Sample cleanup of olive oil samples using the Smartline GPC Cleanup Unit 6500

Abstract
This work describes the implementation of a sample cleaning method for analyzing pesticide residues in olive oil by HPLC. Pesticides were separated from the oily matrix by size exclusion gel permeation chromatography according to US EPA SW-846 Method 3640A. The GPC material used in this study was Bio-Beads SX-3 and the GPC solvent system was cyclohexane/ethyl acetate (1:1, v/v). The optimized GPC purification technique was carried out with a KNAUER Smartline GPC Cleanup Unit 6500 for automated sample cleaning.

Introduction

GPC has been extensively used as an effective post-extraction cleanup procedure for removing high molecular weight interferences such as lipids, pigments, proteins, and polymers from sample extracts. The efficiency of Bio-Beads SX-3 with an organic solvent to separate multi-pesticide residues has been extensively documented [1-3]. The GPC technique is appropriate for both polar and non-polar analytes so it can be effectively used to cleanup extracts containing a broad range of compounds. To demonstrate the flexibility of the sample cleaning method, the olive oil samples investigated were spiked with different types of organic pollutants, including PAHs, phthalates, phenols and triazine. The recovery for all of these compound classes should be higher than 70 %.

Experimental

A glass column was packed with 7 g of pre-swelled absorbent and flushed with cyclohexane/ethyl acetate (1:1, v/v) for an extended period at a flow rate of 5 ml/min. To determine the elution profile of the GPC column, a calibration solution was prepared in cyclohexane/ethyl acetate containing the following analytes: corn oil (25,000 mg/l), bis(2-ethylhexyl)phthalate (1,000 mg/l), methoxychlor (200 mg/l), perylene (20 mg/l) and sulfur (80 mg/l). The solubility of sulfur was enhanced by weighing it out in warm corn oil before mixing it with the other standards.
The calibration solution was injected after solvent flow and column pressure were established. Based on the UV trace, column eluate collection was started just before bis(2-ethylhexal)phthalate elution and after corn oil elution. Eluate collection was stopped after perylene elution. The sample loops of the GPC system were loaded with the diluted oil sample (range 1:1) to be cleaned up automatically at 1.5 ml/min. The various oil sample fractions collected were carefully evaporated under a nitrogen stream, brought up to 1 ml using a suitable solvent and filtered using a 0.45 µm syringe filter. The cleaned extracts could now be analyzed using GC, DC or HPLC techniques (not described here).

Instrumentation

This study used the KNAUER GPC Cleanup Unit 6500 which automates the GPC cleanup process. The system uses a fixed-wavelength UV detector measuring at 254 nm equipped with a semi-preparative measurement cell of 3 mm thickness. An analytical flow cell would require a much less-concentrated solution than the semi-preparative cell and would not be suitable for this purpose. The arrangement of the 15 sample loops and one wash loop avoids cross contamination. Up to 15 oil samples can be loaded in either 1 ml or 5 ml samples loops selected by two 17-port/1-channel switching valves. The pesticide fraction is collected in a round-bottomed flask between the elution of corn oil and sulfur by a third 17-port/1-channel switching valve. Figure 1 shows the chromatogram of the GPC calibration standard eluted with cyclohexane/ethyl acetate (1:1, v/v). Figure 2 shows the elution profile of one olive oil sample containing different types of pesticides.

Smartline GPC Cleanup Unit 6500, system parameters

Column:	Glass column with adjustable plunger (300 x 15 mm ID), 7 g Bio-Beads SX-3
Eluent:	cyclohexane/ethyl acetate (1:1, v/v)
Flow:	1.5 ml/min
Pressure:	12 bar
Detection:	UV at 254 nm
Sample Volume:	1 ml
Temperature:	ambient

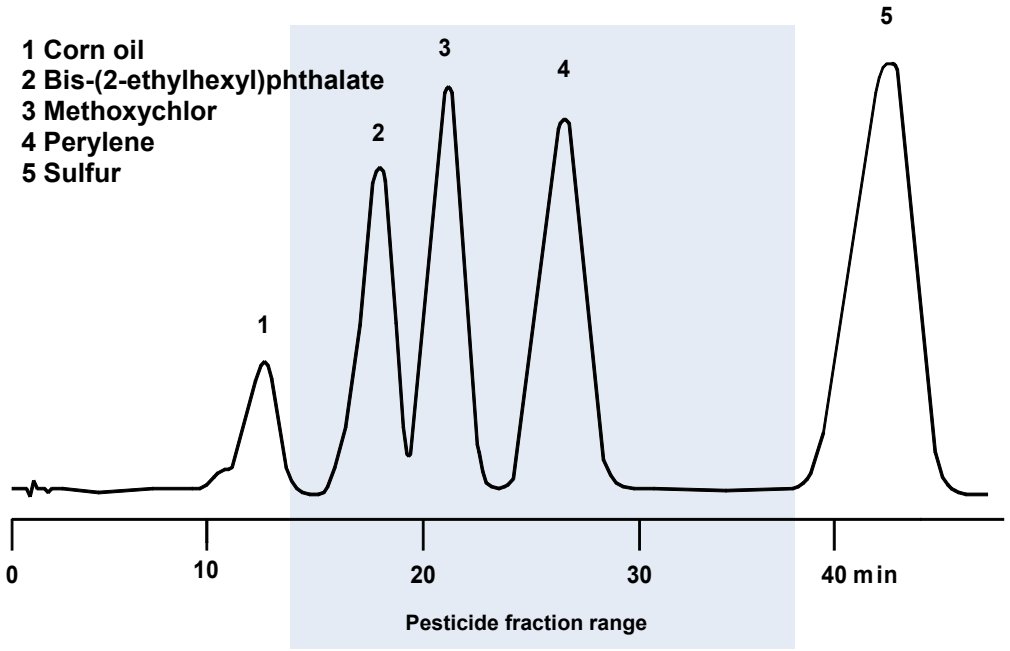

Fig. 1 Chromatogram of US EPA method 3640A calibration standard using cyclohexane/ethyl acetate (1:1, v/v)

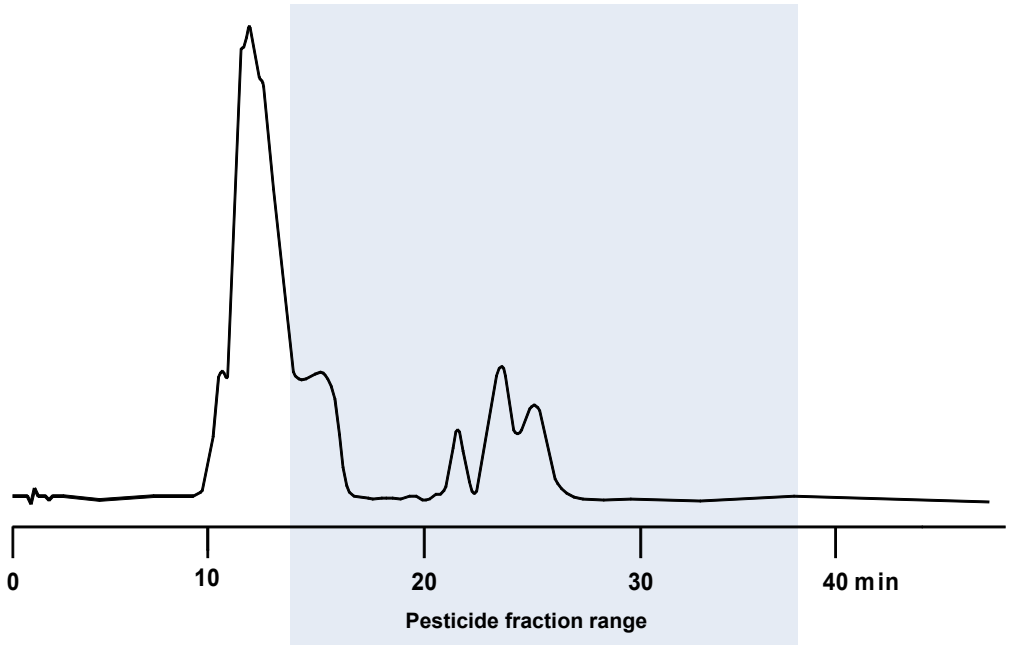

Fig. 2 Chromatogram of olive oil sample, spiked with pesticides

Conclusion

GPC sample preparation is a useful tool for separating small amounts of pesticides from high molecular weight matrices such as olive oil. The KNAUER Smartline GPC Cleanup Unit 6500 is particularly well-suited for sample preparation in pesticide analysis but can also be easily adapted to other laboratory procedures to perform a large variety of GPC sample preparation tasks.

References

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