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Method validation for 243 pesticides and environmental contaminants in meats and poultry by tandem mass spectrometry coupled to low-pressure gas chromatography and ultrahigh-performance liquid chromatography

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ABSTRACT

An easy and reliable high-throughput analysis method was developed and validated for 192 diverse pesticides and 51 environmental contaminants (13 PCB congeners, 14 PAHs, 7 PBDE congeners, and 17 novel flame retardants) in cattle, swine, and poultry muscle. Sample preparation was based on the "quick, easy, cheap, effective, rugged and safe" (QuEChERS) approach using filter-vial dispersive solid-phase extraction (d-SPE) cleanup. Split final extracts were analyzed in parallel by low-pressure (vacuum outlet) GC-MS/MS and UHPLC-MS/MS (10 min each), providing an additional degree of confirmation for 55 overlapping LC- and GC-amenable pesticides. Analyte protectants were utilized to improve sensitivity and decrease matrix effects in GC analysis, and only filtration of initial extracts was enough to avoid ion suppression in UHPLC-MS/MS. The method was validated at three spiking levels (10, 25, and 100 ng/g) at or below established tolerance levels in the sample types. Satisfactory recoveries (70–120%) and RSDs $\leq$ 20% were achieved for 200 analytes. The validated method was successfully applied to the analysis of real-world incurred meat samples, further demonstrating the utility of the method for implementation in regulatory and commercial laboratories.

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1. Introduction

Worldwide meat production totaled 304 million tons in 2012, with an annual average estimated consumption of 42–76 kg per person (Food Outlook. Biannual report on global food markets, 2014). Average meat consumption in the US consists of 55% beef and pork, followed by 37% poultry. (The United States Meat Industry at a Glance) Meat and poultry are important nutritional sources of proteins, essential amino acids, and bioavailable minerals and vitamins. (USDA National Nutrient Database) At the same time, many anthropogenic chemicals, particularly lipophilic pesticides and environmental contaminants, may bioaccumulate in meats resulting in human exposure during consumption. Food of animal origin was estimated to contribute >95% of the human exposure to lipophilic anthropogenic contaminants (Boada et al., 2014). Because many pesticides have been shown to cause various health effects, maximum residue levels (MRLs), called tolerances in the US, have been established to control their residues in meats and protect human health. Depending on toxicity, pesticide tolerances in meats range from 10 to 3000 ng/g, with most set between 10 and 100 ng/g (Global MRL database).

Environmental contaminants, including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and other flame retardants (FRs) represent another group of organic lipophilic chemicals that bioaccumulate in fatty biological tissues. Dioxin-like PCBs and some PBDE congeners are recognized by the Stockholm Convention as persistent organic pollutants (POPs) based on their high persistence, toxicity, and bio-magnification in the food chain. (The Stockholm Convention on Persistent Organic Pollutants. United Nations Environmental Progamme).

Previous reports have shown that meat and poultry are the major dietary sources of PBDEs in the human diet, although the PBDE body burden has been decreasing (Huwe & West, 2011) since

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penta- and octa-PBDE congeners have been banned. Nevertheless, new organic flame retardants (organophoshorous, organochlorine, and organobromine compounds) have been introduced in commerce to comply with fire safety regulations. These new chemicals also possess lipophilic properties, high log K_{ow} values, suggesting their ability to bioaccumulate in animal and human tissues and warranting their monitoring in foods.

Numerous food safety programs operate around the world to help ensure that food is safe for consumption and to reduce environmental impact. Fast, simple, efficient, and cost-effective analytical methods for pesticides and other contaminants are needed in food safety monitoring programs to provide high sample throughput and accurate results. In the USA, USDA Food Safety and Inspection Service (FSIS) conducts routine monitoring of meat (cattle, swine, poultry, sheep, goats) samples to enforce regulatory compliance. The current FSIS sample preparation method for pesticide screening is based on liquid extraction of meat samples with ethyl acetate, followed by freeze-out and solid phase extraction (SPE) clean-up to remove interfering compounds, and involves multiple evaporation steps. (US Department of Agriculture, Food Safety and Inspection Service. Screening for Pesticides by LC/MS/MS and GC/MS/MS. #CLG-PST5.06) Other commonly reported methods for extracting lipophilic pesticides and environmental contaminants from meats are based on pressurized liquid extraction (PLE) with nonpolar organic solvents (*e.g.* hexane, ethyl acetate, dichloromethane) usually followed by cleanup with gel-permeation chromatography (GPC) to remove lipids (Munoz, Munoz, Pineda, Serrahima, & Centrich, 2012; Wu et al., 2011). These methods take much time, solvent, and labor and require specialized equipment.

We have previously developed a high-throughput analytical method for multi-class analysis of selected pesticides and environmental contaminants in fish and shrimp tissues based on QuEChERS extraction and dispersive solid phase extraction (d-SPE) clean-up (Sapozhnikova, 2014; Sapozhnikova & Lehotay, 2013) combined with in-vial filtration (Han, Sapozhnikova, & Lehotay, 2014) using low pressure (LP) gas chromatography (GC) with tandem mass spectrometry (MS/MS).

The goal of this study was to optimize and validate this approach to include FSIS-priority contaminants in FSIS-regulated foods (*e.g.* cattle, swine, and poultry) for transfer to FSIS regulatory field service laboratories for routine monitoring in their National Residue Program (NRP). We sought to develop and validate a simple and fast method for simultaneous determination of multi-class pesticides and diverse environmental contaminants (PCBs, PAHs, PBDEs and flame retardants) in food muscle tissues to provide better quality of results for more chemicals of concern in more samples at higher throughput, less labor, and lower costs.

2. Materials and methods

2.1. Chemicals and materials

Standards of PBDE congeners (#28, 47, 99, 100, 153, 154, and 183), PCB congeners (#77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 170, 180, and 189), and PAHs (acenaphthene, acenaphthylene, anthracene, benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd) pyrene, naphthalene, phenanthrene, and pyrene), flame retardants [dechlorane plus (mixture of syn- and anti-isomers), hexabromobenzene (HBB); hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO), 2,2′,4,4′,5,5′-hexabromobiphenyl (PBB 153), pentabromoethylbenzene (PBEB), pentabromotoluene (PBT), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), 1,2,5,6-tetrabromocyclooctane (TBCO), 1,2-dibromo-4-(1,2-dibromoethyl) cyclohexane (TBECH), tribromoneopentyl alcohol (TBNPA), and tris(1,3-dichloro-2-propyl)phosphate (TDCPP)] were all purchased from AccuStandard (New Haven, CT; USA). Standards of 1,2,4,5-tetrabromo-3,6-dimethylbenzene (TBX), tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl)phosphate (TCPP), and triphenyl phosphate (TPP) were purchased from Sigma–Aldrich (St. Louis, MO; USA), and dechlorane 604 standard was from Santa Cruz Biotechnology (Santa Cruz, CA; USA). Pesticide standards were from the Environmental Protection Agency's National Pesticide Repository (Fort Meade, MD; USA) and ChemService (West Chester, PA; USA).

For use as internal standards (IS), $^{13}C_{12}$-2,2,4,4,5,5-hexachlorobenzene ($^{13}C_{12}$-PCB 153), and a PAH surrogate cocktail 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). 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). FBDE 126 (5-fluoro-2,3,4,4,5-pentabromodiphenyl ether) and *p*-terphenyl-d_{14} used as quality control (QC) standard (added just prior to injection) in GC were purchased from AccuStandard. Phenacetin-ethoxy-1-^{13}C (^{13}C-phenacetin) used as a QC standard for LC was acquired from Sigma–Aldrich. All standards were ≥98% purity.

HPLC-grade acetonitrile (MeCN) was from Fisher Scientific (Pittsburgh, PA; USA) and acetone was from Sigma–Aldrich. Deionized water of 18.2 MΩ-cm was obtained using an E-Pure system from Barnstead/Thermolyne (Dubuque, IA; USA). Formic acid (88% purity) was from Spectrum Quality Products (New Brunswick, NJ; USA). Ammonium formate (HCO_2NH_4) was from Sigma–Aldrich. Anhydrous magnesium sulfate (anh. $MgSO_4$), 99.5% purity, and primary secondary amine (PSA) were purchased from UCT (Bristol, PA; USA). C18 (40 μm) was purchased from Thomas Scientific (Swedesboro, NJ; USA), and zirconium-based Z-Sep sorbent was from Supelco (Bellefonte, PA; USA). Filter vials with 0.45 μm polyvinylidene fluoride (PVDF) filters were from Thomson Instrument Co. (Oceanside, CA; USA). Two types of filter vials were used: 1) for filter-vial d-SPE in GC, screw-cap vials with short plungers allowed room for sorbent in the bottom shell receptacles; and 2) for filtering-only in LC, slit-septa snap-cap filter vials had standard length plungers. A press tray for simultaneous filtering of up to 48 filter vials was provided by Thomson Instrument Co.

A working standard mixture of the environmental contaminants, containing a total of 51 PCBs, PBDEs, PAHs, and FRs and 192 pesticides was prepared at 2.5 ng/μL in MeCN/acetone, except 10-fold lower PCB concentrations (0.25 ng/μL). This mixture was used to prepare spiking and calibration standard solutions in MeCN. The IS solution in MeCN contained a mixture of atrazine-d_5 and fenthion-d_6 at 5 ng/μL, FBDE at 2.5 ng/μL, isotopically-labeled IS for PAHs at 1 ng/μL, and $^{13}C_{12}$-PCB 153 at 0.1 ng/μL. Atrazine-d_5 was used as an IS for all pesticides, and fenthion-d_6 served as a back-up IS.

A mixture of analyte protectants (APs) contained ethylglycerol at 10 μg/μL, gulonolactone and D-sorbitol each at 1 μg/μL, and shikimic acid at 0.5 μg/μL was prepared in 4/1 (v/v) MeCN/water with 0.5% formic acid. Also, this solution contained the QC standard for GC of 0.438 ng/μL *p*-terphenyl-d_{14}. A separate solution of ^{13}C-phenacetin at 4 μg/mL was prepared in MeCN for use as the QC standard in LC.

Ten ≈500 g samples each of cattle, pork, and poultry muscle tissue from different parts of the animal (grown organically), were purchased from local grocery stores. For example, chicken wings, breast, thigh, drumsticks, and whole cornish hens were used as poultry samples. All samples were filleted into skinless and