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Determination of alkylphenols and phthalate esters in vegetables and migration studies from their packages by means of stir bar sorptive extraction coupled to gas chromatography–mass spectrometry

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ABSTRACT

This paper describes a method for the determination of three alkylphenols (APs), 4-*tert*-octylphenol (tOP), 4-*n*-octylphenol (OP) and 4-nonylphenol (NP), and six phthalate esters (PEs), dimethylphthalate (DMP), diethylphthalate (DEP), di-*n*-butylphthalate (DBP), *n*-butylbenzylphthalate (BBP), di-2-ethylhexylphthalate (DEHP) and di-*n*-octylphthalate (DOP), in vegetables using stir bar sorptive extraction (SBSE) in combination with thermal desorption–gas chromatography–mass spectrometry (TD–GC–MS). Ultrasonic radiation was used to extract the analytes from the solid food matrix, and the extract obtained was preconcentrated by SBSE. The different parameters affecting both stages were carefully optimized. The method was applied to analyze commercial vegetables, in the form of plastic packed salads and canned greens, as well as the corresponding filling liquids of the canned food. Quantification of the samples was carried out against aqueous standards using an internal standard (anthracene). The analysis of a 2 g vegetable sample provided detection limits between 12.7 and 105.8 pg g^{-1} for OP and DEHP, respectively. Migration studies from the plastic packages of the vegetables samples analyzed were carried out. DEP, DBP and DEHP were found to have migrated from the bags to the simulant and the same compounds were quantified in lettuce, corn salad, arugula, parsley and chard, at concentration levels in the 8–51 ng g^{-1} range. However, OP and NP were found in only two vegetable samples and one filling liquid, but neither was detected in any package. The proposed method provided recoveries of 83–118%.

1. Introduction

Fruits and vegetables play a relevant role in human diet, since their intake ensures an adequate supply of biologically active compounds such as vitamins, minerals, antioxidants and fibers. The importance of a suitable nutrition, along with the growing interest for fresh vegetables, has resulted in the development of a market share for fresh ready-to-eat (RTE) vegetables (bagged salads, pre-packed mixed vegetables, etc.) [1,2]. These products receive some technological processing before their commercial distribution, taking into account that they are usually consumed without any washing or additional preparation [1]. Consequently the potential chemical contaminants that may be incorporated to the food until its consumption have to be controlled.

Endocrine disrupter chemicals (EDCs) are compounds of known toxicity even at low concentrations, which are able to mimic or block the action of natural hormones affecting the normal biological function in animals and humans [3]. Alkylphenols (APs) and phthalate esters (PEs) are EDCs commonly used in different industrial areas. APs are degradation products of alkylphenol polyethoxylates (APEOs), which are added as non-ionic surfactants to cleaning agents, including food detergents. The use of detergents containing nonylphenolethoxylates or octylphenolethoxylates to wash vegetables prior to their package can led to contamination by their degradation products, whose estrogenic activity has been proved [3–5] and their sale and use legislated in 2005 [6]. PEs are a numerous group of chemicals used in everyday life because of their wide variety of applications, being the most important by far as plasticizer agents in polymer industry [7]. The migration of PEs from plastic materials to the food in contact to has been widely reported [8–12], even in the case of polyethylene film bags [13] similar to those used in RTE vegetables. The residual moisture of vegetables and the large surface/weight ratio may facilitate the migration of these species. Considering the toxicological effects of PEs, they were forbidden in materials to come into contact with foodstuffs [14].

Gas chromatography (GC) and liquid chromatography (HPLC) coupled to mass spectrometry (MS) are the most widely used techniques in trace analysis of APs and PEs. The low quantification limits required for the analysis of these compounds in different types of samples, environmental, biological or food matrices, makes

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necessary the inclusion of enrichment methods in the whole procedure. Environmentally friendly sample preparation techniques have replaced classical methods. In this sense, the application of solid-phase microextraction (SPME) [15–19], stir bar sorptive extraction (SBSE) [20–28] and several liquid–liquid microextraction techniques (LLME) as dispersive liquid–liquid microextraction (DLLME) [29–32], ultrasound-assisted emulsification-microextraction (USAEME) [33] and solidification of floating organic microdrop (SFDME) [34,35] satisfies the primary objectives of green analytical chemistry, and have been employed for the determination of APs or PEs in water or aqueous samples. The determination of APs and PEs in vegetables requires an extraction step prior to preconcentration. Steam distillation [5,36], Soxhlet extraction [37,38] and bath ultrasound assisted [39–42] have been applied in these complex matrices.

This paper deals with the determination of nine EDCs, three APs, 4-*tert*-octylphenol (tOP), 4-octylphenol (OP) and 4-nonylphenol (NP) and six PEs, dimethylphthalate (DMP), diethylphthalate (DEP), di-*n*-butylphthalate (DBP), *n*-butylbenzylphthalate (BBP), di-2-ethylhexylphthalate (DEHP) and di-*n*-octylphthalate (DOP), in RTE vegetables, which to the best of our knowledge has not been carried out by extracting them by ultrasounds applied by means of a probe directly immersed into the sample mixture and preconcentration and separation by the combination SBSE–TD–GC–MS.

2. Experimental

2.1. Reagents

A standard stock solution containing six phthalates esters, dimethylphthalate (DMP), diethylphthalate (DEP), di-*n*-butylphthalate (DBP), *n*-butylbenzylphthalate (BBP), di-2-ethylhexylphthalate (DEHP) and di-*n*-octylphthalate (DOP), in methanol at 200 mg L^{-1} per compound was purchased from Supelco (Bellefonte, PA, USA). 4-*Tert*-octylphenol (tOP), 4-*n*-octylphenol (OP) and 4-*n*-nonylphenol (NP), with purities in the range 97–99.7%, were also provided by Supelco. Anthracene (IS), with a purity of 99.5% was supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). Stock solutions of the alkylphenols (1000 mg L^{-1}) were prepared by dissolving the commercial products, without previous purification, in methanol. Solutions were kept at −10 °C in the dark. Working standard solutions were prepared daily by diluting with Milli-Q water.

Sodium chloride was obtained from Sigma (St. Louis, MO, USA). Analytical-reagent grade methanol, acetonitrile and acetone were purchased from Lab-Scan (Dublin, Ireland). Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

Stir bars (Twisters supplied by Gerstel, Mullheim an der Ruhr, Germany) with PDMS coating film (0.5 mm thick, 10 mm length, 24 μL) were used. Prior to use, the stir bars were conditioned in an empty thermal desorption tube at 275 °C for 0.5 h with helium at a flow desorption rate of 50 mL min^{-1}. The stir bars could be used more than 50 times after a suitable reconditioning process (as recommended by the manufacturer). All analyses were performed in 15 mL glass vials and the solutions were stirred with a fifteen position magnetic stirrer (Velp Scientifica, Usmate, Italy). Coated stir bars were thermally desorbed using an automated TDU-2 thermal desorption unit (Gerstel) connected to a programmed temperature vaporization (PTV) injector CIS-4 (Gerstel) by a heated transfer line. The CIS-4 was equipped with a deactivated empty glass liner with baffles. This injection system was mounted on an Agilent 6890N (Agilent, Waldbronn, Germany) gas chromatograph coupled to an Agilent 5973 quadrupole mass selective spectrometer equipped with an inert ion source.

Table 1
Retention times and target and qualifier ions for the analytes.

Compound	t_R, min	T	Q_1 (Q_1/T %)	Q_2 (Q_2/T %)
DMP	6.8	163	194 (6.3)	
tOP	7.2	135	107 (13.1)	206 (3.6)
DEP	7.3	149	177 (23.8)	222 (2.3)
OP	8.0	107	206 (14.9)	
IS	8.2	178	152 (9.8)	
NP	8.45	107	220 (15.2)	
DBP	8.73	149	223 (6.3)	278 (<1)
DEHP	9.9	149	167 (31.2)	279 (11.2)
BBP	10.5	149	206 (26.1)	312 (<1)
DOP	11.76	149	279 (8.4)	390 (<1)

The analytes were desorbed in the splitless mode and applying the following desorption temperature program: start temperature 50 °C, increased to 250 °C at 200 °C min^{-1} and held 10 min. Meanwhile, the desorbed compounds were trapped on the baffled liner in the CIS-4 injector, which was maintained at 25 °C by means a Peltier unit. After the thermodesorption step, the PTV CIS-4 was heated at 250 °C min^{-1} to 275 °C, which was maintained for 5 min. Other conditions in the TDU were: vent flow 30 mL min^{-1} and vent pressure 13.5 psi. Injection into the GC was performed in the splitless mode (9 min splitless time). Analytes were separated on a DB-17MS (50% diphenyl–50% dimethylpolysiloxane, Agilent) capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness). The oven temperature was programmed as follows: start at 75 °C for 0.5 min, increase to 200 °C at 25 °C min^{-1}, increase to 275 °C (held 5 min) at 50 °C min^{-1}, in a total run time of 12 min. The helium carrier gas in column was maintained at a constant flow of 1 mL min^{-1}. The transfer line, ion source and quadrupole analyzer temperatures were maintained at 300, 230 and 150 °C, respectively. The mass spectrometer was operated using electron-impact (EI) mode (70 eV). The compounds were quantified in the selected ion monitoring (SIM) mode in order to improve the detection limits using the target ion (Table 1). Identification was confirmed by the retention time of the target ion and the qualifier-to-target ion ratios for each compound.

An ultrasonic probe processor UP 200H (Dr. Hielscher, Germany) was used to extract the analytes from food samples.

2.3. Samples and analytical procedure

A total of twelve vegetable samples, eight packed in plastic bags (lettuce, corn salad, arugula, parsley, red cabbage, carrot and two different samples of chard) and the rest canned (artichokes, mushroom, corn and peas), were obtained from a local supermarket. All analyses were carried out before the expiry date printed on the package. Vegetables were placed in glass beakers, frozen at −18 °C and then chopped to produce a homogenized product. The packaging bags were cut with scissors into small pieces (~30 mg).

All glassware used was washed with acetone, rinsed with hexane and dried at 80 °C for at least 2 h, in order to avoid phthalate contamination. Direct extraction by immersing the stir bar into a suspension containing the vegetable solid sample is not possible and so a previous extraction step was carried out, submitting the reconstituted liquid extract to the SBSE procedure. For this, 10 mL of methanol was added to 2 g of vegetable (1 g for samples with high analytes content) in a 15 mL vial. The mixture was sonicated for 1 min (30% amplitude, 0.5 cycles per second) by means of a probe directly immersed into the sample mixture. The mixture was centrifuged for 5 min at 2000 rpm and the supernatant was concentrated to almost dryness using a rotator vacuum evaporator at