APPLICATION

A Simple and Rapid Extraction Method for Chlorinated Pesticides in Poultry Fat Using Solid Phase Extraction and GC/ECD

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Pesticide contamination is not limited to produce items. Animals used for food consumption are also exposed to contaminants at levels that can pose harm to the human population. Presented is a method developed using SPE and GC/ECD for pesticides analysis from poultry fat. This method improves upon the traditional procedure resulting in a 75 % reduction in laboratory space, reduction of hazardous waste, and a 50 % reduction in labor consumption, leading to greater laboratory productivity.

Introduction

Chlorinated hydrocarbons (CHC) are persistent in the environment and are known to cause adverse health effects. Several of these compounds are endocrine disruptors and may be linked to a variety of sexual, developmental, behavioral, and reproductive problems. Many of them are known to have carcinogenic effects as well.

In an effort to ensure adherence to various United States pesticides regulatory acts, as well as provide the world with trusted and safe food products, Tyson Foods, Inc. instituted a pesticide screening program to test all flocks of chickens pre-slaughter to verify that all incoming poultry is clear of pesticide residues. This laboratory program is registered with and monitored by the United States Department of Agriculture Food Safety and Inspection Service Accredited Laboratory Program (USDA-FSIS-ALP). The official methodology currently used to extract CHC is listed under USDA-FSIS Determinative Method CHC2. Traditionally under this method, glass chromatography columns are manually prepared using glass wool, alumina, and sodium sulfate which are then suspended in petroleum ether. In an effort to reduce the labor force and space needed to analyze this method, Phenomenex Strata® Alumina-N solid phase extraction (SPE) cartridges were used to develop a new extraction procedure that delivers reliable and accurate results. In this study, we demonstrate a simple, rapid extraction for CHCs in poultry fat using SPE and fast GC/ECD analysis with a Phenomenex Zebron™ ZB-MultiResidue™-1 column.

Reagents and Chemicals

The analytical standards 2,4,5,6-tetrachloro-m-xylene (Internal Standard 1), Hexachlorobenzene (HCB), Alpha-BHC, Lindane (gamma-BHC), Heptachlor, Aldrin, Dursban® (Chlorpyrofos), Heptachlor Epoxide, Dieldrin, p,p'-DDE, Endrin, p,p'-TDE, p,p'-DDT, o,p-DDT, Methoxychlor, Mirex, and Decachlorobiphenyl (Internal Standard 2) were purchased from SPEX CertiPrep® (Metuchen, NJ, USA). Matrix spikes were used to validate the method at various concentrations with 2,4,5,6-tetrachloro-m-xylene and Decachlorobiphenyl being used as internal standards to signify the beginning and end of compound elution order. Distilled water was obtained using a Millipore Direct-Q® 3 UV system (Millipore, Bedford, MA, USA). Methanol, petroleum ether, hexane, and ethyl ether were all obtained from Thermo Fisher Scientific (Pittsburg, PA, USA).

An OA-SYS heating system (Organomation Associates, Inc., Berlin, MA, USA) was used to evaporate organic solvents at 30 °C. Samples were analyzed with an Agilent® 7890A GC equipped with a micro electron capture detector (ECD) (G2397A ECD-Ni63) (Agilent Technologies, Palo Alto, CA, USA). Chromatographic analysis was carried out on an Phenomenex ZB-MultiResidue-1 (30 m 0.32 mm x 0.50 µm) column.

Sample Preparation

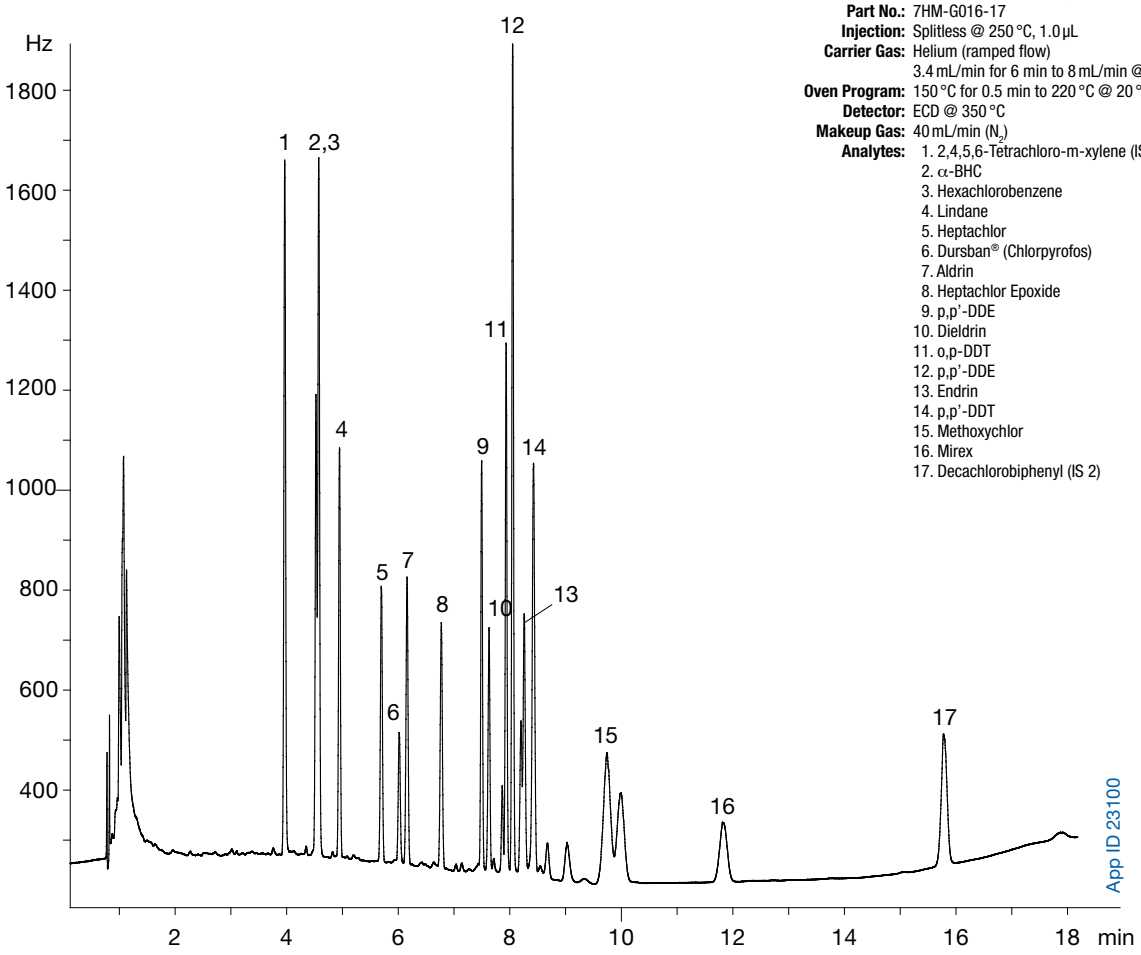
Pre-treatment:

1. Render poultry fat pads using a microwave in 1 minute intervals ensuring the sample does not exceed 100 °C.
2. Weigh 1 gram of rendered fat into a 10 mL volumetric flask and bring to volume with hexane containing internal standards 1 and 2 for SPE.
3. Vortex or shake volumetric flasks to ensure proper mixing. Sample is now ready for SPE.

Solid Phase Extraction (SPE) Protocol

1. Before extracting samples, condition Strata Alumina-N SPE cartridges (Phenomenex part no. 8B-S313-KDG) by placing on vacuum chamber with pump and filling each cartridge to volume with 86/14 methanol/water solution.
2. Slowly rinse the solution through the cartridge at a rate of approximately 10 mL/minute until bedding is dry.
3. Fill cartridges to volume with petroleum ether to remove traces of methanol/water solution and drip at a rate of approximately 10 mL/minute.
4. Place glass culture tubes inside vacuum chamber to catch sample eluent and replace manifold to ensure a tight vacuum seal.
5. Pipet 1 mL sample into cartridge.
6. As sample drains through alumina bed, fill cartridge to volume with 1.5/98.5 Ethyl Ether/Petroleum Ether solution to ensure analyte elution.
7. Once samples have been collected, transfer glass culture tubes into nitrogen evaporator and evaporate to dryness.
8. Reconstitute samples with 2 mL Hexane, vortex for 30 seconds, and transfer to autosampler vial for injection.

Figure 1.
GC/ECD analysis of CHC extraction



Columns: Zebron™ ZB-MultiResidue -1
Dimensions: 30 m x 0.32 mm x 0.50 µm
Part No.: 7HM-G016-17
Injection: Splitless @ 250 °C, 1.0 µL
Carrier Gas: Helium (ramped flow)
3.4 mL/min for 6 min to 8 mL/min @ 5 mL/min, hold 12.08 min
Oven Program: 150 °C for 0.5 min to 220 °C @ 20 °C/min to 310 °C @ 6 °C/min
Detector: ECD @ 350 °C
Makeup Gas: 40 mL/min (N_2)
Analytes:
1. 2,4,5,6-Tetrachloro-m-xylene (IS 1)
2. α-BHC
3. Hexachlorobenzene
4. Lindane
5. Heptachlor
6. Dursban® (Chlorpyrofos)
7. Aldrin
8. Heptachlor Epoxide
9. p,p'-DDE
10. Dieldrin
11. o,p-DDT
12. p,p'-DDE
13. Endrin
14. p,p'-DDT
15. Methoxychlor
16. Mirex
17. Decachlorobiphenyl (IS 2)

Results and Discussion

A method validation was performed and submitted to USDA-FSIS personnel to prove fitness of use. This validation relied on USDA listed tolerance values as the minimum requirement for acceptability. All analyte standards and spiked matrix samples were analyzed at levels below these minimum requirements to ensure lower than required detection limits were obtainable. Three specific residues, Lindane, Dieldrin, and p,p'-DDT were selected as representative residues for validation. Four samples of poultry fat were each separately spiked at three concentration levels, 0.05 µg/mL, 0.1 µg/mL, and 0.3 µg/mL. Additionally, eight matrix spikes were performed to establish the limit of detection. For each residue validated, precision, accuracy, linearity, internal standard recovery, method detection limit, method quantitation limit, limit of detection, and limit of quantitation were calculated. The limit of detection for the extraction was 1.6 µg/L and the limit of quantitation was established as 4.0 µg/L based on the three residues Lindane, Dieldrin, and p,p'-DDT. After review, USDA-FSIS approved all methodology changes based upon the data provided.

The GC method was optimized on a Zebron™ ZB-MultiResidue-1 column, which is a proprietary mid-polar phase. This phase offered high efficiency and selectivity required to resolve all 17 components in the pesticide mix. The run time was 19 minutes, with the last peak in the chromatogram, Decachlorobiphenyl, eluting at 15.59 minutes. This gave room to hold the column for 3.4 more minutes at the final temperature of 310 °C to bake the column after each run. As shown in **Figure 1**, all the peaks eluted at 310 °C, which is well below the column's maximum temperature. This aspect enhances the lifetime of the column. The MS certified, engineered self cross-linked phase not only gave enhanced separation for the pesticide mix but also provided the lowest bleed on ECD.

The presented method is selective in terms of extraction, separation, and detection: the Strata® Alumina-N SPE sorbent is a polar phase that extracts polar compounds, such as chlorinated pesticides, from food matrices; the GC analysis using the Zebron ZB-MultiResidue-1 column offers the selectivity and sensitivity necessary to separate the 17 pesticide compounds; and the ECD is selective to electronegative compounds and gives high peak response to chlorinated pesticides. The selectivity in each stage of analysis resulted in bringing the detection limits to µg/L level. The SPE sample preparation and GC analytical method were optimized by Tyson Foods, Inc. Further optimization of the method can be done based on specific analytical goals. Carrier gas in constant flow mode is recommended for quantitative analysis using ECD. To further enhance lifetime, the GC oven program can be extended for 5 minutes and a Z-Guard™ column can be used in front of the Zebron ZB-MultiResidue-1 column to prevent non-volatile impurities from attacking the analytical column.

The practical implications of this methodology revision are quite significant due to the space and labor saving aspects. With no reduction in sample volume, the residue section of the Tyson Foods, Inc. Food Safety and Research Laboratory was able to effectively cut labor by 50 percent and space required by 75 percent while maintaining compliance with program protocols. The Strata Alumina-N SPE cartridges greatly increased efficiency by providing excellent results at a fraction of the time required to manually prepare columns under the traditional method. This increase in efficiency allowed flexibility to reassign labor previously tied up for pesticide residue analysis to focus on other areas of need throughout the laboratory. Additionally, these products allow for a greener method by requiring much less organic solvents and chemicals to extract CHC residues. This contributes to a reduction in hazardous waste which impacts both safety and budgetary issues.

Conclusion

The Strata Alumina-N SPE method decreases the amount of labor and reagent that is associated with the USDA-FSIS CHC2 method. This analysis can be performed in an 8 foot vent hood freeing up valuable bench top space for an expanding laboratory. The combination of these findings make for decreased costs involved with reagent use and disposal as well as the labor and time associated with the analysis while providing accurate and reliable results.

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Ordering Information

Strata® Alumina-N SPE Cartridges

Format	Sorbent Mass	Part Number	Unit
Tube			
	500 mg	8B-S313-HBJ	3 mL (50/box)
	1 g	8B-S313-JCH	6 mL (30/box)
Giga™ Tube			
	2 g	8B-S313-KDG	12 mL (20/box)

Zebron™ ZB-MultiResidue™ -1 GC Columns

ID(mm)	df(µm)	Temp. Limits °C	Part No.
20-Meter			
0.18	0.18	-60 to 320/340	7FD-G016-08
30-Meter			
0.25	0.25	-60 to 320/340	7HG-G016-11
0.32	0.25	-60 to 320/340	7HM-G016-11
0.32	0.50	-60 to 320/340	7HM-G016-17
0.53	0.50	-60 to 320/340	7HK-G016-17

Zebron ZB-MultiResidue -2 GC Columns

ID(mm)	df(µm)	Temp. Limits °C	Part No.
30-Meter			
0.25	0.20	-60 to 320/340	7HG-G017-10
0.32	0.25	-60 to 320/340	7HM-G017-11
0.53	0.50	-60 to 320/340	7HK-G017-17

Note: If you need a 5 in. cage, simply add a (-B) after the part number, e.g., 7HG-G016-11-B or 7HG-G017-10-B. Some exceptions may apply. Agilent 6850 and some SRI and process GC systems use only 5 in. cages. See p. 101.

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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