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Technical Report

Determination of Phthalate Esters in Vegetable Oils Using Direct Immersion Solid-Phase Microextraction and Fast GC Coupled with Triple Quadrupole MS

SPME followed by fast GC-QqQ MS for phthalates determination in vegetable oils

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Abstract:

A simple, low-solvent consuming and sensitive solid-phase microextraction method followed by fast GC-QqQ MS determination was developed for the qualitative and quantitative analysis of phthalates in vegetable oils. Multiple reaction monitoring (MRM) acquisition mode was applied to improve sensitivity. A rapid liquid-liquid extraction was performed using acetonitrile to remove the bulk of triglycerides, before immersing the fiber. A preliminary comparison between a polydimethylsiloxane (PDMS) and a Carbopack Z/PDMS fiber was carried out both in head-space (HS) and direct immersion extraction modes. PDMS in direct immersion extraction mode showed the best performance. The method was fully validated, obtaining good performance characteristics, and applied to analyze different vegetable oil samples.

Keywords: SPME, fast GC, phthalates, vegetable oils

1. Introduction

Phthalates (PAEs) are a group of synthetic compounds mainly used as plasticizers, classified as endocrine-disrupting chemicals and potential human-cancer causing agents. PAEs present in plastic materials can be lost over time, since they are not chemically bound to the polymeric matrix. They can be found in high amounts in foods, deriving mainly from direct migration from packaging films, especially into fatty food due to the liphophilic nature of PAEs. No regulations about PAEs content in food are available, but Directive 2007/19/EC refers to PAEs migration from food contact material setting a list of substances allowed in such materials. Due to PAEs ubiquity, their analytical determination is very challenging; thus, a minimal sample manipulation is highly desirable. Many methods have been proposed to analyze PAEs in foods and vegetable oils, mainly followed by a gas chromatographic-mass spectrometric (GC-MS) determination. The main point of sample preparation is basically the removal of triacylglycerols (TAGs) and free fatty acids interferences. Head space solid-phase microextraction (HS-SPME) has been investigated to minimize sample manipulation in the preparation step for PAEs analysis in vegetable oils, but not very low quantification limits were achieved. The aim of this work was to optimize a rapid and simple SPME method, minimizing sample manipulation, followed by a fast GC-triple quadrupole (QqQ) MS determination.

2. Experimental

2-1. Reagents and materials

HPLC-grade acetonitrile (ACN), acetone and *n*-hexane (*n*-Hex) were used as solvents. A stock solution (10,000 mg/L) was prepared mixing dimethyl phthalate (99%, DMP), diethyl phthalate (99.5%, DEP), dipropyl phthalate (DPP), diisobutyl phthalate (DiBP), dibutyl phthalate (99%, DBP), benzylbutyl phthalate (>98%, BBP), dicyclohexyl phthalate (99%, DCHP), dietylhexyl phthalate (DEHP), diisononyl phthalate (99%, DiNP), diisodecyl phthalate (>98%, DiDP). A sample of extra virgin olive oil (EVO) was spiked with 2 mg/kg of the PAEs standard solution, and it was used for method optimization, because of the lack of edible oil or fat standards certified for plasticizers. Special care was taken with solvents, all the glassware, caps and septa before use.

2-2. Extraction methods comparison

HS-SPME: 1 g oil + 1 mL of methanol (matrix modifier) in a 4-mL vial, and incubation for 60 min at 40°C under continuous stirring. Extraction in HS mode by exposing both the PDMS and the Carbopack Z/PDMS fibers for 20 min.

Direct Immersion-SPME: 0.5 g oil + 3 mL of ACN (30 s vortex and centrifuge) of which 2.5 mL was back-extracted in 4 mL of *n*-Hex. The Carbopack Z/PDMS fiber was then directly immersed into 1.5 mL of *n*-Hex (in a 2-mL vial) for 30 min under stirring. PDMS fiber cannot be immersed in *n*-Hex, therefore a further comparison was carried out by immersing both fibers directly in ACN.

A comparison between the LOQ values (mg/kg, calculated as 10 times S/N), using PDMS and Carbopack Z (Car Z)/PDMS both in HS and in Direct Immersion modes is reported in Table 1.

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Table 1 Comparison between the LOQ values (S/N) using PDMS and Carbopack Z (Car Z)/PDMS both in HS and in Direct Immersion modes.

PAE	Abbreviation	HS		Direct Immersion		
				n-Hex	ACN	
		PDMS	Car Z/PDMS	Car Z/PDMS	Car Z/PDMS	PDMS
Dimethyl phthalate	DMP	0.187	2.066	0.052	0.816	0.053
Diethyl phthalate	DEP	0.575	1.300	0.053	0.261	0.044
Dipropyl phthalate	DPP	1.673	—	0.207	0.485	0.043
Diisobutyl phthalate	DiBP	0.364	0.229	0.038	0.093	0.019
Dibutyl phthalate	DBP	0.110	0.167	0.621	0.250	0.018
Benzyl butyl phthalate	BBP	1.564	—	1.842	0.550	0.111
Dicyclohexyl + Diethyl hexyl phthalate	DCHP	—	—	2.148	0.990	0.044
Diisononyl phthalate	DEHP	0.668	1.085	0.336	0.409	0.026
Diisodecil phthalate	DiNP+DiDP	—	—	—	3.569	0.138

2-3. Samples and sample preparation

Eight vegetable oils, namely 2 EVOs, 1 olive oil, 1 peanut oil, 2 sunflower oils, 1 soybean oil, and 1 mixed seeds oil, were purchased from a local market in Messina (Italy).

The optimized procedure is: 0.5 g oil was weighed into a 10-mL glass centrifuge tube, added with 3 mL of ACN and intensively shaken in a vortex mixer for 30 s. After centrifugation (10 min at 3000 rpm), 1.5 mL of the ACN extract was collected into a 2-mL vial and extracted by immersion of a PDMS fiber (100 µm thickness, provided by Sigma-Aldrich/Supelco, USA) for 20 min under stirring (500 rpm).

2-4. Instrumentation (Shimadzu)

- GC-2010 Plus Gas Chromatograph.
- GCMS-TQ8040 Triple Quadrupole Mass Spectrometer.

2-5. Chromatographic method

Column	: SLB-5ms 10 m × 0.1 mm ID × 0.1 µm *df* column [silphenylene polymer virtually equivalent in polarity to poly (5% diphenyl/95% methylsiloxane)] (Supelco, Milan, Italy)
GC oven	: from 90°C (5 min) to 310°C at 30°C/min from 310°C to 350°C (3 min) at 50°C/min
Carrier gas	: Helium
Inlet pressure	: 378.8 kPa (constant linear velocity mode)
Injection	: splitless (5 min sampling time), then split 1:20

2-6. Software

- GCMSsolution software ver. 4.20

Table 2 MRM transitions and collision energies (CE).

Compound	Time window		MRM 1	CE	MRM 2	CE
	Start	End	*m/z*		*m/z*	
DMP	6.00	7.50	163>77	20	163>133	10
DEP	7.50	8.15	149>65	25	149>93	15
DPP	8.15	8.85	149>65	25	149>93	20
DiBP	8.85	9.15	149>65	25	149>93	15
DBP	9.15	10.00	149>65	25	149>93	20
BBP	10.00	10.75	149>65	20	149>93	15
DCHP + DEHP	10.75	11.15	149>65	20	149>93	20
DiNP	11.15	14.00	149>65	20	293>167	5
DiDP	11.15	14.00	149>65	20	307>167	5

2-7. MS parameters

MS ionization mode	: electron ionization
Acquisition mode	: multiple reaction monitoring (MRM)
Acquisition frequency	: 10 Hz
Mass range	: 45–360 *m/z*
MS loop time	: 0.1 s
Ion source temperature	: 220°C
Interface temperature	: 280°C
Collision gas	: argon (200 kPa)
MRM transitions	: Table 2

3. Results and discussion

3-1. Method optimization

The low-polarity column employed allowed a proper separation of all target PAEs, except for the DiNP and DiDP, which consisted of partially co-eluted humps (quantified together) due to the presence of many isomers; therefore they were quantified together as a sum. A MRM chromatogram of a spiked EVO sample is shown in Fig. 1. The extraction step was optimized focusing on fiber sorption times, desorption time and temperature, and oil/ACN. In particular, different fiber sorption times, namely 10, 20 and 30 min, were tested. The extraction efficiency and the repeatability values (n=3) were much better using 20 min sorption time.

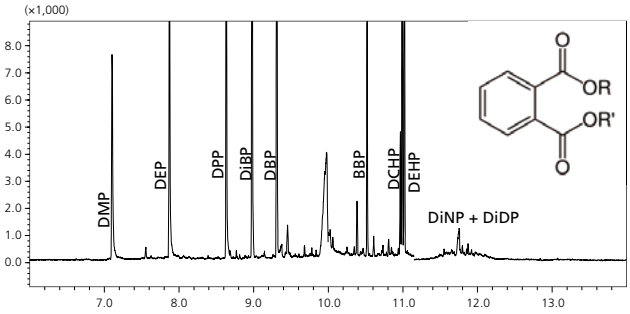

Fig. 1 MRM chromatogram of an EVO spiked with PAEs.

Moreover, considering a 16 min total GC run, plus a few minutes of cooling of the system prior to be ready for the following analysis, 20 min of fiber sorption time was the perfect choice to synchronize the sample preparation time with the instrument run time (Fig. 2).

Desorption of the fiber in the injector was performed testing different temperatures, namely 250, 270 and 280°C. Desorption at 280°C gave higher signals and guaranteed no carry-over effect (Fig. 3).

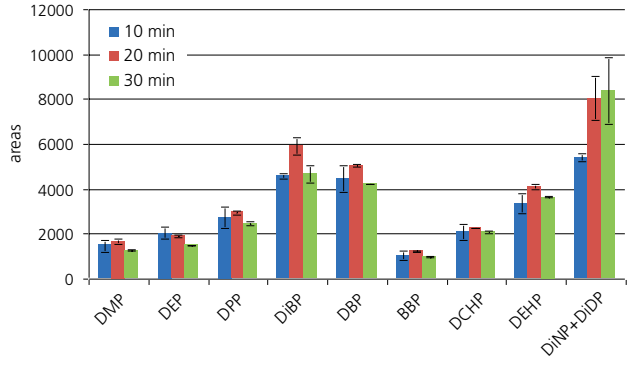

Fig. 2 Graphical comparison among areas obtained by using different fiber exposition times.

Finally, different amounts of oil, namely 0.5, 1 and 2 g, were extracted with 3 mL ACN to assess the better oil/solvent ratio. Data suggested a less favorable partition ratio between oil and solvent using 1 and 2 g of oil in 3 mL of ACN. Therefore, 0.5 g was maintained as the sample amount to minimize sample consumption, optimize the partition ratio, and extend the dynamic range.

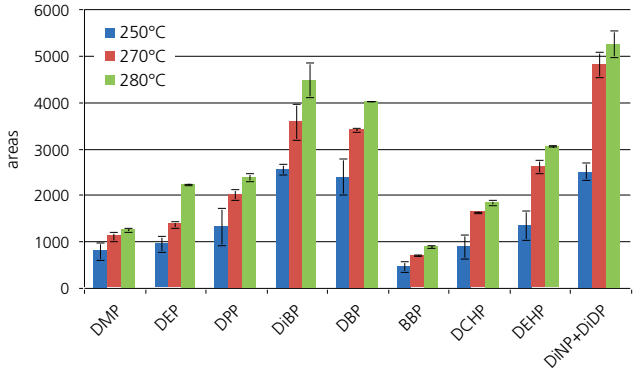

Fig. 3 Graphical comparison among areas obtained by using different desorption temperatures.

3-2. Method validation

Linearity was calculated constructing a 6-point calibration curve (n=3) by analyzing a spiked EVO sample at increasing concentrations (0.02, 0.05, 0.1, 2, 5 and 10 mg/kg). Intra- and inter-day repeatability (CV%) was calculated analyzing a spiked EVO sample, at a level of 2 mg/kg for each PAE.

Accuracy was determined as relative error deviation (A%) between the values observed in the spiked sample (4 mg/kg) and the expected values. All the figures-of-merits are summarized in Table 3 along with the LOQ values, evaluated both as 10 times the signal-to-noise ratio (S/N) and according to the EuraChem Guidelines.

The evaluation of LOQ values by using S/N is manly related to the specific method applied, while the LOQs calculated according to the EuraChem Guidelines include also the unavoidable blank problem due to the ubiquity occurrence of PAEs.

Table 3 *Figures-of-merits* of the proposed method.

PAE	Linearity R^2	Intra-day repeatability CV% (n=3)	Inter-day repeatability CV% (n=6)	Accuracy A% (n=3)	LOQ (mg/kg) S/N	LOQ (mg/kg) EuraChem
DMP	0.9989	4.7	7.0	2.1	0.018	0.055
DEP	0.9994	4.0	5.4	8.0	0.020	0.057
DPP	0.9989	7.4	7.9	7.7	0.018	0.035
DiBP	0.9962	2.3	5.9	10.2	0.015	0.523
DBP	0.9989	3.2	4.8	7.6	0.018	0.064
BBP	0.9991	9.0	9.6	9.7	0.047	0.156
DCHP	0.9994	1.0	6.1	–1.0	0.015	0.025
DEHP	0.9991	7.8	8.0	–1.3	0.016	0.292
DiNP+DiDP	0.9970	9.5	11.9	–11.8	0.144	0.157

Table 4 PAEs (mg/kg) found in different vegetable oils analysed with the optimized method.

Samples	EVO1 (aluminium)	EVO2 (glass)	Olive (glass)	Sunflower1 (plastic)	Sunflower2 (plastic)	Soybean (plastic)	Peanut (plastic)	Mix seeds (plastic)
DMP	<LOQ[a]	<LOQ[a]	<LOQ[a]	0.04*	<LOQ[a]	<LOQ[a]	<LOQ[a]	<LOQ[a]
DEP	0.23	<LOQ[a]	<LOQ[a]	0.07	0.33	0.63	0.32	0.89
DPP	<LOQ[a]	<LOQ[a]	<LOQ[a]	0.24	0.019*	<LOQ[a]	0.02*	<LOQ[a]
DiBP	0.95	<LOQ[a]	0.94	<LOQ[a]	0.59	3.22	1.29	3.91
DBP	0.127	<LOQ[a]	<LOQ[a]	<LOQ[a]	<LOQ[a]	0.21	0.05*	0.62
BBP	<LOQ[a]	<LOQ[a]	<LOQ[a]	0.30	<LOQ[a]	<LOQ[a]	<LOQ[a]	0.099*
DCHP	<LOQ[a]	<LOQ[a]	<LOQ[a]	0.14	<LOQ[a]	<LOQ[a]	<LOQ[a]	<LOQ[a]
DEHP	0.62	0.31	2.34	<LOQ[a]	0.11*	0.45	0.58	2.51
DiNP+DiDP	5.99	7.21	1.074	<LOQ[a]	<LOQ[a]	0.21	<LOQ[a]	0.31
Total	7.92	7.52	4.36	0.80	1.05	4.71	2.27	8.33

LOQ[a]: calculated as 10 times the signal-to-noise ratio; *: values below the LOQ calculated according to EuraChem Guidelines.

3-3. Real-world samples

The Direct Immersion-SPME-GC QqQ MS optimized method was applied to analyze PAEs in different kinds of vegetable oil samples. Table 4 shows all the PAEs amounts (expressed as average of two replicates), subtracted from the contamination deriving from the sample preparation procedure, along with information on packaging material.

The olive-derived oils were more contaminated than seed oils (except for the mixed seed oil sample), in agreement with data reported in literature. For these samples, the main contribution was due to DiNP+DiDP, confirming their growing diffusion as DEHP substitutes.

4. Conclusions

A rapid, easy and sensitive method for PAEs analysis in vegetable oils was developed. After a rapid LLE extraction method to remove the bulk of TAGs, the application of the PDMS fiber in direct immersion mode, gave good performance characteristics, in terms of linearity repeatability, accuracy, a limit of quantification. No detrimental effect on the selectivity uptake neither on the separation performance of the column were observed even after more than 300 analyses.

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