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Evaluation of a recent product to remove lipids and other matrix co-extractives in the analysis of pesticide residues and environmental contaminants in foods

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ABSTRACT

This study demonstrates the application of a novel lipid removal product to the residue analysis of 65 pesticides and 52 environmental contaminants in kale, pork, salmon, and avocado by fast, low pressure gas chromatography – tandem mass spectrometry (LPGC–MS/MS). Sample preparation involves QuEChERS extraction followed by use of EMR-Lipid ("enhanced matrix removal of lipids") and an additional salting out step for cleanup. The optimal amount of EMR-Lipid was determined to be 500 mg for 2.5 mL extracts for most of the analytes. The co-extractive removal efficiency by the EMR-Lipid cleanup step was 83–98% for fatty samples and 79% for kale, including 76% removal of chlorophyll. Matrix effects were typically less than ±20%, in part because analyte protectants were used in the LPGC–MS/MS analysis. The recoveries of polycyclic aromatic hydrocarbons and diverse pesticides were mostly 70–120%, whereas recoveries of nonpolar polybrominated diphenyl ethers and polychlorinated biphenyls were mostly lower than 70% through the cleanup procedure. With the use of internal standards, method validation results showed that 76–85 of the 117 analytes achieved satisfactory results (recoveries of 70–120% and RSD ≤ 20%) in pork, avocado, and kale, while 53 analytes had satisfactory results in salmon. Detection limits were 5–10 ng/g for all but a few analytes. EMR-Lipid is a new sample preparation tool that serves as another useful option for cleanup in multiresidue analysis, particularly of fatty foods.

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1. Introduction

In the residue analysis of pesticides and environmental contaminants in food matrices, sample preparation (extraction and cleanup) should be designed to fully extract the analytes while minimizing the amount of matrix components in the final extracts. QuEChERS (which stands for "quick, easy, cheap, effective, rugged, and safe") is a streamlined sample preparation concept typically employing acetonitrile (MeCN) for extraction and dispersive solid-phase extraction (d-SPE) for cleanup in a variety of applications [1–3]. In residue analysis of pesticides [4], veterinary drugs [5], environmental contaminants [6], and mycotoxins [7] in diverse food and environmental samples, QuEChERS has gained much popularity because it enables laboratories to meet growing demands for high sample throughput and cost-effective operations while still achieving reliably satisfactory results.

Food matrices are highly complex and consist of numerous components with different physical and chemical properties [8]. Many food types have a fat composition >2% (termed fatty foods) [9], including dairy products, nuts, grains, seafoods, meats, eggs, avocado. In fatty matrixes, both lipophilic and hydrophilic contaminants may be present [10], and analytical methods should be devised to have a wide polarity range, particularly for pesticides. Environmental contaminants, including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and polybrominated diphenyl ethers (PBDEs) and other flame retardants (FRs), represent lipophilic pollutants with low solubility in water and high K_{ow} values. Some of them are identified as persistent organic pollutants (POPs) due to their toxicity, persistency, and ability to bioaccumulate in fatty biological tissues [11], warranting their monitoring in fatty foods.

Although MeCN does not readily dissolve fat, QuEChERS still can yield satisfactory performance in fatty foods [12–16]. Less polar

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extraction solvents, such as hexane, acetone, ethyl acetate (EtOAc), and dichloromethane, can yield complete extraction efficiencies for lipophilic analytes, but extensive, expensive, and wasteful cleanup steps such as gel-permeation chromatography (GPC) is nearly always needed in those cases [17]. EtOAc may be used in QuEChERS to better extract lipophilic residues from fats [18], but reduced co-extraction of lipids is actually a benefit of using MeCN, thus requiring less post-extraction cleanup. Partitioning into MeCN is a consistent physicochemical property for each analyte, thus lower recoveries of lipophilic analytes can be normalized *vs.* internal standards or compensated by known recovery factors [14,19,20]. Even so, several studies demonstrated that QuEChERS using MeCN yielded complete extraction of many lipophilic POPs from fatty fish samples [21–23].

The reduced co-extraction of lipids by MeCN in QuEChERS enables use of simpler and less costly cleanup than GPC, such as d-SPE using different adsorbents, *e.g.* primary secondary amine (PSA), octadecylsilane (C_{18}), graphitized carbon black (GCB), and a commercial zirconia-based sorbent (Z-Sep) [20,24,25]. However, even a small amount of lipid in the final extracts can damage chromatographic columns and coat instrument surfaces, especially in gas chromatography – (tandem) mass spectrometry [GC–MS(/MS)]. Backflushing is one way to avoid build-up of lipid and other less volatile contaminants in GC systems [26,27], but post-extraction cleanup is still needed to reduce co-extractives, matrix effects, sensitivity losses, carry-over, and need for instrument maintenance. Several post-QuEChERS cleanup procedures have been reported for fatty foods, including freeze-out steps [28], liquid-liquid partitioning with hexanes [29], (d)-SPE [4,20], and even GPC [30], but each technique is limited in effectiveness and/or efficiency.

Recently, a vendor introduced a unique product known as "enhanced matrix removal" (EMR)-Lipid [31]. The structure of EMR-Lipid is a proprietary secret, and it does not function as a solid adsorbent in (d-)SPE, but it dissolves to saturation in extract solution, and its mechanism is said to involve both size exclusion and hydrophobic interactions. Long-chain hydrocarbons associated with lipids fit within the EMR-Lipid structure, where they are trapped. The lipid-EMR-Lipid complex is either precipitated out of solution or remain in the aqueous phase during the final salting-out step. In any case, the manufacturer claims that EMR-Lipid selectively removes lipids from QuEChERS extracts of fatty foods such as avocado and animal tissues, without loss of common pesticide, veterinary drug, or PAH analytes [32–36].

The aim of this study was to evaluate cleanup efficiency and method performance of the EMR-Lipid product for 65 pesticides and 52 environmental contaminants, plus 15 internal standards and a quality control standard, in 4 different food matrices (avocado, kale, pork, and salmon). The pesticides were chosen from US Environmental Protection Agency (EPA) priorities, including some typically problematic pesticides. The environmental contaminants included 15 EPA priority PAHs, 14 PCB congeners, 7 common PBDE congeners, and 16 novel FRs. We intended to optimize the final method and compare results with our previous validation studies using QuEChERS sample preparation and fast, low-pressure (LP)GC–MS/MS analysis, including use of analyte protectants [20,37].

2. Experimental

2.1. Chemicals and materials

Deionized water of 18.2 MΩ-cm was obtained with an E-Pure Model D4641 from Barnstead/Thermolyne (Dubuque, IA; USA), and HPLC-grade MeCN was from Fisher Scientific (Pittsburgh, PA; USA). Toluene and ammonium formate (HCO_2NH_4) were purchased from Sigma-Aldrich (Saint Louis, MO; USA). Anhydrous magnesium sulfate (anh. $MgSO_4$) and primary secondary amine (PSA) were from UCT (Bristol, PA; USA). C_{18} (40 μm) was purchased from Thomas Scientific (Swedesboro, NJ; USA), and zirconium-based Z-Sep sorbent was from Supelco (Bellefonte, PA; USA).

Pesticide standards were obtained from ChemService (West Chester, PA; USA), Dr. Ehrenstorfer GmbH (Augsburg; Germany), and the Environmental Protection Agency's National Pesticide Repository (Fort Meade, MD; USA). Standards of 16 FRs, 7 PBDE congeners (#28, 47, 99, 100, 153, 154, and 183), 14 PCB congeners (#77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 170, 180, and 189), and 15 PAHs were purchased from AccuStandard (New Haven, CT; USA), Sigma-Aldrich, and Santa Cruz Biotechnology (Santa Cruz, CA; USA).

For use as internal standards (IS), atrazine-d_5 (ethyl-d_5) and fenthion-d_6 (o,o-dimethyl-d_6) were from C/D/N Isotopes (Pointe-Claire, Quebec; Canada). $^{13}C_{12}$-PCB 153, $^{13}C_{12}$-*p,p'*-DDE, TCEP-d_{12} and a PAH surrogate mixture containing acenaphthylene-d_8, benzo[a]pyrene-d_{12}, benzo[g,h,i]perylene-d_{12}, fluoranthene-d_{10}, naphthalene-d_8, phenanthrene-d_{10}, and pyrene-d_{10} were purchased from Cambridge Isotope Laboratories (Andover, MA; USA). 5′-Fluoro-3,3′,4,4′,5-pentabromodiphenyl ether (FBDE 126), and *p*-terphenyl-d_{14} were purchased from AccuStandard. $^{13}C_{18}$-TPP was from Wellington Laboratories (Guelph, Ontario, Canada). All standards were ≥98% purity.

The standard solution of the 52 environmental contaminants was prepared at 5 ng/μL in MeCN and acetone and a little toluene for all except that the concentrations of PCB congeners were 10 times lower (0.5 ng/μL). Another standard mixture of 65 pesticides at 5 ng/μL in MeCN with 0.05% formic acid was prepared, and the two solutions were combined (1/1, v/v) to make a working standard mixture of all targeted analytes at 2.5 ng/μL, except the PCBs were 0.25 ng/μL. The working standard also served as the high level spiking solution and was used to prepare the medium (0.625 ng/μL) and low (0.25 ng/μL) level spiking solutions, and also for preparation of calibration standard solutions.

The IS standard solution was prepared in MeCN and acetone, and contained: 2.5 ng/μL of FBDE 126, TCEP-d_{12} and $^{13}C_{12}$-p,p'-DDE, 5 ng/μL of atrazine-d_5 and fention-d_6, 2 ng/μL of isotopically labeled PAHs, 1.25 ng/μL of $^{13}C_{18}$-TPP, and 0.5 ng/μL $^{13}C_{12}$ PCB 153.

Analyte protectants (APs) containing 10 mg/mL ethylglycerol, 1 mg/mL each of gulonolactone and d-sorbitol, and 0.5 mg/mL shikimic acid was prepared in 4/1 (v/v) MeCN/water with 0.5% formic acid [38]. As a quality control (QC) standard, *p*-terphenyl-d_{14} was prepared into the APs mixture at 0.438 ng/μL.

Kale, salmon, avocado, and pork samples were purchased from local organic grocery stores. These matrices contain lipids and/or pigments such as chlorophyll that serve as good representative sample types for evaluation of the EMR-Lipid product. The samples were comminuted with dry ice using a Robotcoupe (Ridgeway, MS; USA) RSI 2Y1 chopper and stored in glass jars at −20 °C until analysis. A Glas-Col platform pulse mixer (Terre Haute, IN; USA) and a Thermo Fisher Sorvall Legend RT centrifuge (Waltham, MA; USA) were used for extraction and centrifugation, respectively.

Bond Elut EMR-Lipid was from Agilent Technologies (Little Falls, DE; USA) consisting of 1 g EMR-Lipid material in a 15 mL polypropylene tube and 2 g mixture of 4/1 (w/w) anh. $MgSO_4$/NaCl in a second 15 mL centrifuge tube of the same type.

2.2. Fast LPGC–MS/MS analysis

The 117 targeted analytes plus 15 IS and QC standard were analyzed using LPGC–MS/MS, which involved an Agilent 7890A/7000B gas chromatograph/triple-quadrupole mass spectrometer with electron ionization (EI) at 70 eV. The separation was achieved on a 15 m × 0.53 mm i.d. × 1 μm film thickness Agilent DB–5 ms analyt-

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