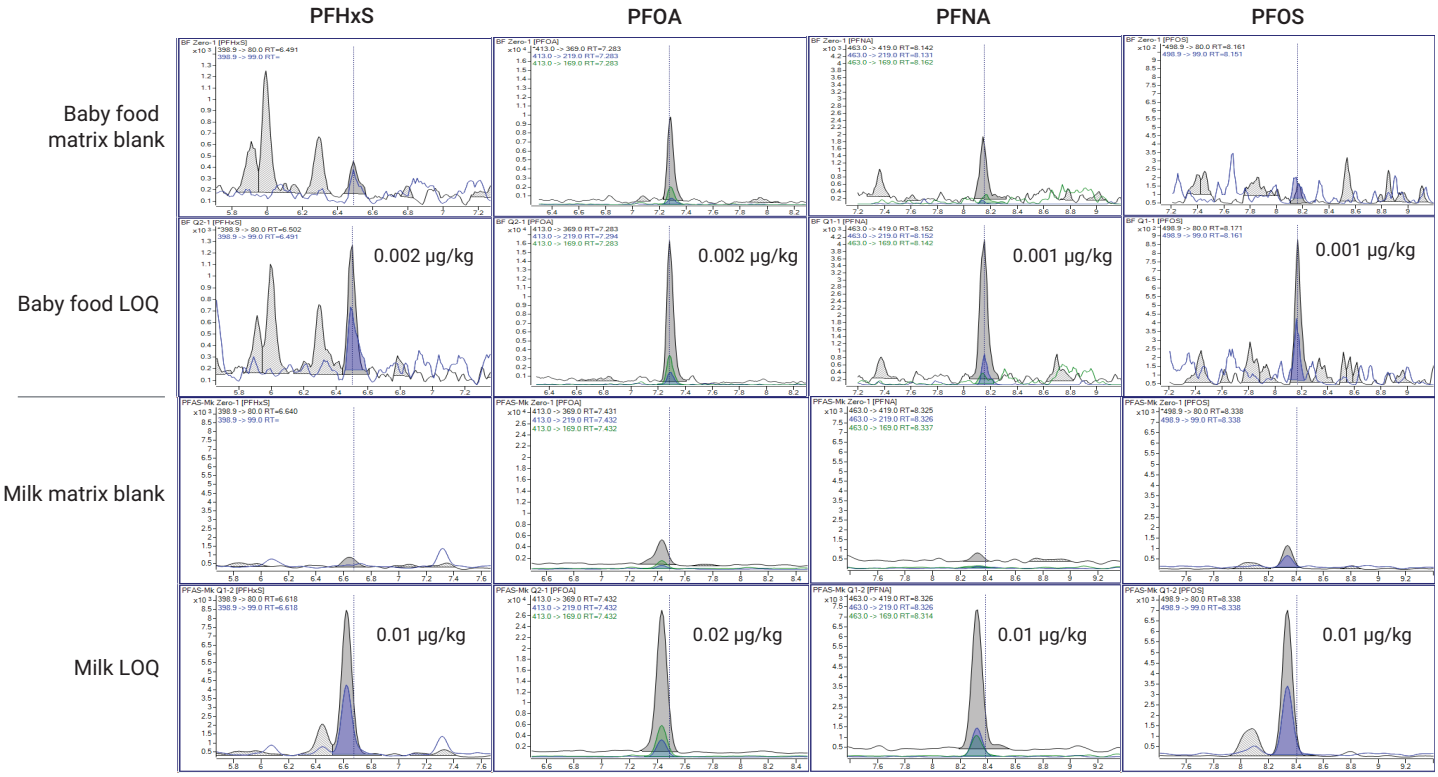

Figure 4-9. Baby food and milk matrix blank and spiking LOQ chromatograms for the four critical PFAS targets, PFHxS, PFOA, PFNA, and PFOS.

References

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6. Giardina, M. The Determination of 40 Per- and Polyfluoroalkyl Substances in Biosolids: Using Dual-phase Blended Bond Elut PFAS WAX/Carbon S Solid Phase Extraction Cartridges Followed by Post-Extraction Matrix Reduction with Captiva EMR PFAS II. *Agilent Technologies application note*, publication number 5994-8753EN, **2025**.

Multiclass Multiresidue Mycotoxins Analysis in Food and Feed

5.1 Introduction of application

Mycotoxins are toxic metabolites generated by certain fungi that can grow on many agricultural commodities in the field and during processing, storage, and transportation. Consumption of foods or feeds contaminated with mycotoxins can cause public health concerns.[1] Many regulatory agencies have established maximum residue limits (MRLs) for mycotoxins in foods and feeds to closely monitor and control dietary exposure.[2,3]

Common mycotoxins include aflatoxins, fumonisins, deoxynvalenol and metabolites, HT-2 and T-2 toxins, ochratoxin A, zearalenone, and patulin. Among these classes of mycotoxins, aflatoxins are a group of compounds with planar structural features, which can be sensitive to the use of carbon material during sample preparation. Fumonisins are a group of acidic compounds containing four carboxylic acid groups, which are highly sensitive to the use of acid scavenger sorbents such as primary-secondary amines (PSA). Deoxynvalenol is highly polar and typically shows chromatographic challenges. Ochratoxin A also has planar features, which can be sensitive to carbon material use. Even with just 20 to 40 mycotoxins as typical targets, mycotoxins analysis presents significant challenges for analytical methods.

A variety of detection techniques have been developed for mycotoxins analysis, including enzyme-linked immunosorbent assays (ELISA), LC coupled with diode array detection (DAD) or fluorescence detection (FLD), LC/MS/MS, as well as other techniques. Among various detection techniques, LC/MS/MS has been used more in recent years, which is attributed to the high sensitivity and selectivity for simultaneous detection of multiclass multiresidue mycotoxins. However, matrix effect using MS detection can be significant due to the limited sample preparation that can be used. Therefore, a large panel of isotopically labeled internal standards (ISTD) are usually used to mitigate matrix effect; this is called a stable isotopic dilution assay (SIDA).[4]

The sample matrices for mycotoxins are usually dry plant-origin or processed food and feed, including many crops, nuts, pet food, cheese, butter, and infant formula. Many regulatory agencies have established MRLs for mycotoxins in foods and feeds to closely monitor and control dietary exposure to humans and animals.[5–7] The MRL varies depending on the targeted mycotoxins and food or feed matrices, ranging from low to high ppb levels but usually with the lowest level down to single-digit ppb for aflatoxin B1 (AB1) due to its toxicity.[1]

Sample preparation methods for mycotoxins analysis include SIDA, the immunoaffinity method[8], and the QuEChERS extraction method.[9] The SIDA method applies a direct solvent/water mixture extraction without any matrix cleanup. This simple and reliable method has been widely used in many mycotoxins testing labs. However, the method relies heavily on the use of isotopic ISTDs for sample matrix correction and instrument detection selectivity. Complex food or feed samples are extracted without further cleanup of matrix co-extractives, which can cause significant flow path and MS source contamination and carryover issues. Immunoaffinity columns have also widely been used as a cleanup method after sample extraction, providing highly efficient and selective cleanup. However, the method is based on class-specific antibody use with a specific mycotoxin class. Therefore, the method can be limited to a single or a few classes of mycotoxins. QuEChERS extraction followed by dispersive SPE (dSPE) cleanup has been used in food sample preparation, providing the advantages of method simplicity and extraction efficiency. However, the dSPE cleanup may not provide efficient matrix cleanup, and it may also cause the loss of sensitive mycotoxins such as fumonisins and aflatoxins.

5.2 Captiva EMR product recommendations

Given the sensitive classes of mycotoxins such as aflatoxins and fumonisins, the multiclass multiresidue analysis for mycotoxins can be highly sensitive to carbon material and PSA sorbents. Therefore, it is not recommended to use Captiva EMR products with a high percentage of Carbon S and PSA sorbent for mycotoxins analysis, including all Captiva EMR with Carbon S cartridges. Captiva EMR–Lipid cartridges fit well for fatty food matrices such as infant formula, cheese, butter, and oils. Captiva EMR Mycotoxins cartridges were developed with a formula optimized for complex dry plant-origin or processed food and feed such as dry crops and feed, herbals, and pet food. Captiva EMR–Lipid HF was introduced later, but given the demonstrated equivalence of this product with Captiva EMR–Lipid products, Captiva EMR–Lipid HF can also be applied to mycotoxins analysis in fatty food matrices and provide the benefits of easy elution flow under gravity.

Table 5-1 shows the ordering information for Captiva EMR–Lipid cartridges, EMR–Lipid HF cartridges, and Captiva EMR Mytocoxins cartridges. Figure 5-1 shows the recommendation of EMR passthrough cleanup for mycotoxins analysis in dry food and feed matrices.

Table 5-1. Captiva EMR–Lipid, EMR–Lipid HF, and EMR Mycotoxins cartridges for order.

Product Name	Cartridge Format (mL)	Sorbent Bed Mass (mg)	Part Number
Captiva EMR–Lipid	3	300	5190-1003
Captiva EMR–Lipid	6	600	5190-1004
Captiva EMR–Lipid HF	3	300	5610-2235
Captiva EMR–Lipid HF	6	600	5610-2236
Captiva EMR Mycotoxins	3	300	5610-2233
Captiva EMR Mycotoxins	6	600	5610-2234

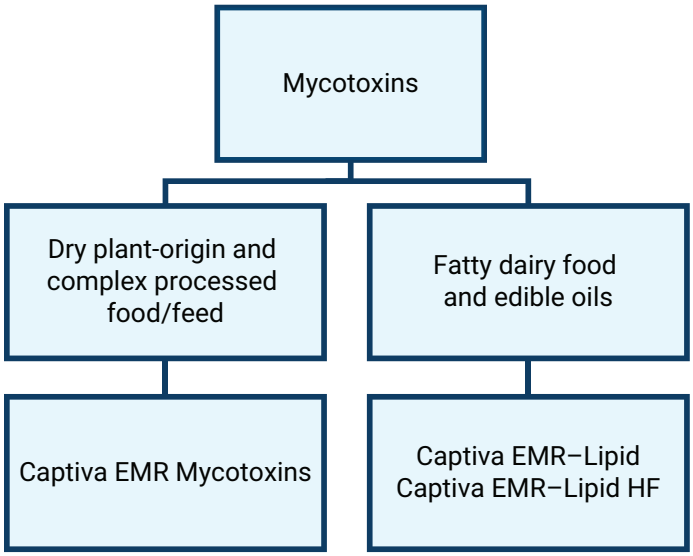

Figure 5-1. EMR passthrough cleanup for mycotoxins analysis in dry food and feed matrices.

5.3 Method development and comparison

QuEChERS extraction demonstrated to be an efficient method for sample extraction for mycotoxins. For typical dry samples, the sample size is usually 2 to 5 g. An aliquot of water or acidic buffer (7.5 to 10 mL) is added to homogenize and equilibrate the sample. The sample mixture is then extracted using acidified ACN. Compared to traditional acidic solvents, such as ACN with 1% acetic acid used in QuEChERS extraction, the stronger acid, formic acid, and higher concentration, 2%, were used to acidify ACN for mycotoxins extraction. The stronger acidic extraction preserves fumonisins from degradation and prevents loss during sample preparation. Figure 5-2 shows the comparison of using weak and strong acidified ACN for mycotoxins extraction.

After QuEChERS extraction, the crude extract is mixed with 10 to 20% water. For cleanup on Captiva EMR–Lipid or EMR–Lipid HF, 20% water is usually mixed. And, for cleanup on Captiva EMR Mycotoxins, 10% water is mixed. Since the dry matrix crude extract contains fatty co-extractives, the addition of water often results in a hazy sample mixture due to the emulsion effect. Sample loading volume is typically 2 to 2.5 mL on 3 mL cartridges, and 4 to 4.5 mL on 6 mL cartridges. Both Captiva EMR Mycotoxins and EMR–Lipid HF cartridges are designed for smooth elution flow under gravity. However, sample elution on Captiva EMR–Lipid cartridges may require the use of external force such as vacuum or positive pressure. After sample cleanup on EMR cartridges, the eluent is then diluted with water to approximately 1:1 water/organic before injection.

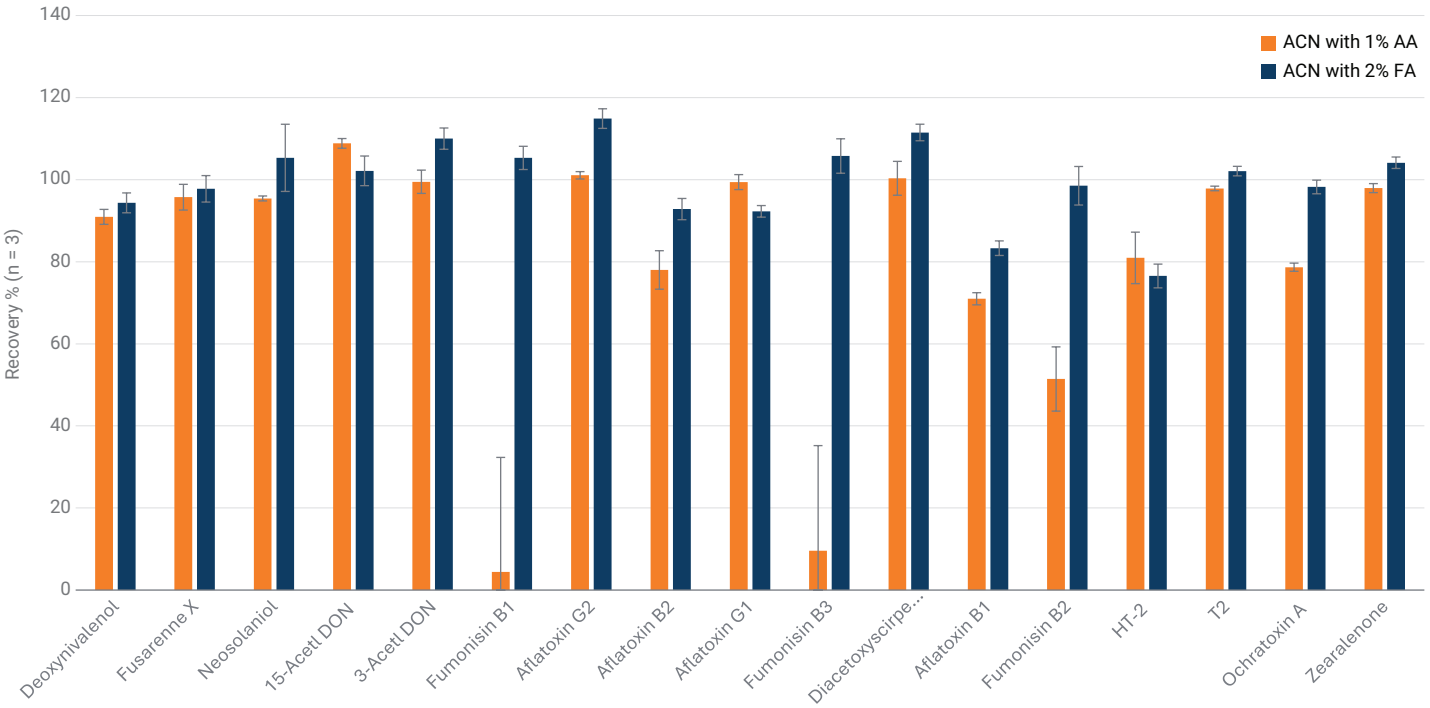

Figure 5-2. Mycotoxins recovery with QuEChERS extraction using different acidified ACN for extraction.

The entire sample preparation method offers a simplified procedure compared to other methods, which saves time and effort for sample preparation. Figure 5-3 shows the procedure comparison of EMR, SIDA, and QuEChERS plus dSPE and SPE cleanup methods.

5.4 Performance review

The method provided acceptable recoveries and repeatability for 19 mycotoxins in complex dry crops and processed feed matrices using Captiva EMR Mycotoxins cleanup. Figure 5-4 shows the statistical summary for mycotoxins recoveries in corn and pet foods, while Figure 5-5 shows the results for RSDs. The recovery was within 78 to 112%, and RSDs were below 20%.

Compared to another matrix cleanup method after QuEChERS extraction, which uses a typical commercial SPE cartridge plus special dSPE for mycotoxins analysis, the EMR mixed-mode passthrough cleanup provided a simplified matrix cleanup procedure with fewer cleanup steps (one cleanup versus two cleanups) and a simpler cleanup procedure (removing steps such as uncapping and capping of dSPE tubes, vortexing, centrifugation, and multiple sample transfers). It provides equivalent matrix removal efficiency but improved sensitivity for mycotoxins recovery including fumonisins, OTA, and CPA.

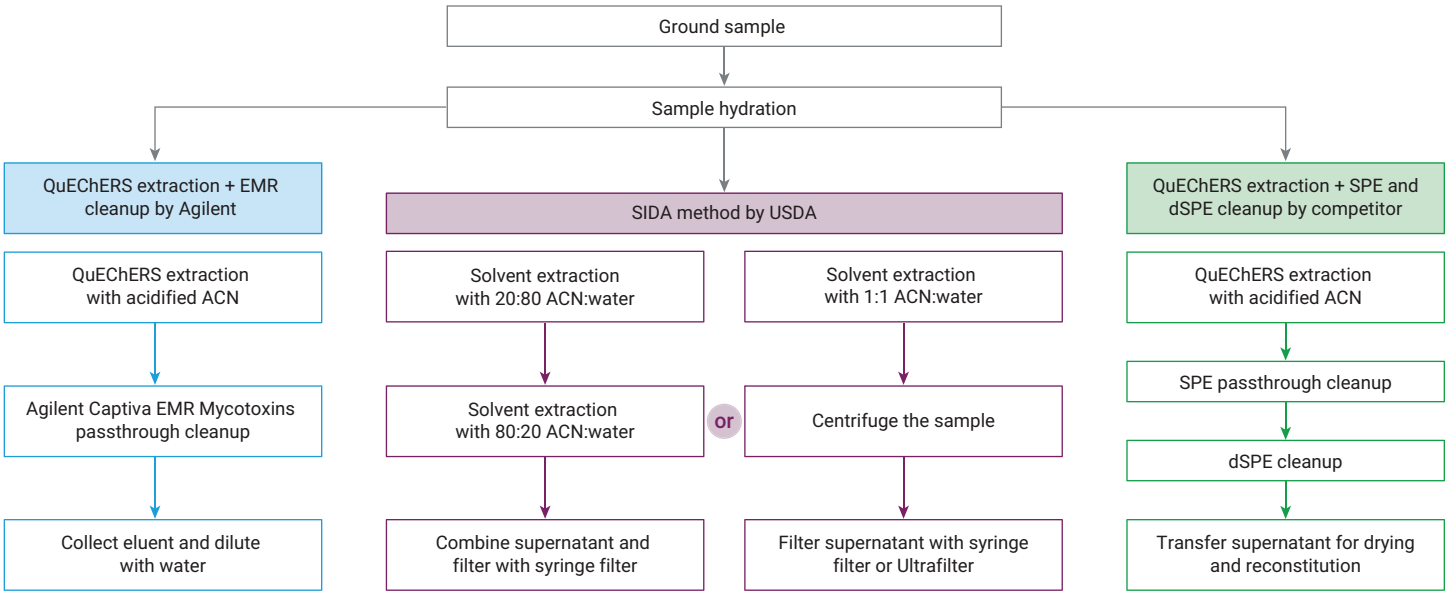

Figure 5-3. Method procedure comparison.

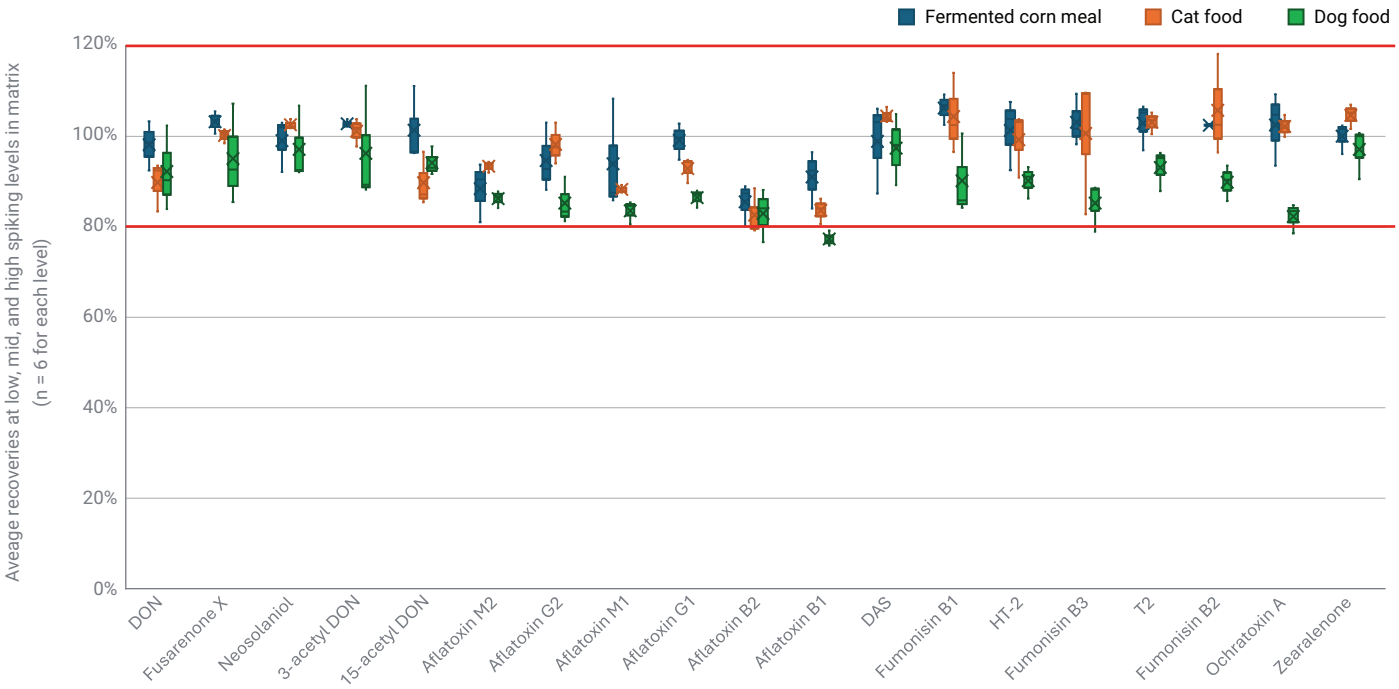

Figure 5-4. Statistical summary for the recovery of 19 mycotoxins in corn and pet food matrices.

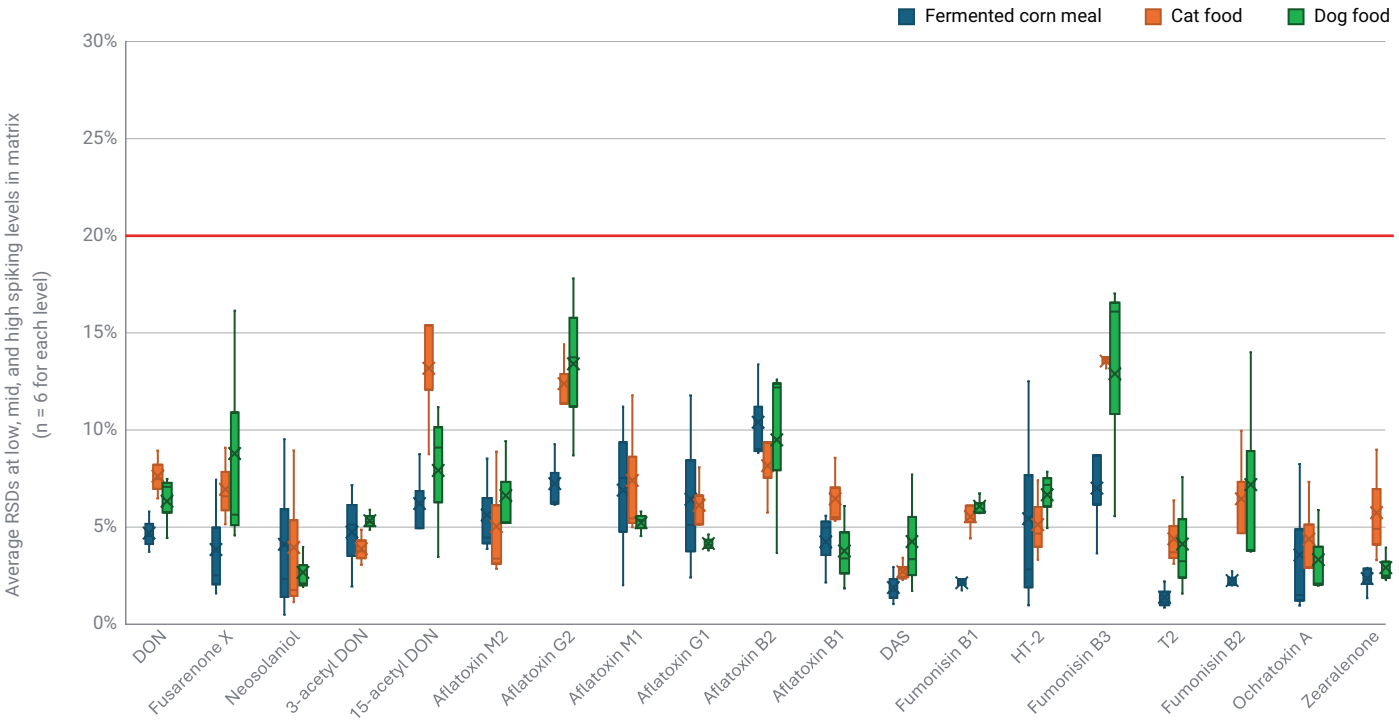

Figure 5-5. Relative standard deviations (RSDs) for 19 mycotoxins.

Figure 5-6 shows the mycotoxin recovery during post-QuEChERS extraction sample cleanup. Compared to the other method using sequential SPE plus dSPE cleanups, the EMR mixed-mode passthrough cleanup significantly improved the recovery of sensitive mycotoxins, especially for FB1, FB2, and FB3. The use of the other cleanup method caused almost complete loss of these targets, while the EMR passthrough cleanup provided > 90% recovery.

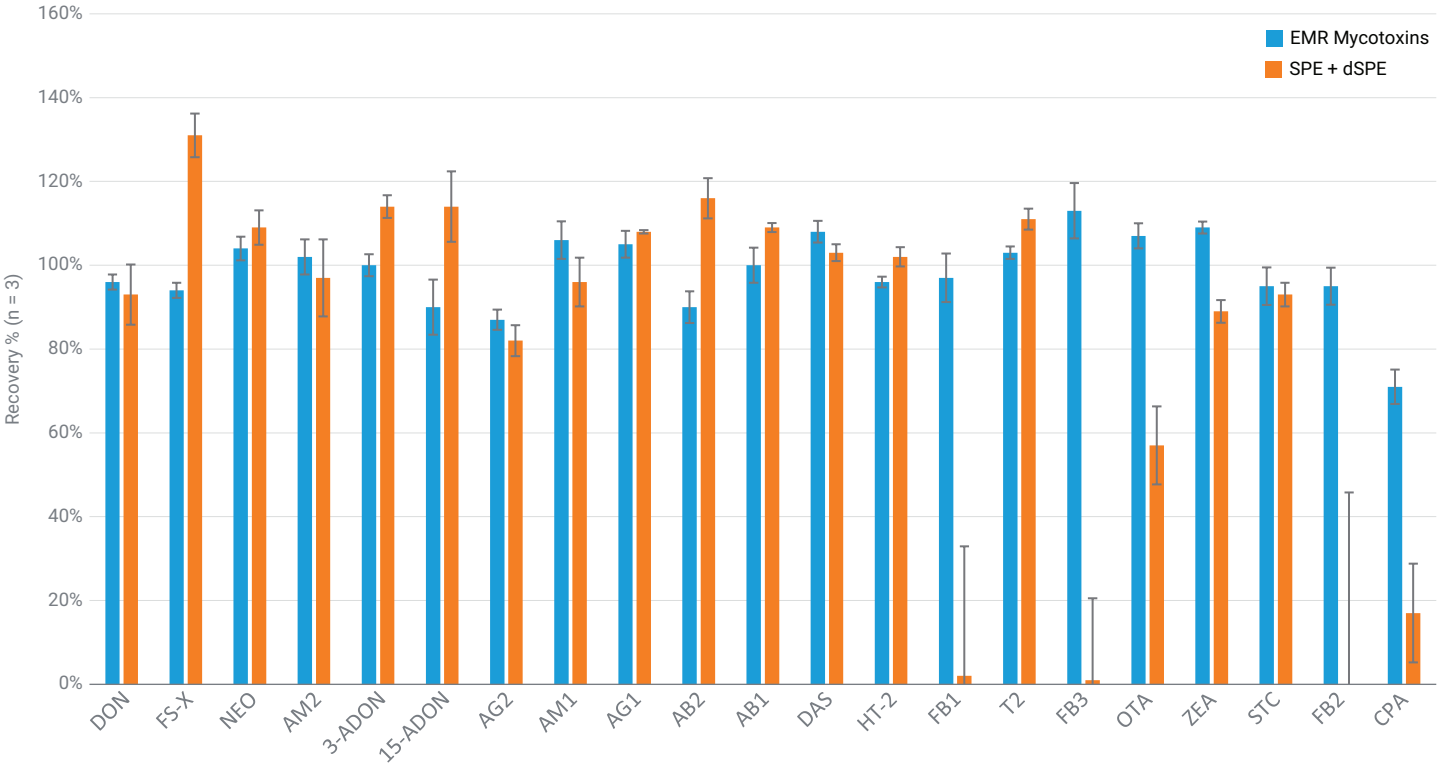

Figure 5-6. Post-QuEChERS extraction cleanup method comparison for mycotoxin recovery using (blue) Agilent Captiva EMR Mycotoxins passthrough cleanup versus (orange) SPE plus dSPE cleanup.

The QuEChERS extraction followed with EMR passthrough cleanup provided significantly improved matrix cleanup efficiency, especially compared to the SIDA method. Figure 5-7 shows the final sample extract cleanliness comparison for fermented corn matrix on (A) final sample appearance, (B) final sample dried residue, and (C) the LC/Q-TOF scan chromatography background. Results confirmed that EMR mixed-mode passthrough cleanup on Captiva EMR Mycotoxins cartridges is excellent for complex dry plant-origin and processed food and feed matrices.

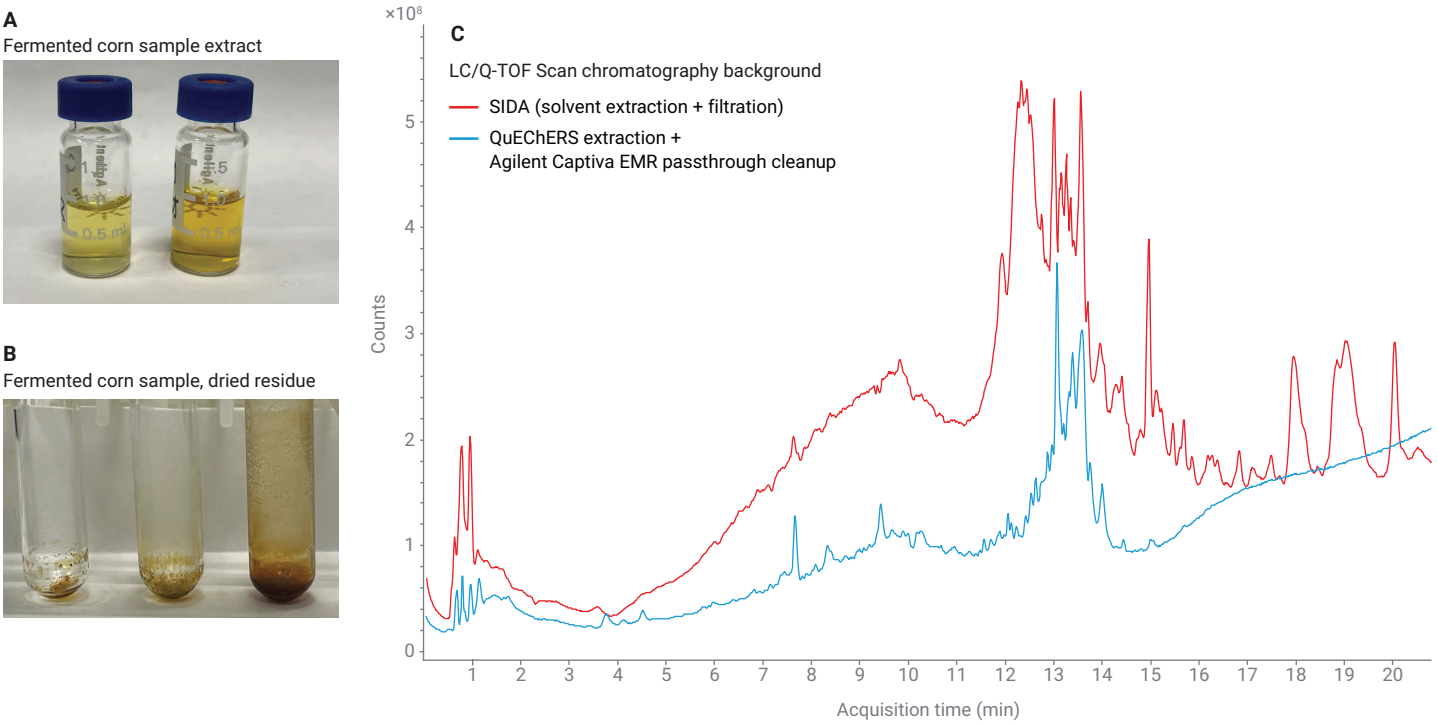

Figure 5-7. Fermented corn powder matrix cleanliness comparison between the SIDA method and a method using QuEChERS extraction followed by Agilent Captiva EMR Mycotoxins cartridge passthrough cleanup. (A) Final sample extract using the SIDA method (right) and the new method (left); (B) dried residue for sample prepared by the SIDA method (right), QuEChERS extraction (middle), and QuEChERS extraction followed with EMR passthrough cleanup (left); (C) LC/Q-TOF scan chromatography background for sample prepared by the SIDA method (red) and the EMR method (blue).

Figure 5-8 shows the GC/MS full scan chromatographic background comparison for an infant formula sample with and without Captiva EMR–Lipid passthrough cleanup, demonstrating the excellent cleaning efficiency provided by EMR passthrough cleanup on Captiva EMR–Lipid cartridges for fatty food matrix without significant complexity.

The cleaner sample extract resulted in reduced matrix effect on the mycotoxins. The reduced matrix effect is demonstrated by representative targets using the new EMR method versus the traditional SIDA method. All targets shown in Figure 5-9 use equivalent standard spiking in the sample matrix, but different sample preparation methods. The results indicate significant differences on the matrix effect of these compounds. For DON, neosolaniol, ZEA, aflatoxin M2 (AM2), and HT-2, matrix effect shows as matrix ion suppression; while for fumonisin B3 (FB3), matrix effect shows as matrix enhancement. For samples prepared using the new method, the cleaner final sample extract demonstrates higher responses for targets impacted by matrix ion suppression, and lower response for fumonisin due to less matrix enhancement.

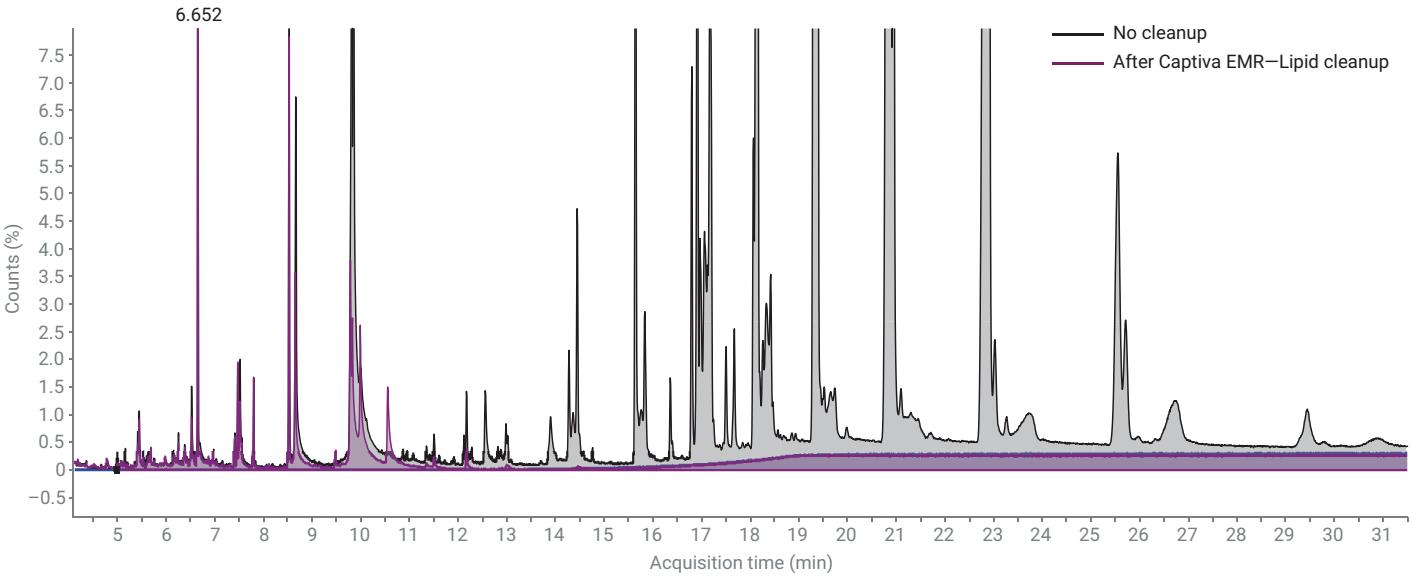

Figure 5-8. Matrix removal evaluation using GC/MS full scan chromatogram comparison of infant formula sample before and after Agilent Captiva EMR–Lipid cleanup.

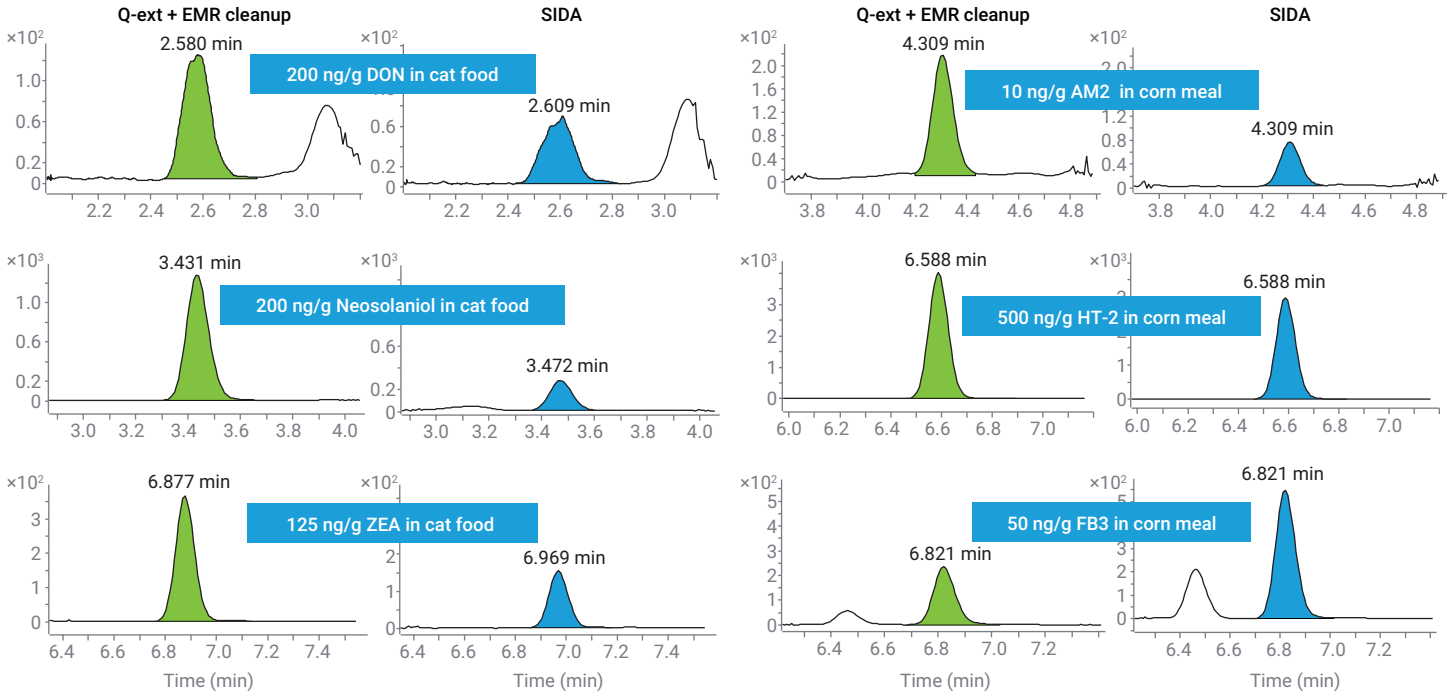

Figure 5-9. Representative mycotoxins matrix effect comparison for QuEChERS extraction followed with EMR passthrough cleanup versus the SIDA method.

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Multiresidue PAHs Analysis in Food and Other Applications

6.1 Introduction of application

Polycyclic aromatic hydrocarbons (PAHs) are a large class of ubiquitous and toxic compounds characterized by a thermodynamically stable fused aromatic ring structure. These compounds are naturally found in crude oils and coal but can also be formed through food processing. PAH compounds can be classified, according to the number of condensed aromatic rings, as light (two to three rings) or heavy (four to six rings). The heavy PAHs are more stable and toxic than the lighter ones. The U.S. Food and Drug Administration (FDA) requires PAH analysis at low-ppb levels in seafood.[1] The European Commission (EC) specifies the criteria for the methods of analysis of four heavy PAH compounds: benzo(a)pyrene, benzo(a)anthracene, benzo(b) fluoranthene, and chrysene down to a limit of quantitation (LOQ) of 0.9 µg/kg and limit of detection (LOD) of 0.3 µg/kg for each PAH.[2]

PAHs are hydrophobic compounds with a Log P value ranging from 3 to 5 for light PAHs and from 5 to 7 for heavy PAH compounds. They are also difficult to ionize under the typical soft ionization mechanism used in LC/MS detection. This group of compounds is thus more suitable for GC/MS and GC/MS/MS detection. Heavy PAH compounds require temperature gradient ramping to high temperature (> 300 °C) for elution and also > 300 °C at the interface for transferring to the MS detector. A high ion source temperature is also needed for detection of heavy PAHs.

PAHs are highly lipophilic compounds and tend to bio-accumulate in fatty foods such as fish, meat, oil, and milk. The main challenge for the analysis of PAHs in fatty food matrices is to isolate the analytes of interest from the bulk presence of lipid compounds in the food matrix. This challenge includes the efficient extraction of PAHs from a fatty matrix, then the selective removal of the unwanted fatty matrix co-extractives. To increase the extraction efficiency, sample extraction normally involves using a large volume of more hydrophobic solvent with multiple extractions and longer extraction time. Captiva EMR–Lipid cartridge passthrough cleanup then follows, providing highly efficient and selective lipid/fat removal.

6.2 Captiva EMR product recommendations

PAH compounds usually have a planar structural feature, and thus can be highly sensitive to the carbon material. Therefore, it is not recommended to use Captiva EMR products with a high percentage of Carbon S sorbent for PAHs analysis, including Captiva EMR–HCF1 and 2, Captiva EMR–GPF, and Captiva EMR–GPD. Captiva EMR–LPD contains a very small amount of Carbon S sorbent and can be usable for some pigmented fatty matrices. Since common fatty food matrices are not highly pigmented, Captiva EMR–Lipid is thus the main recommendation for PAHs analysis in food, including both 3 mL and 6 mL cartridges, depending on the desired sample loading capacity. For high-fat food sample matrices, Captiva EMR–Lipid HF cartridges were developed to improve the ease of sample elution under gravity.

In addition to Captiva EMR–Lipid cartridges, Bond Elut Jr PSA cartridges can be useful for fatty matrices with high abundance of fatty acids. PSA sorbent provides an excellent supplementary cleaning for fatty acid removal. The convenient Jr cartridge format easily combines with typical Captiva EMR–Lipid cartridges, so samples can be cleaned with a one-time passthrough of Captiva EMR–Lipid and Bond Elut Jr PSA cartridges. Figure 6-1 shows the hyphenation of Bond Elut Jr PSA cartridges with Captiva EMR–Lipid cartridges for pumpkin seed oil passthrough cleanup.

Table 6-1 shows the ordering information for Captiva EMR–Lipid cartridges, EMR–Lipid HF cartridges, and Bond Elut Jr PSA cartridges. Figure 6-2 shows the recommendation of EMR passthrough cleanup for PAHs analysis in fatty food matrices.

Table 6-1. Agilent Captiva EMR–Lipid and EMR–Lipid HF cartridges ordering information.

Product Name	Cartridge Format	Sorbent Bed Mass	Part Number
Captiva EMR–Lipid	3 mL	300 mg	5190-1003
Captiva EMR–Lipid	6 mL	600 mg	5190-1004
Captiva EMR–Lipid HF	3 mL	300 mg	5610-2235
Captiva EMR–Lipid HF	6 mL	600 mg	5610-2236
Bond Elut Jr PSA	Jr	500 mg	12162042B
Bond Elut Jr PSA	Jr	1,000 mg	12166050B

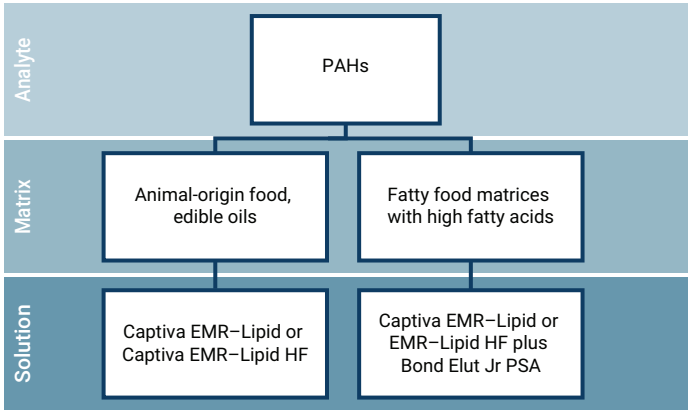

Figure 6-2. EMR passthrough cleanup for PAHs analysis in fatty food matrices.

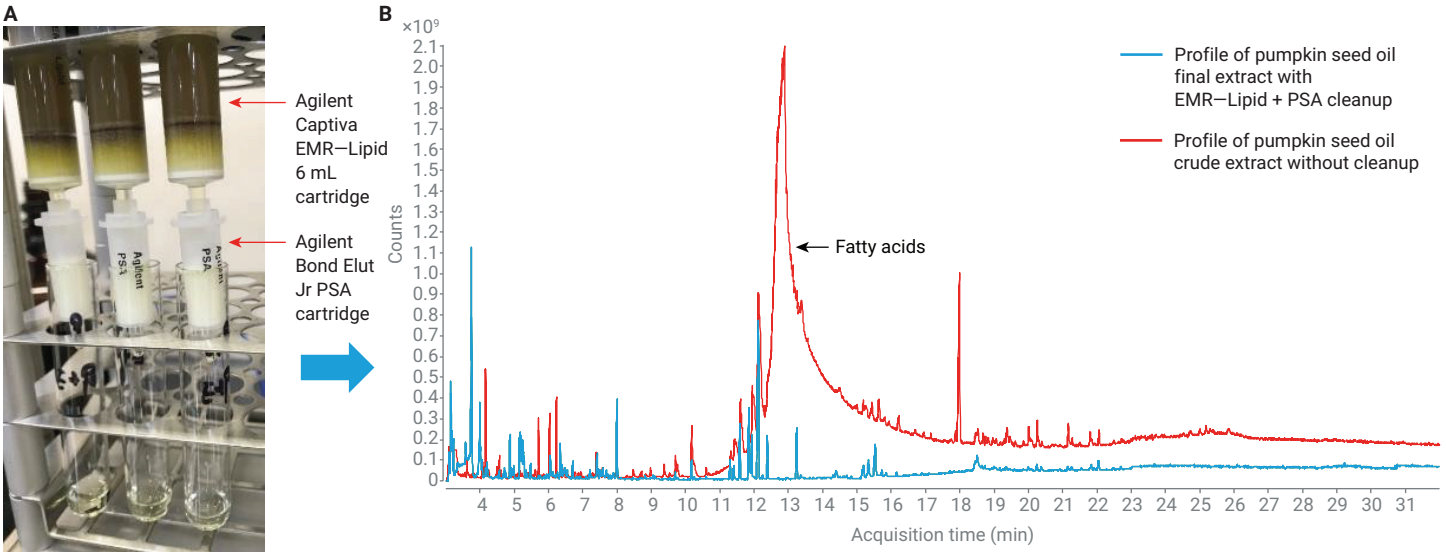

Figure 6-1. Agilent Captiva EMR–Lipid hyphenated with Agilent Bond Elut Jr PSA cartridges for pumpkin seed oil extract passthrough cleanup. (A) Hyphenated cartridge setup. (B) GC/MS full scan comparison of a pumpkin seed oil matrix sample, demonstrating cleaning efficiency.

6.3 Method development and comparison

Due to the highly lipophilic properties of PAH compounds, the analysis of PAHs in food is focused on fatty and processed food matrices such as edible oils, meat and fish, infant formula, etc. The sample preparation procedure usually involves sample extraction, matrix cleanup, and post-treatment.

Direct solvent extraction is usually used for sample extraction, as traditional QuEChERS extraction can cause the loss of highly hydrophobic PAH targets due to low extraction efficiency. In addition, the extraction of highly hydrophobic PAH targets from a fatty matrix is challenging and requires specific strategies to enhance the extraction efficiency. These strategies include the use of a stronger extraction solvent mixture (80:20 ACN:EtOAc), longer extraction times, and multiple extractions. For dry food matrices that must be dissolved, such as infant formula, it is also important to use a small amount of water. Figure 6-3 shows the impact of water being used to dissolve infant formula powder prior to extraction.

After extraction, the crude extract is cleaned using EMR mixed-mode passthrough cleanup. Prior to sample passthrough cleanup, the crude sample extract needs to be premixed with 10 to 20% water. For crude extracts of highly fatty food matrices, such as oil, that contain high amounts of lipid co-extractives, it is recommended to use a 10% water premixing ratio to prevent the loss of PAHs during passthrough cleanup due to reduced solubility in the sample mixture. After sample elution, an additional elution using 90:10 extraction solvent:water is then applied, which can improve PAHs recovery. These strategies are critical to achieve acceptable PAHs recovery from fatty food matrices. Figure 6-4 shows the optimization of sample extraction and EMR passthrough cleanup steps for PAH recovery improvements.

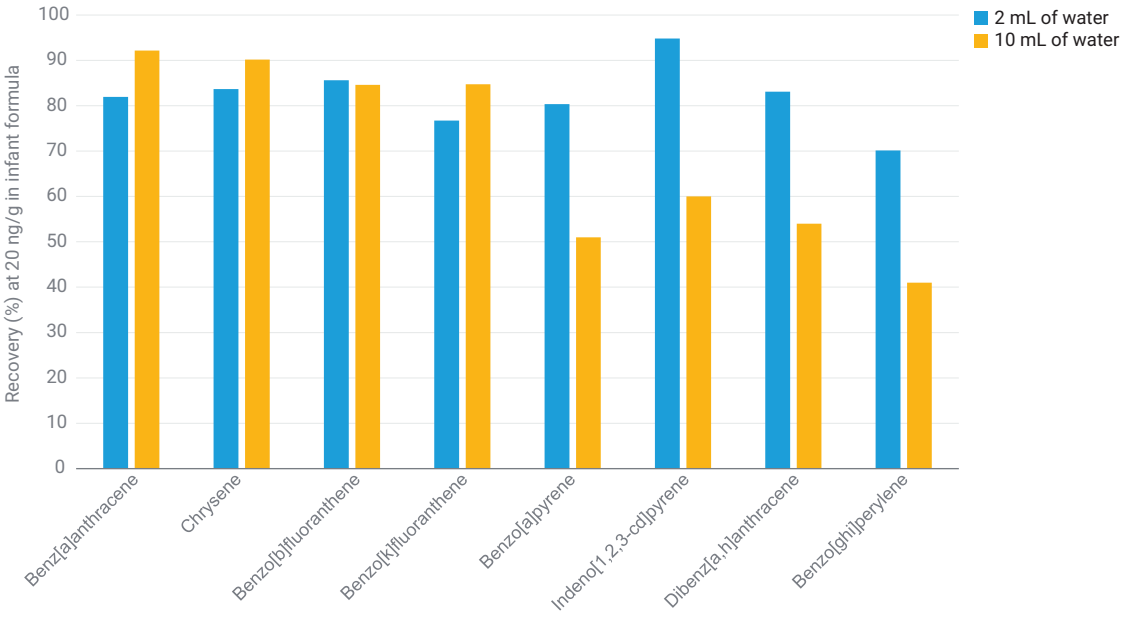

Figure 6-3. Comparison of PAH target recoveries for different amounts of water used to dissolve infant formula powder before solvent extraction.

After sample cleanup with EMR passthrough cleanup, appropriate sample post-treatment is necessary prior to sample analysis. For samples that use GC/MS or GC/MS/MS detection, it is critical to remove water residue completely. Three post-treatment methods can be applied for water residue removal after EMR passthrough cleanup, including salt partition using anhydrous $MgSO_4$, drying and reconstitution, and hydrophobic solvent back-extraction.

Table 6-2 shows three methods' general methodology, pros and cons, and their suitability for different applications. For hydrophobic PAH targets, the isooctane back-extraction provides a convenient and fast solution to remove water residue from EMR eluent. In addition, this post-treatment also results in switching to a GC-friendly solvent and partial sample concentration, which benefits sample analysis using GC/MS/MS and GC/MS detection.

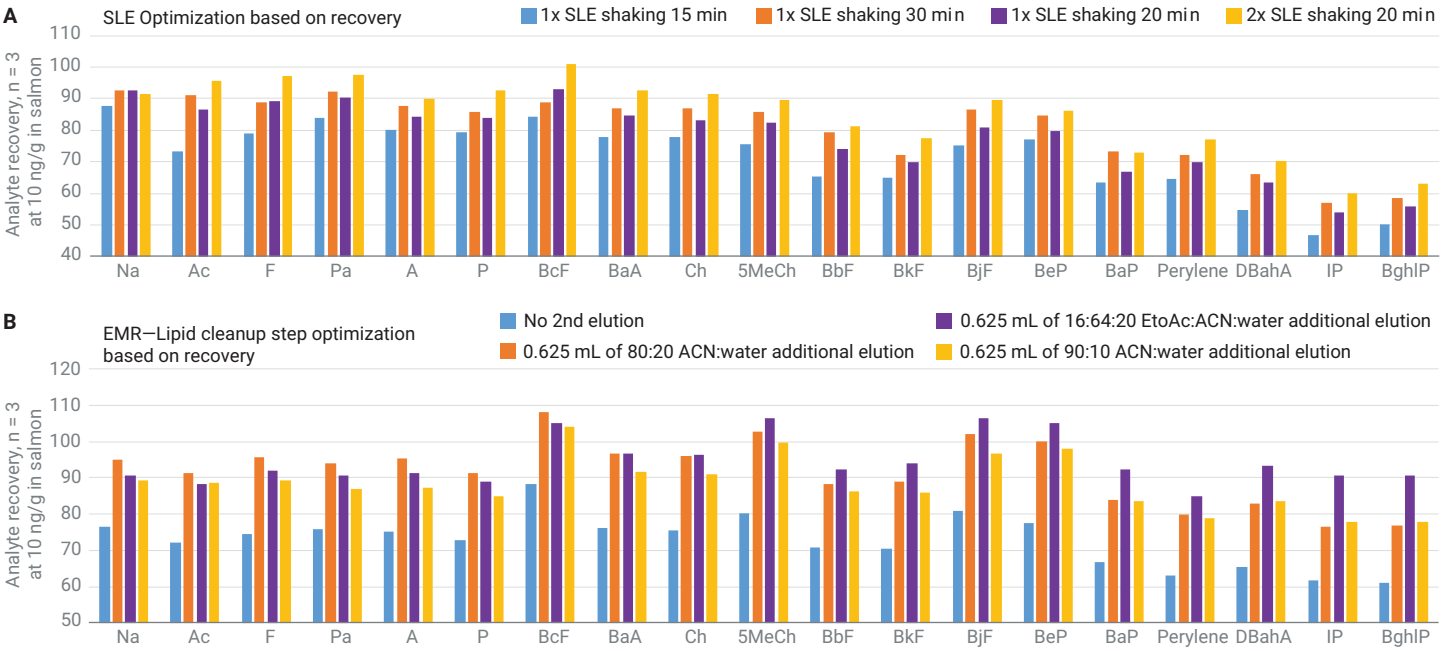

Figure 6-4. Sample extraction and EMR passthrough cleanup optimization for PAH targets recovery improvements.

Table 6-2. Sample post-treatment methods on EMR eluent for water residue removal.

Method for Water Removal	General Methodology	Advantages	Disadvantages	Suitability
Salt Partition by Anhydrous $MgSO_4$	– Add 700 mg of anhydrous $MgSO_4$ per 1 mL of EMR—Lipid eluent. – Vortex vigorously and centrifuge.	Usually no significant analyte loss	– Labor-intensive operation – Time-consuming – Inability to swap for a GC-amenable solvent	Multiclass multiresidue analysis
Dry and Reconstitute	– Use sample evaporation equipment to dry EMR eluent (TurboVap, CentriVap). – Reconstitute in a GC-amenable solvent. – Mix thoroughly.	– Relatively easy operation – Feasible sample concentrating and solvent swapping	– Time-consuming – Volatile analyte loss – Possible degradation of labile compounds	– Nonvolatile and stable analytes – Samples can be relatively low volume – Concentrating is required to reach low LOQ
Hydrophobic Solvent Back-Extraction	– Add water to the EMR eluent to approximately 1:2 organic:water. – Add isooctane (equivalent to the organic volume or slightly less). – Vortex thoroughly for 10 minutes, then centrifuge.	– Relatively easy operation – Feasible solvent swapping and partial sample concentrating – Further removal of dissolved polar matrix co-extractives	– Loss of polar to medium-polar compounds – Possible leaking during sample mixing	Hydrophobic compounds with log P ≥ 3

6.4 Performance review

The sample cleanup method was validated in multiple fatty food matrices including edible oils, fatty meat (beef) and fish (salmon), and infant formula. The method demonstrated acceptable absolute recoveries (50 to 120%) and excellent repeatability (RSD < 15%). The use of stable labeled internal standards delivered excellent method quantitation accuracy and precision for PAHs in edible oils, as demonstrated in Figure 6-5.

The method provided acceptable LOQs (0.9 ng/g in food) for critical PAH targets that meet the EU Commission requirements. It also demonstrated excellent matrix removal, providing > 90% matrix co-extractives residue removal in edible oil and beef matrices, and > 60% in salmon matrix. The lower matrix removal in salmon matrix is due to inefficient fatty acids removal using the passthrough cleanup with Captiva EMR–Lipid cartridges only. It is believed that the more recently developed passthrough cleanup using hyphenated Captiva EMR–Lipid and Bond Elut Jr PSA cartridges can improve salmon matrix cleanup. Figure 6-6 shows the matrix dried co-extractives residue before and after EMR passthrough cleanup in multiple food matrices.

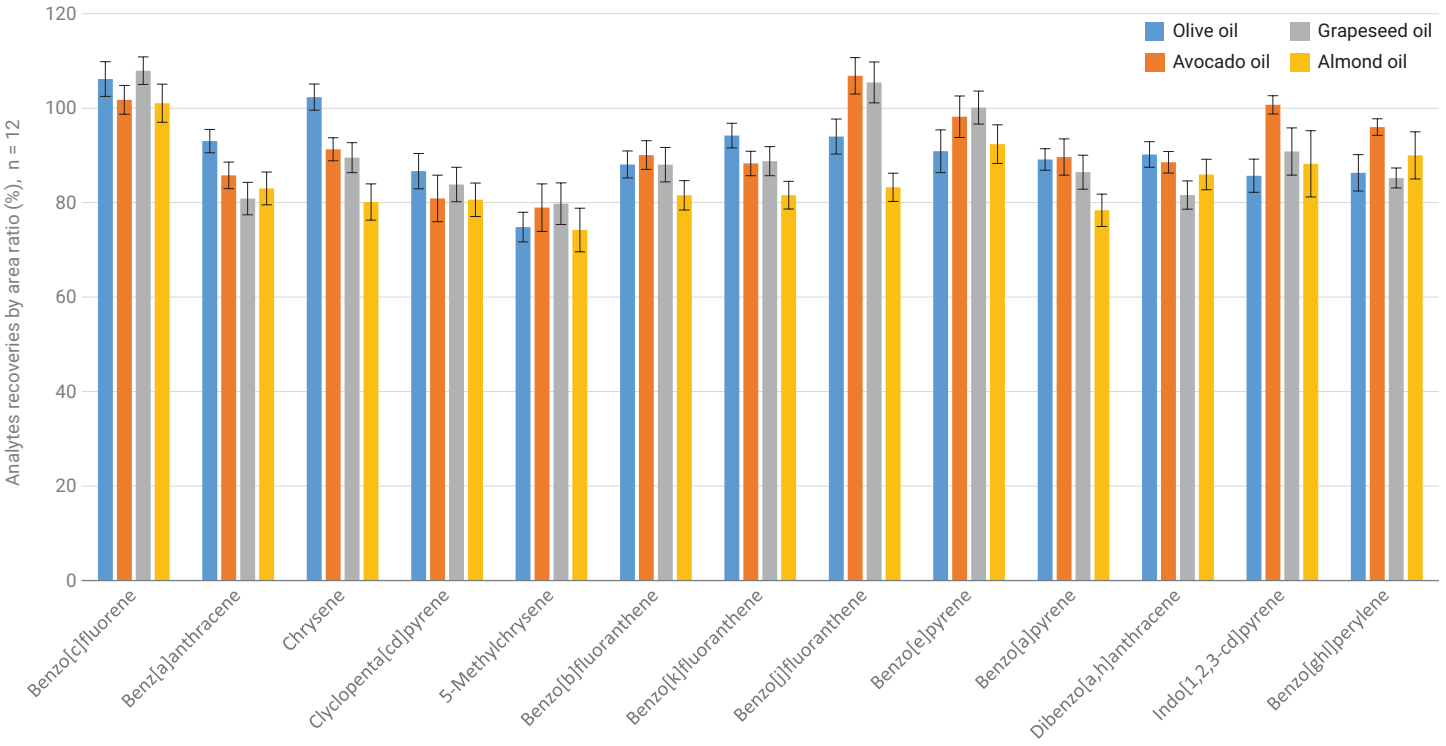

Figure 6-5. Method quantitation accuracy and precision for PAHs in edible oils.

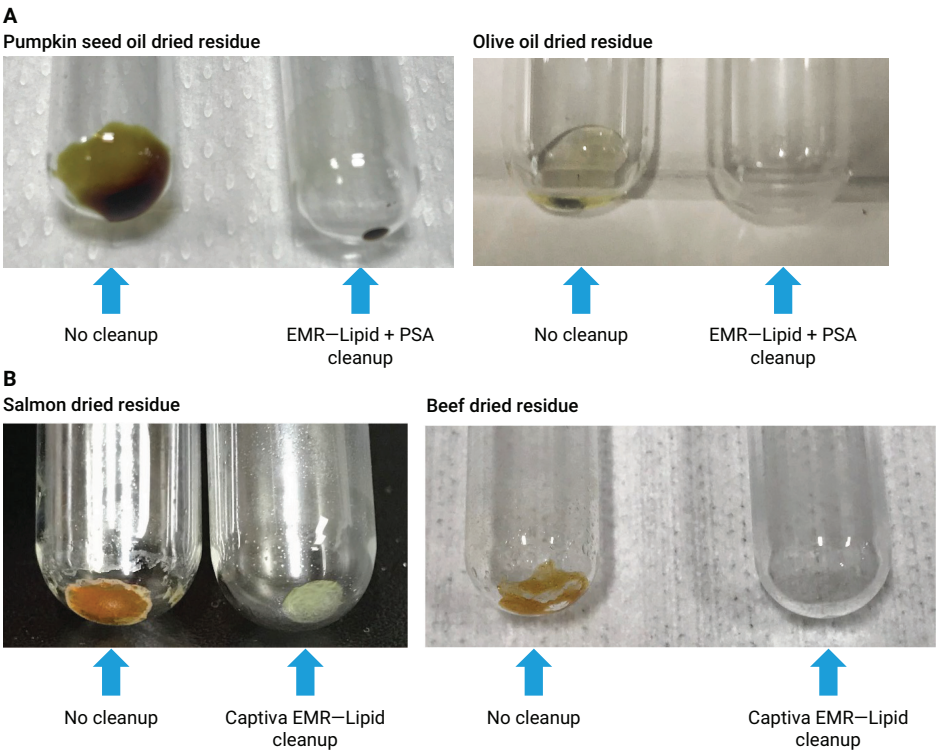

Figure 6-6. Matrix co-extractives residue comparison with versus without EMR passthrough cleanup for fatty foods, including (A) edible oils, and (B) beef and salmon.

6.5 Other applications

EMR passthrough cleanup has been applied to many other applications for food analysis, as well as personal care products analysis. A unique application was demonstrated for UV filters in sunscreen. Sunscreen samples were extracted by isopropanol (IPA) followed with simple membrane filtration, then tested with HPLC/UV detection for essential compounds used as UV filters. Due to the significant matrix co-extractives from sample matrices, the accumulated matrix interferences on the LC column caused dramatic retention time shifting, peak shape deterioration, and inconsistency of target responses over consecutive injections. Irreproducible chromatograms were observed with just three sunscreen sample injections, even with an intensive high organic wash between injections. The analytical LC column could be damaged with as few as six sunscreen sample injections. Overlaid chromatograms on the left and middle of Figure 6-7 were collected with three injections of sunscreen samples A and B prepared with the original method. Compared to the reference standard chromatogram (blue), the sunscreen samples showed dramatic retention shifting and peak shape and response deterioration.

To resolve the significant chromatographic deterioration resulting from complex sample matrix, Captiva EMR–Lipid passthrough cleanup was used after sample extraction. The problem was resolved completely. Overlaid chromatograms on the right of Figure 6-7 show six consecutive injections of sunscreen samples A, B, and C, with neither retention time shifting nor other chromatographic issues. The results demonstrate that adding Captiva EMR–Lipid passthrough cleanup to the sample preparation process enables the sequential, robust analysis of multiple sunscreen and lip balm samples on the same LC column. Acceptable levels of quantitative accuracy and reproducibility were achieved for the active ingredients analysis in all samples.

Captiva EMR–Lipid passthrough cleanup used in the analysis of THC and CBD in chocolates is another important application, delivering excellent target recoveries (104 to 110%) and matrix effect (~ 100%).

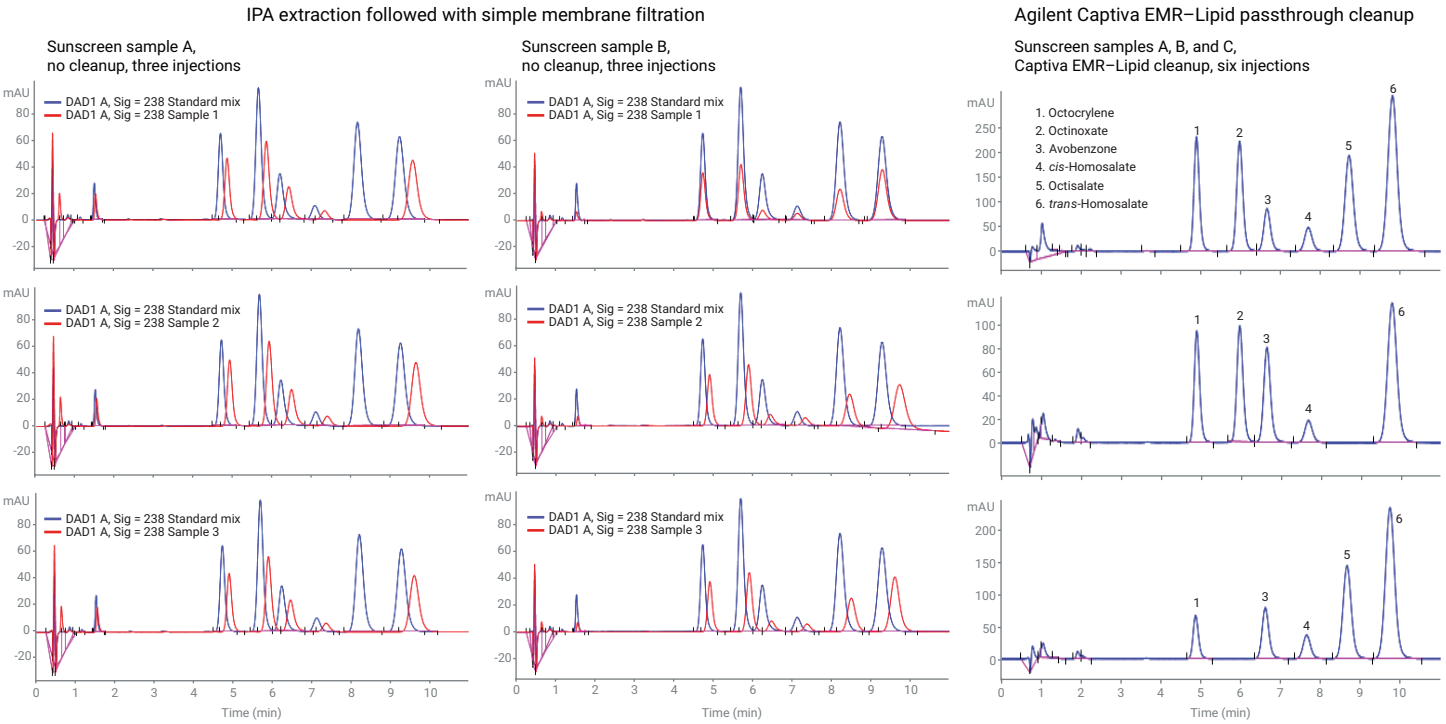

Figure 6-7. Chromatographic comparison for active ingredients analysis in sunscreen samples, prepared by IPA extraction followed by simple membrane filtration (left and middle) versus Agilent Captiva EMR–Lipid passthrough cleanup (right).

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