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GPC CLEANUP OF OLIVE OIL SAMPLES

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SUMMARY

This work describes a sample cleaning method for analyzing pesticide residues in olive oil in preparation for an HPLC analysis. Pesticides were separated from the oil matrix by size exclusion/gel permeation chromatography (GPC) according to US EPA SW-846 method 3640A. The GPC material used in this study was BioBeads 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 AZURA GPC Cleanup System for automated sample cleaning.

INTRODUCTION

GPC is extensively used as an effective post-extraction cleanup procedure for removing high molecular weight interferences such as lipids, proteins, and polymers from sample extracts. The efficiency of BioBeads 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 compounds.

RESULTS

Fig. 1 shows the chromatogram of the GPC calibration standard eluted with cyclohexane/ethyl acetate (1:1, v/v). The three detected pesticides were baseline separated and could be identified easily. **Fig. 2** shows the elution profile of one olive oil sample containing different types of pesticides. It can be seen that all pesticides were detected with the US EPA method 3640A. Compared to the measurement of the standard solution, the spiked sample showed less matrix effects. This means that all interfering high molecular elements were removed during clean up. The recovery for all of these compound classes was higher than 70 %.

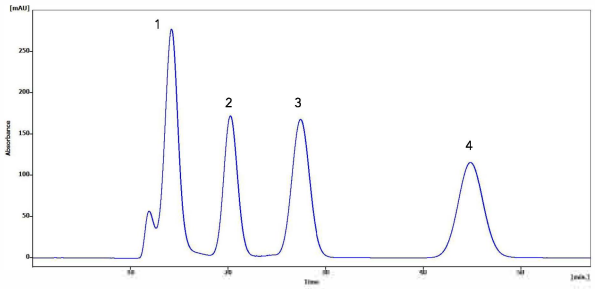

Fig. 1 Chromatogram of US EPA method 3640A calibration standard containing 1. Corn oil matrix, 2. Bis-(2-ethylhexyl)phthalate, 3. Methoxychlor, 4. Perylene

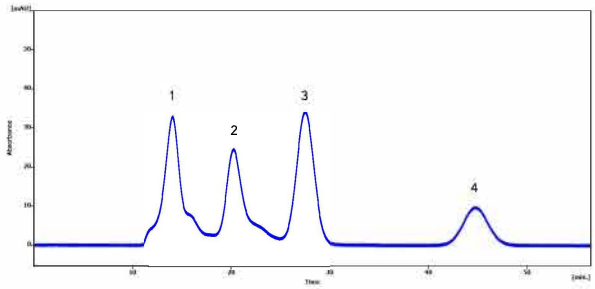

Fig. 2 Chromatogram of olive oil sample, spiked with pesticides: 1. Olive oil matrix, 2. Bis-(2-ethylhexyl) phthalate, 3. Methoxychlor, 4. Perylene

MATERIALS AND METHOD

This study used the KNAUER AZURA GPC Cleanup System which automates the GPC cleanup process. The system comprising of the two AZURA ASM 2.1L Assistant modules with different valves, a pump, and a UV detector. The compunkds were detected at 254 nm wavelength with the AZURA UVD 2.1 S UV detector with 10 Hz data rate. The two 16-port multiposition valves used here enabled the loeading of up to 15 oil samples (1 mL or 5 mL samples loops). Moreover, the pesticide fraction was collected in a round-bottomed flask between the elution of corn oil by a third 16-port multiposition valve.

The glass column with BioBeads SX-3 was 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 g/L), bis(2-ethylhexyl)phthalate (1 g/L), methoxychlor (0.2 g/L), and perylene (0.02 g/L). The calibration solution was injected after solvent flow and column pressure were established. The eluates were collected based on the UV traces of the four eluates. For further analysis purposes with GC, DC or HPLC techniques (not described here), the various oil sample fractions collected were carefully evaporated under a nitrogen stream, dispensed in 1 mL of a suitable solvent and filtered using a 0.45 µm syringe filter.

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 AZURA GPC Cleanup System 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. The arrangement of the 15 sample loops and one wash loop avoids cross contamination hence allowing a robust sample preparation procedure.

REFERENCES

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[3] Guardia-Rubio, M; Fernandez-De Cordova, M.L.; Ayory-Canada, M.J. and Ruiz-Medina, A. Simplified pesticide multi-residue analysis in virgin olive oil by gas chromatography with thermoionic specific, electron-capture and mass spectrometric detection. J. of Chrom. A (2006) Volume 1108, 231-239

Additional information:

VFD0153

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ADDITIONAL MATERIALS AND METHODS

Tab. A1 Sample preparation

Standards	**Prepared and diluted with Cyclohexane/ethyl acetate (1:1, v/v)**
1. Corn oil	25 g/L
2. Bis-(2-ethylhexyl)phtalate	1 g/L
3. Methoxychlor	0.2 g/L
4. Perylene	0.02 g/L

Tab. A2 Method parameters

Eluent A	Cyclohexane/ethyl acetate (1:1, v/v)		
Isocratic	Time [min]	% A	% B
	0	100	0
	60	100	0
Flow rate	5 mL/min	System pressure	0.6 bar
Column temperature	25°C	Run time	60 min
Injection volume	1 mL	Injection mode	Full loop
Detection wavelength	254 nm	Data rate	10 Hz
		Time constant	0.10s

Tab. A3 System configuration & data

Instrument	**Description**	**Article No.**
Assistant 1	AZURA ASM 2.1L, **left:** single variable wavelength UV detector **middle:** 6 port 2 position injection valve, 1/16" connectors **right:** Pump with pressure sensor, 10 mL pump head, SSt	AYCAEABA
Assistant 2	AZURA ASM 2.1L, **left:** 16 port multi position valve, 1/16" connectors **middle:** 16 port multi position valve, 1/16" connectors **right:** 16 port multi position valve, 1/16" connectors	AYGAGAGA
Flow cell	UV, 3mm, 2 µL	A4042
GPC tubing guide	16 sample loops with 1 ml	A5329-2
Software	ClarityChrom	A1670-9
Column	BioBeads SX-3	B41
Injection valve	Manual injection valve 6-Port/3-position, 1/16" connectors	AVI26BC