Determination of Chlorinated Pesticides in Poultry Fat Using GPC and Optional Alumina Clean-up with GC-ECD Detection

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Introduction

Poultry Fat is routinely monitored for the presence of chlorinated pesticides. This insures that consumers are not exposed to unacceptable levels of these pesticides and that unauthorized use of these pesticides is detected. Determination of chlorinated pesticides in poultry fat requires post-extraction clean-up steps to effectively remove lipids and other co-extractives prior to analysis by gas chromatography (GC) or GC/MS. Failure to remove these compounds results in decreased column life, contamination of the ion source and decreased analytical performance.

Gel permeation chromatography (GPC) is a common tool for the post-extraction removal of high molecular weight interferents prior to pesticide analysis. GPC removes these interferents via a size separation mechanism that uses organic solvents and a hydrophobic gel (a cross-linked divinylbenzene-styrene copolymer) to separate the interferents from the lower molecular weight compounds of interest. The interferents are discarded to waste and the fraction containing the pesticides is collected for further clean-up and analysis. GPC clean-up may be followed by additional clean-up procedures such as adsorption chromatography using alumina, Florisil™ or silica.

This application note describes the use of the Gilson Automated GX-271 GPC Clean-up System to perform the post-extraction clean-up of poultry fat followed by an optional alumina clean-up step performed in a special filter rack placed on-line to the GPC eluent. Pesticide recoveries are reported and representative chromatograms are shown.

Methods – Sample Preparation

- Poultry fat prepared according to AOAC International Methods 970.52, 984.21 and USDA-FSIS Method CHC3-19
- Poultry fat was spiked with 10 ppb of the following: Lindane, Heptachlor, Aldrin, Heptachlor epoxide, alpha-Chlordane, Methoxychlor, Internal Standard 1: TCMX, Internal Standard 2: DCBP

Clean-up Conditions - Hardware

- Instrument: Gilson Automated GX-271 GPC Clean-up System
- GPC Column: Low pressure glass column filled with 60 g Envirobeads S-X3 resin in 1:1 dichloromethane:cyclohexane
- Evaporation flasks fitted with fritted filter with grooved 24/40 joint (Adams & Chittenden part no. BUCH30C24G)
- Special Collection Rack Code 1340

Automated GX-271 GPC Clean-up System with Detector

GPC Clean-up Conditions

- Mobile phase: 50:50 dichloromethane: cyclohexane
- Flow rate: 5 mL/min
- Start fraction collection: 24 min
- Fraction collection time: 26 min
- Total run time: 55 min
- Total injection of fat on column: 1 g
- Software: Gilson TRILUTION® LC version 1.4 with pre-installed GPC Clean-up Methods

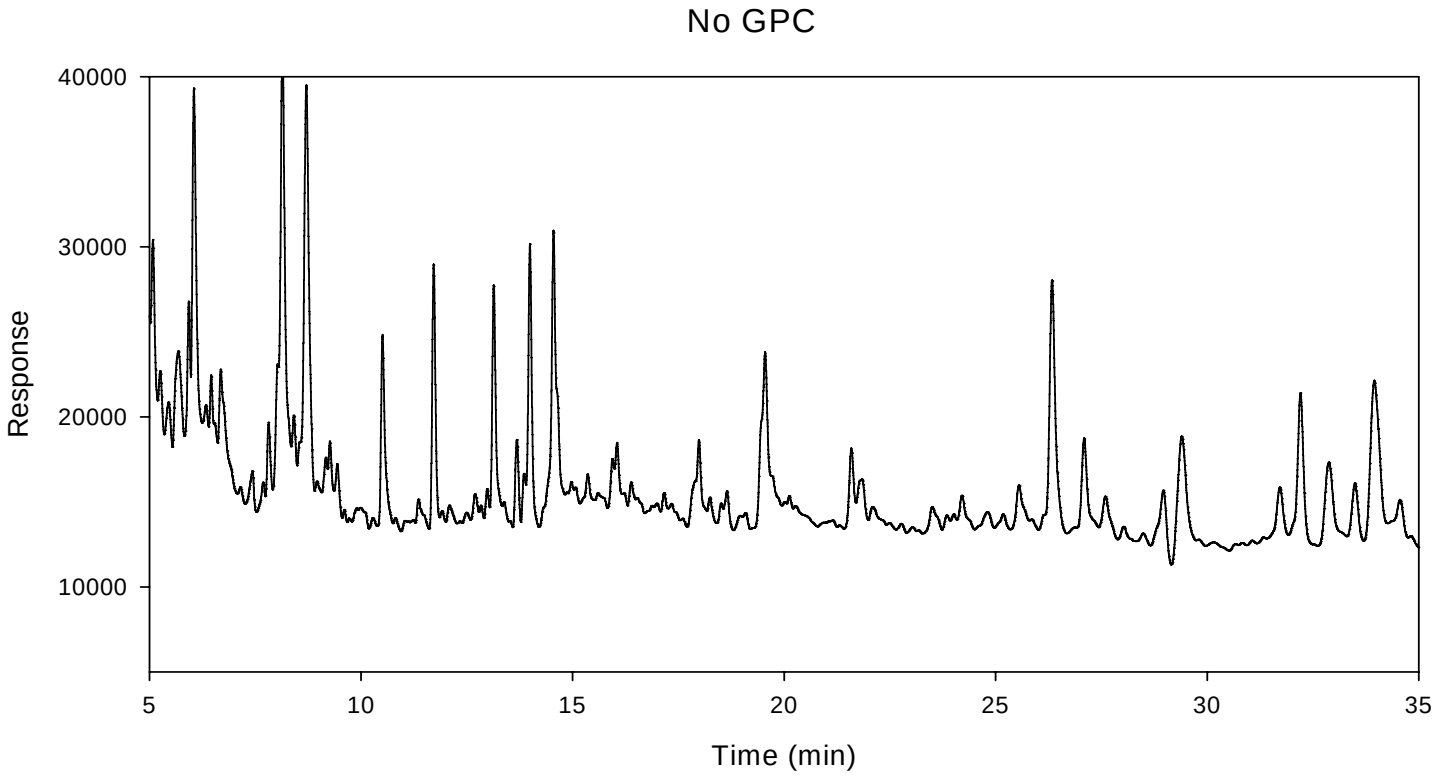

VWR
250 ml

Alumina Clean-up Step

- Alumina, Neutral Brockman Activity 1 (60-325 Mesh) [1344-28-1]-Fisher A950-500
- 2 g Total Bed Weight
- Collected GPC fraction passes over alumina bed as it elutes from the GPC column
- Cleaned extract transferred and dried down using RapidVap N2 System and reconstituted in appropriate GC mobile phase
- A “keeper” (300 µL of 2% heavy paraffin oil in isooctane) was added to the GPC collection flasks prior to GPC collection.

GC Conditions

- Agilent® Series 5890 GC with ECD Detector
- Supelco Equity-5 column (30 m x 0.53 x 0.25)
- Splitless Injection, 3 µL
- Manual Flow Control


No GPC
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Response
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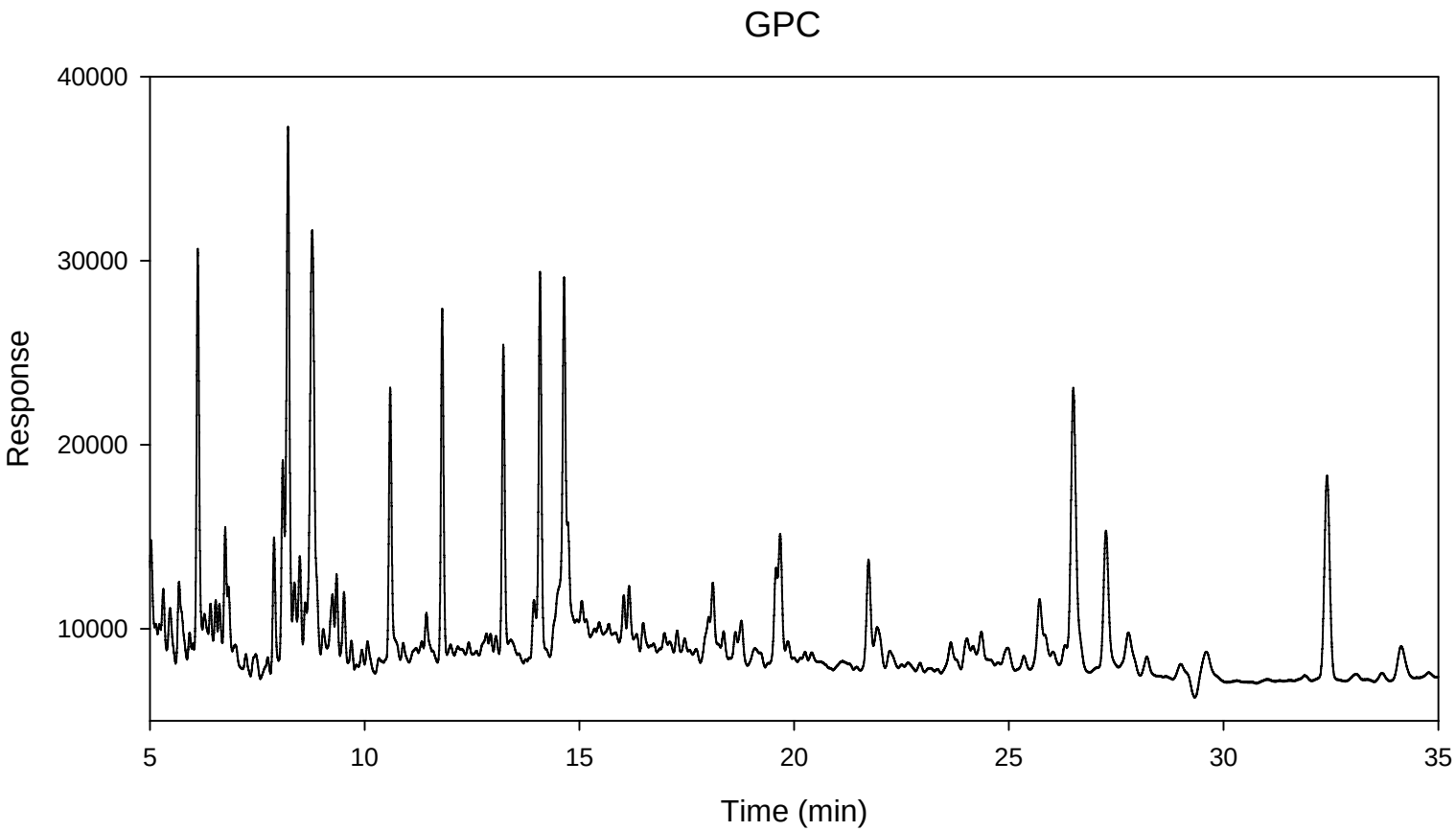
Time (min)


GPC
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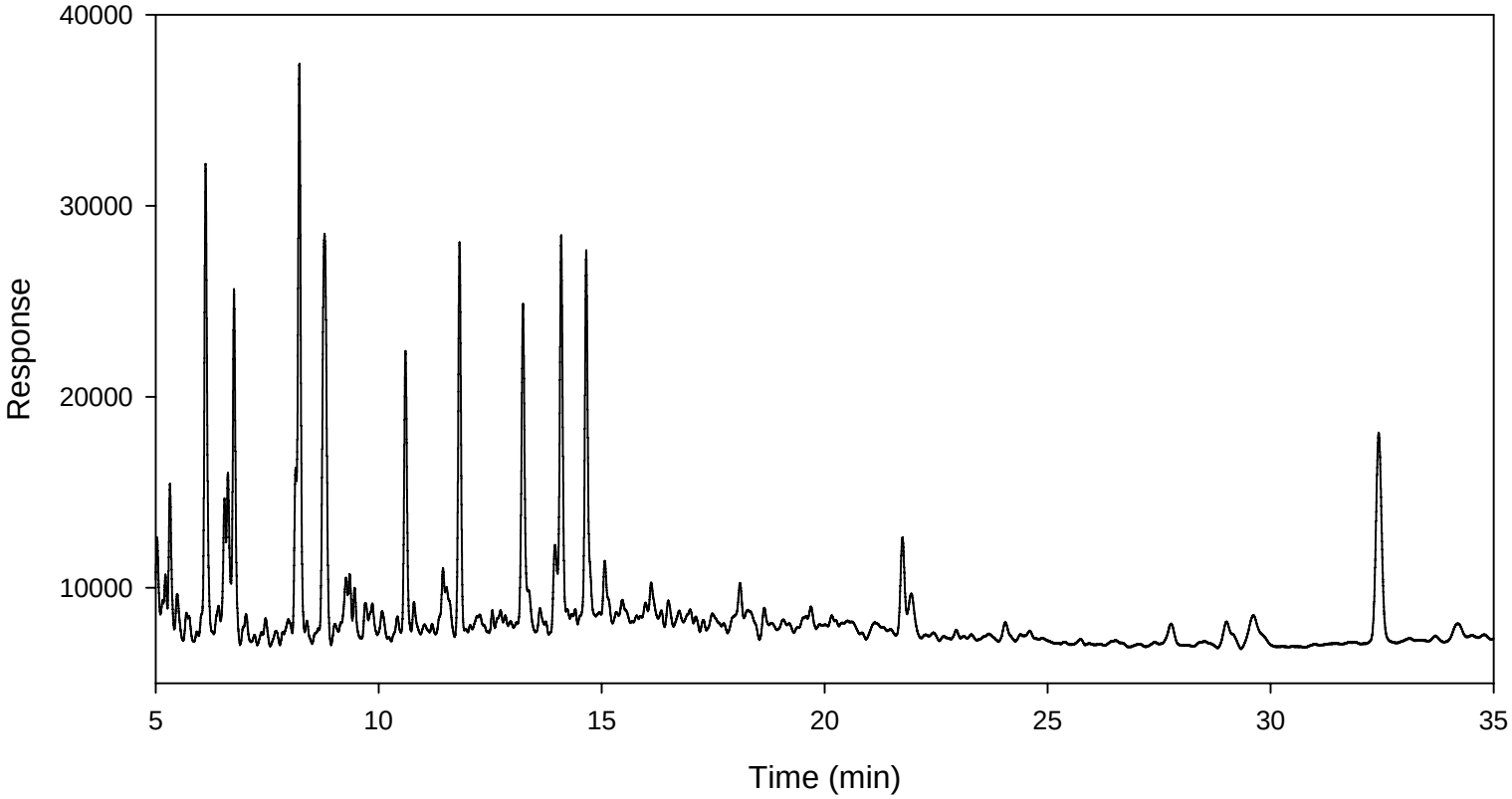
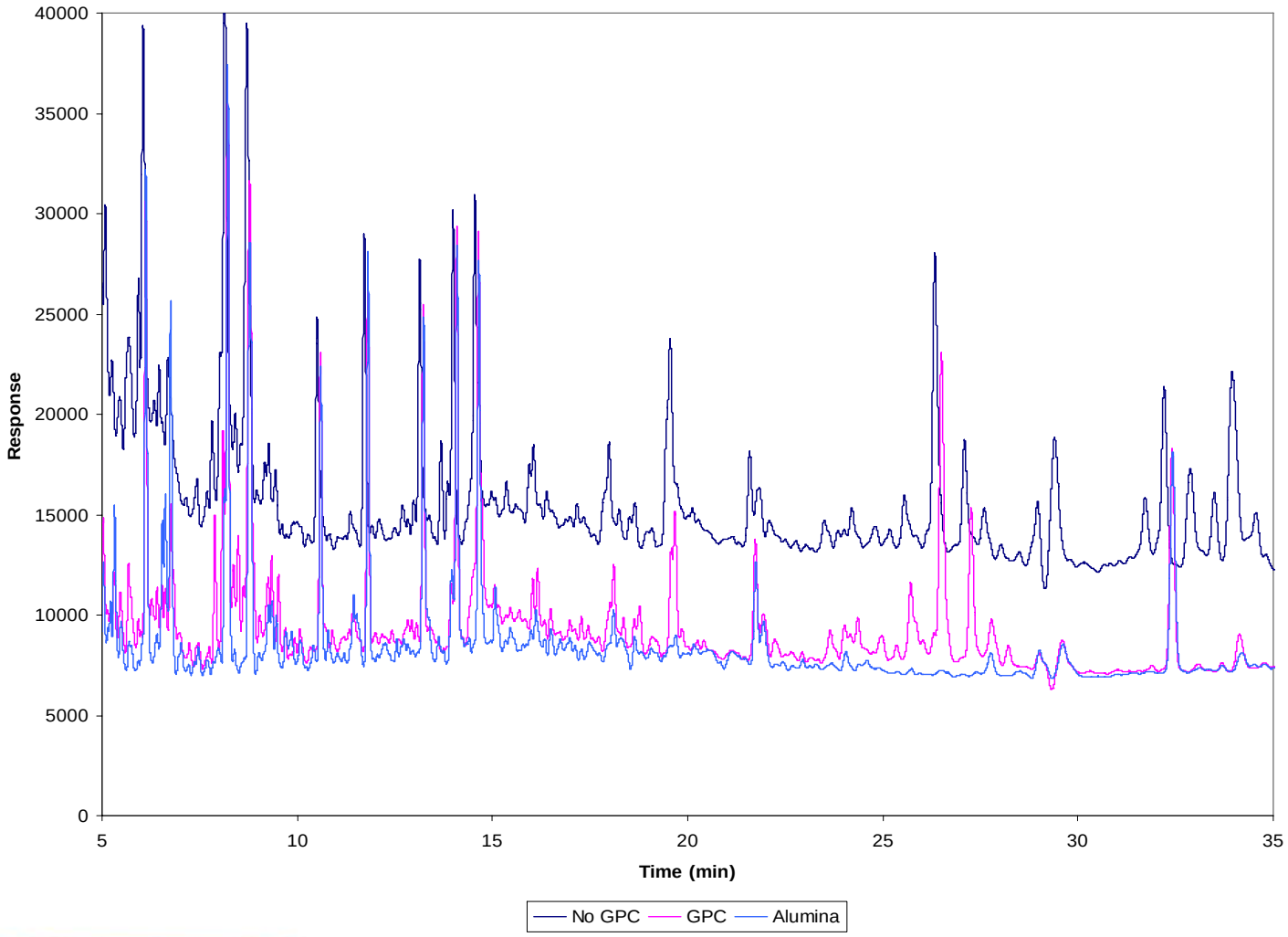
Time (min)

GPC with Alumina Bed




40000
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Response
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20
25
30
35
Time (min)
No GPC
GPC
Alumina

Retention Times of Pesticides Shown in Chromatograms Above

- TCMX (IStd 1): 6.17 min
- Lindane: 8.29 min
- Heptachlor: 10.68 min
- Aldrin: 11.89 min
- Heptachlor epoxide: 13.32 min
- Gamma-Chlordane: 14.18 min
- Alpha-Chlordane: 14.74 min
- Methoxychlor: 21.87 min
- DCBP (IStd 2): 32.59 min
- ***** Analysis was performed on different days with Manual flow control so slight variations in retention times occurred as noted on Slide 13 (chromatogram overlays).*

Compound	Observed Recovery (%)	Observed Reproducibility %CV	CHC3 Expected Recovery (%)	CHC3 Reproducibility %CV
Lindane	108	3	70-120	20
Heptachlor	96	2	70-120	20
Aldrin	96	2	70-120	20
Heptachlor epoxide	98	1	70-120	20
Gamma-Chlordane	115	3	70-120	20
Endosulfan I	79	5	70-120	20
Alpha-chlordane	101	2	70-120	20
Methoxychlor	93	3	70-120	20
2,4'-DDE	95	16	ND	ND
4,4'-DDE	97	14	70-120	20
2,4'-DDD	95	7	ND	ND
4,4'-DDD	93	6	70-120	20
2,4'-DDT	75	11	70-120	20
4,4'-DDT	93	10	70-120	20
TCMS (Internal Standard 1)	87	8	ND	ND
DCBP (Internal Standard 2)	103	4	ND	ND

Summary And Conclusions

- The utilization of post-extraction GPC clean-up offers a decrease in chromatographic baseline and signal resulting from matrix effects. This results in high recovery of organochlorine pesticides and lower detection and quantitation limits
- There was no significant difference in recovery between use of GPC alone and GPC with alumina clean-up
- The ability to add additional sorbent phases such as alumina, silica, or Florisil™ may allow for lower detection and quantitation limits
- The utilization of post-extraction GPC clean-up increased the lifetime of the GC column as well as other GC consumables such as inlet liners, seals and septa.
- The Gilson GX-271 GPC Clean-up System allowed for the automation of the GPC Clean-up process for poultry fat extracts as well as the use of additional clean-up steps. The unit could also be readily upgraded to perform solid phase extraction clean-up and liquid handling steps
- Poultry fat extraction and clean-up provides an excellent model for use with other difficult matrices, such as animal and plant tissues and soil.