Talanta 127 (2014) 59–67

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Comparison between solid phase microextraction (SPME) and hollow fiber liquid phase microextraction (HFLPME) for determination of extractables from post-consumer recycled PET into food simulants

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ARTICLE INFO

Article history:
Received 11 November 2013
Received in revised form
8 March 2014
Accepted 17 March 2014
Available online 1 April 2014

Keywords:
Solid phase microextraction
Hollow fiber liquid phase microextraction
Recycled PET
Food contact

ABSTRACT

Hollow fiber liquid phase microextraction (HFLPME) and solid phase microextraction (SPME) methods for pre-concentration of contaminants (toluene, benzophenone, tetracosane and chloroform) in food simulants were investigated. For HFLPME 1-heptanol, 2-octanone and dibutyl-ether were studied as extracting solvents. Analysis by gas chromatography coupled to mass spectrometry (GC–MS), flame ionization (GC–FID) and electron capture detectors (GC–ECD) were carried out. In addition, the methods were employed to evaluate the safety in use of a PET material after the recycling process (comprising washing, extrusion and solid state polymerization (SSP)) through extractability studies of the contaminants using 10% (v/v) ethanol in deionized water and 3% (w/v) acetic acid in deionized water as food simulants in different conditions: 10 days at 40 °C and 2 h at 70 °C. The HFLPME preconcentration method provided increased sensitivity when compared to the SPME method and allowed to analyze concentration levels below 10 μg surrogate per kg food simulant. The results of the extractability studies showed considerable reductions after the extrusion and SSP processes and indicated the compliance with regulations for using recycled PET in contact with food.

1. Introduction

The generation of large amounts of plastic waste and improper disposal has become the target of environmental discussions [1]. In this context, PET is worth mentioning: it is one of the main polymers used in the Brazilian market to produce packaging of quick disposal and consequently is one of the most abundant plastics in solid urban waste [2,3].

One of the alternatives to reduce this environmental impact is by recycling this polymer [1,4,5]. However, the legislation that regulates the use of food contact materials restricts the use of recycled plastic due to the possible migration of contaminants present in the recycled material into food [6–10]. For this reason the efficiency of decontamination of the recycling technology must be assessed through a challenge test. The test consists of contaminating the polymer with a few substances (surrogates), that mimic a broader range of potential contaminants that may be present in the post-consumer PET due to consumer packaging misuse, in standardized conditions of time and temperature [6,11]. Final articles manufactured with recycled PET (bottles, trays) must comply with migration tests established by the legislation of food contact materials [6,12,13].

However, when having the recycled material before manufacturing the final bottle or tray intended for food packaging, migration studies are not possible and extractability tests can be applied. Using the powdered material represents the worst scenario for safety in the use of the recycled material. It is assumed that migration from a manufactured article will always be lower than extractability from the powdered material, so extractables by food simulants provide the required information about the feasibility of the material for food contact and thus, these tests can be used for compliance.

For this purpose, sophisticated analytical procedures have been proposed to determine different types of packaging migrants at trace concentration levels [14–17]. The analysis of extractables or migrants in fatty simulants is not usually a problem, but this is not the case for aqueous simulants, where an additional extraction step is necessary. Without a doubt, the sample treatment techniques, mainly those concerning extraction and preconcentration of the sample are the bottleneck of the analytical process.

Regarding the use of analytical techniques able to quantify trace analyte levels in food simulants, several methods are used,

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http://dx.doi.org/10.1016/j.talanta.2014.03.042
0039-9140/

such as liquid–liquid extraction [18,19], solid phase extraction [20] and solid phase microextraction (SPME). The SPME technique is one of the most consolidated because of its simplicity and sensitivity and the fact that it is solvent-free.

Among the latest proposed techniques, two phases hollow fiber liquid-phase microextraction (HF-LPME) has been shown as an efficient and attractive technique to enhance the sensitivity [21–23]. In this technique the extraction of the analyte present in an aqueous phase (donor phase) takes place through the pores of a hollow fiber (semipermeable membrane) containing an organic solvent (acceptor phase) immiscible with water. The membrane is immersed in the aqueous solution sample containing the analyte and the two phases (aqueous and organic) are maintained in physical contact through the pores of the membrane. By means of chemical affinity, the compounds present are transferred from the aqueous phase into the organic phase. Finally, the organic solvent containing the analytes is injected directly into a gas chromatograph and the substances are analyzed [23].

The main advantages of this technique are high versatility, selectivity, economic and especially the high concentration rate of the compounds obtained. One of the latest innovations of HFLPME was reported by Pezo et al. [16] with the development of an automatic multiple dynamic system, used to determine the migration of essential oils in aqueous simulants. In this system, the authors were able to identify compounds at ng g^{-1} level with enrichment factors above 300.

In this study, post industrial PET resin was contaminated with a series of surrogates according to a US-FDA 2006 protocol [6] to simulate the worst case of misuse of PET packaging. Then, the samples were recycled. The recycling process involved washing, extrusion and re-polymerization by solid state polymerization (SSP). In order to explore the feasibility of the final recycled material for being in contact with food, extractability tests with the standard aqueous food simulants were carried out. After the extractability tests, the amount of surrogates released by the samples into the food simulant were preconcentrated from the aqueous phases using SPME and HFLPME techniques and quantification was performed by gas chromatography with different detectors.

2. Experimental

2.1. PET contamination process

To simulate the worst case of misuse of PET packaging, post-industrial PET flakes (PET) supplied by Mossi & Ghisolfi were subjected to a challenge test according to the US-FDA Guidance 2006 [6]. The surrogates used for the cocktail were 10% (v/v) chloroform (Vetec, 99%. CAS no. 67663), 10% (v/v) toluene (Vetec, 99.5%, CAS no. 108-88-3) 1% tetracosane (w/w) (Aldrich, 99%, CAS no. 16416-32-3) and 1% (v/v) benzophenone (Acros Organics, 99%, CAS no. 16416-32-3) dissolved in 78% (v/v) n-heptane (Neon, 99%, CAS no. 142-82-5), where v/v means volume of surrogate per unit volume of entire cocktail, and w/w means mass of surrogate per unit mass of entire cocktail. Chloroform represents volatile polar substances, toluene represents volatile non-polar substances, benzophenone non-volatile polar substances and tetracosane non-volatile non-polar ones. A total of 5 kg of flakes in square form were immersed in the cocktail inside a steel reactor. The system was designed to provide a uniform contamination of the total sample. It was hermetically sealed and maintained with constant stirring at 40 °C for 14 days.

2.2. PET recycling processes

The contaminated PET (PETc) was cleaned by conventional methods. Firstly, samples were washed with water for 10 min, with 1% of sodium hydroxide solution for 5 min and again with water for another 10 min using a washer developed by 3R-Residue Recycling Center, Brazil [24]. After that, the samples were dried in a conventional oven for 6 h at 160 °C. Finally, the material was granulated in a single-screw extruder (AX Plastics) (D=16 mm, L/D=26). The extruder used in this work did not have a vacuum system or vent, was operated at 90 rpm screw speed, 1 kg/h and 50 MPa, approximately which simulated a medium to high shear rate. The barrel zone settings were previously studied to melt the polymer. The zone profiles used were zone 1 – 220 °C, zones 2 and 3 – 255 °C. Afterwards reprocessed PET samples (PETr) were repolymerized via SSP (PETp) in a reactor. Basically it consists of a rotatable drum wrapped by heating blankets. The experimental conditions were previously optimized and 8 h at 190 °C under vacuum application was selected to remove sub-products generated by the esterification reactions as water and others cause degradation of the PET.

2.3. Analysis of extractables

In order to evaluate the transference of contaminants from PETc, analysis of extractables was performed using 10% (v/v) ethanol in deionized water and 3% (w/v) acetic acid in deionized water as food simulants according to Commission [25] for plastic materials and articles intended to come into contact with foodstuffs.

Representative samples of PETc flakes, PETr and repolymerized PET (PETp) pellets were ground with a cryogenic mill, using liquid nitrogen, to reduce their size and increase their contact surface area. The final material was a powder of PET. Because of the reactors used in this work, the PET flakes contamination and the recycling process were homogeneous, as demonstrated by the low Relative Standard Deviation (RSD) values obtained in the analysis of independent replicates. About 0.3 g of all the ground samples were added to 20 mL of each food simulant inside glass vials hermetically sealed and then maintained in different conditions: 10% (v/v) ethanol and 3% (w/v) acetic acid (10 days at 40 °C and 2 h at 70 °C). Three independent replicates for each sample were prepared. After the extractability tests from PET samples, the solutions were filtered using Nylon filters (Chromafil®Xtra PET-20/25) with pore size of 0.20 μm and a diameter of 25 mm, extracted by HF-LPME or SPME and the quantitative analysis was carried out.

2.4. Analytical procedures

The surrogates were preconcentrated from the aqueous phase by HFLPME and analyzed by gas chromatography with different detectors. Flame ionization detector (GC–FID) was used to analyze toluene, benzophenone and tetracosane because it is a good and sensitive detector for hydrocarbon ions. Electron capture detector (GC–ECD) was used for chloroform because of the high sensitivity of ECD to halogenated compounds. When SPME was applied gas chromatography with mass spectrometry detector, SPME-GC–MS was used to analyze all surrogates.

Pentachlorobenzene, purchased from Chem Service (CAS no. 608-93-5) was used as an internal standard in all calibration curves and chromatographic analysis. None of the polymers were dissolved or affected by the solvents, thus, the matrix effect in these cases can be considered negligible. Besides, the microextraction techniques, either SPME or HF-LPME, provide very clean extracts (HF-LPME) or pure compounds desorbed from the fiber (SPME), which avoid the matrix influence.

2.4.1. Selection of the organic solvent for extraction by HF-LPME and analysis by GC–FID and GC–ECD

A fundamental step in the optimization process in HF-LPME is the selection of the organic solvent (acceptor phase). It should have adequate solubility for the analytes of interest, low solubility in water to prevent the loss in the aqueous phase, low volatility to prevent the loss of the organic phase during extraction and compatibility with the capillary semipermeable membrane [26–28]. Based on these considerations, 1-heptanol (Aldrich 98% CAS no. 111706), 2-octanone (Sigma Aldrich, 98%, CAS no. 111137) and dibutyl-ether (Fluka, 99%, CAS no. 14296) were studied as extracting solvents. All evaluated solvents were purified before use by filtration over silica gel 60 (0.063–0.200 mm, Merck).

Stock solutions of about 1000 μg/g containing benzophenone (Sigma Aldrich 99%), toluene (99.5%, Sigma-Aldrich Chemistry), chloroform (Scharlau, 98%) and tetracosane (Fluka, 99%) were prepared in each organic solvent (1-heptanol, 2-octanone and dibutyl-ether). To calculate the enrichment factor, calibration curves in each organic solvent solution with different concentrations were prepared from the stock solution.

Microextractions were performed with a programmable multisyringe pump Aladdin AL-8000 from World Precision Instruments (Stevenage, UK). For more details of the system see Reference [16]. The syringe pump was fitted with six Hamilton (Bonaduz, Switzerland) microsyringes (100 μL capacity). Polymeric hollow fibers consisted of polypropylene hydrophobic membranes Accurel PP 150/330 (0.2 μm nominal pore size, 150 μm wall thickness, 600 μm inner diameter), purchased from Membrana (Wuppertal, Germany). The hollow fibers were cut into lengths of 11 cm to improve the surface ratio of mass transfer. One end of the fiber was connected to a 2 mL vial containing the organic solvent, using a small curved needle, in the ∩ form. The other end of the fiber was connected to the microsyringe. The membrane was completely immersed in a 20 mL vial containing the aqueous simulant solutions as illustrated in Fig. 1.

All microextraction assays were performed at 50 °C, 300 rpm, with an extraction velocity of 2.5 μL min^{-1}. Extraction volume was fixed at 50 μL and it was transferred to a 200 μL vial. The extracted compounds were analyzed by GC–FID and GC–ECD.

Quantitative analyses were carried out with a Trace GC Ultra from Thermo Electron Corp equipped with an AS 3000 auto sampler and FID. A DB-1 column was used (60 m × 25 mm × 25 μm film thickness). 1 μL was injected and carried out in splitless mode. Carrier gas was helium (99.99%) at a constant flow rate of 1.0 mL/min. The temperature ramp was previously studied for each solvent to adequate peaks separation: when 2-octanone was used as the organic solvent the temperature ramp was as follows: 40 °C for 1 min, 1 °C/min to 60 °C then 30 °C/min to 200 °C and 20 °C/min to 320 °C and held for 3 min. For 1-heptanol as organic solvent the initial temperature was 40 °C, 0.5 °C/min to 50 °C then 40 °C/min to 150 °C and 20 °C/min to 320 °C and held for 3 min. For dibuthyl-ether: 40 °C for 1 min, 0.5 °C/min to 50 °C, 50 °C/min to 150 °C and 20 °C/min to 320 °C and held for 3 min.

For chloroform a GC Hewlett-Packard HP 5890 model with a ^{63}Ni ECD equipped with a HP 7673 autosampler was used. 1 μL was injected and split mode (1:10) was used. The analytical column was VF-5MS (5% phenyl methyl siloxane; 30 m × 0.25 mm × 0.25 μm). The interface temperature was 250 °C. The same temperature program was used for all organic solvents: 40 °C for 0.5 min, 10 °C/min to 90 °C and 25 °C/min to 300 °C.

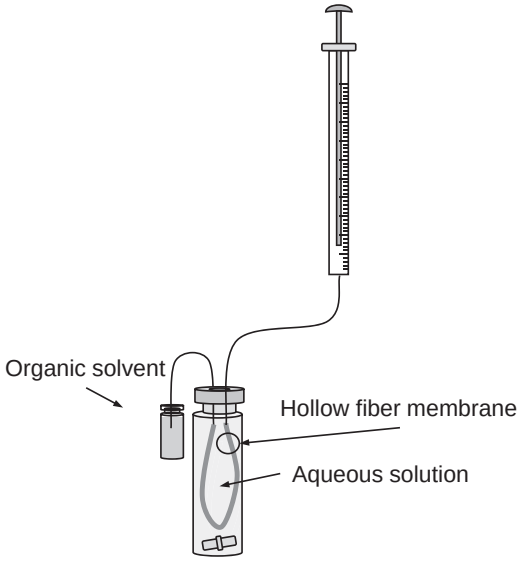

Fig. 1. Experimental set-up used for HF-LPME.

2.4.2. Analysis by SPME-GC–Ms

Stock solutions containing all contaminants described were prepared in 10% (v/v) ethanol in deionized water (Scharlau, grade absolute, CAS no. 64-175) and 3% (w/v) acetic acid in deionized water (Sigma Aldrich, 99.8%, CAS no. 64-197). Due to the insolubility of these compounds in water, firstly a stock solution containing chloroform, toluene and benzophenone of about 1000 μg/g was prepared in ethanol and from that a stock solution of 5 μg/g. For tetracosane a stock solution of about 5000 μg/g was prepared in tetrahydrofuran (THF) and from that a stock solution of 1000 μg/g in ethanol. Diluted solutions were used for the preparation of the calibration curve in 10% (v/v) ethanol in deionized water and 3% (w/v) acetic acid in deionized water.

For SPME analysis, 20 mL vials containing the solutions were stabilized at 80 °C for 2 min and after optimization a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber was exposed to the sample inside the vial for 10 min at 80 °C while stirring (500 rpm). After extraction, the loaded fiber was automatically transferred to the injection port of the GC–MS chromatograph, where desorption took place for 2 min at 250 °C. GC–MS determinations were performed using a Hewlett-Packard model 6890N GC (Agilent Technologies. Santa Clara, CA) equipped with a 5975B inert XL mass spectrometry and a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) which allows automatic SPME analysis. The mass spectrometer was operated in electron impact mode (70 eV) and the masses were scanned over an *m/z* range of 55–400 amu. Data were collected and processed using the MSD Chem-Station software (Agilent Technologies). A HP-5MS (Agilent Technologies) analytical column of 30 m × 0.25 mm and 0.25 μm film thickness was used. The temperature program for the gas chromatography was as follows: 40 °C for 4 min, 10 °C/min to 270 °C and held for 1 min. The injector temperature was 250 °C and 1 μL was injected in the splitless mode. The carrier gas was helium (99.999% purity, 1.0 mL/min) supplied by Carburos Metálicos (Barcelona, Spain). Compounds of interest were then quantified in the SIM mode, once their characteristic masses had been identified from their full spectra.

3. Results and discussion

3.1. Selection of the microextraction solvent

Firstly, calibration curves were prepared for each surrogate in all organic solvents. The quantification limits (LOD) and detection limits (LOQ) were calculated using the relations: LOD = 3 × SD/*a* and LOQ = 10 × SD/*a*, where SD is a standard deviation of 10 blank injections and *a* the angular coefficient of the analytical curves.

Inside the linear dynamic range (LDR), in μg/kg, the linearity (R^2) values were higher than 0.9100 for all contaminants.

It was not possible to analyze toluene by GC–FID. The eluted peak for all microextraction solvents was very wide and it was impossible to separate the solvents from the toluene peak. Different strategies were applied to solve this problem. Solvents were purified before use as described above; different types of chromatographic columns (HP-5 30 m × 0.25 mm × 0.25μm, DB-1 30 m 0.25 mm × 0.25 μm and DB-1 60 m × 0.25 mm × 0.25 μm) and different temperature programs minimizing the temperature ramp to increase the separation of the compounds were also tested. However, LOD for toluene was above 100 μg/kg when working in these conditions. Consequently, the determination of this compound by GC–FID was not efficient and it was quantified by SPME-GC–MS.

The surrogates were extracted from food simulants through the pores of the semipermeable hollow fiber membrane with three different organic solvents (dibutyl-ether, 2-octanone and 1-heptanol) covering a wide range of chemical characteristics. One of the main advantages of HF-LPME is the high enrichment of the compounds in the organic phase. Thus, the enrichment factor and recovery for each aqueous food simulant using each organic solvent were determined. Due to the insolubility of contaminants in water, firstly a stock solution containing about 1000 μg/g of each contaminant was prepared in ethanol. 20 mL of the aqueous food simulants (donor phase) with a concentration of about 1 μg/g of the contaminants were extracted into 50–100 μL of the organic solvent (acceptor phase). Theoretically a maximum value of about 400–200 could be expected for the enrichment factor. Fig. 2 shows the enrichment factor calculated using the three organic solvents after microextraction for each aqueous simulant. The theoretical enrichment factor for a 100% recovery is indicated by the dashed lines.

The concentration of surrogates after microextraction was calculated by interpolation of the chromatographic signal in an independent calibration curve for each organic solvent previously prepared. The enrichment factor (E) was calculated using equation $E = C_{org}/C_i$ [29], where C_{org} is the concentration of contaminants in the acceptor phase after microextraction and C_i is the initial surrogate concentration in the aqueous simulants (1 μg/g).

Generally, low solubility in water, polarity and partition coefficient (K) between aqueous solution and the organic solvent have an important influence on the mass transfer between the aqueous and the organic solvent. It is assumed that an increase in K for the analyte increases the enrichment factor [23,29].

The enrichment factors of the surrogates using 1-heptanol as the acceptor phase were the lowest, among all organic solvents evaluated. The solubility of 1-heptanol in water is higher than that of the other solvents, so it could reduce the mass diffusion rates of surrogates present in the aqueous phase into the organic phase. Besides, microextraction temperature was 50 °C, so the solubility in water was favored. Not only 1-heptanol but the other solvents evaluated showed some solubility in water at this temperature because at the end of each experiment the characteristic smell of each solvent was detected in the aqueous phase.

Better enrichment factors were found for the other two organic solvents. The enrichment factor of benzophenone for both food simulants when using dibutyl-ether as the extraction solvent was almost 200 times. Both benzophenone and dibutyl-ether present an oxygen group with free electrons and have similar polarities, so the transfer of benzophenone from the aqueous phase to the organic one was favored. Tetracosane is a non-polar molecule, so the affinity by the aliphatic non-polar group of the dibutyl-ether has a good interaction too. For the acidic food simulant, the best enrichment factor for chloroform was obtained with 2-octanone. This result was not expected because the chemical affinities between carboxylic acid (donor phase) and ketone (acceptor phase) are favorable and therefore it would result in lower enrichment factor for chloroform. It is noteworthy that for dynamic extractions by pumping a continuous flow of organic solvent the equilibrium law cannot be applied like for static mode. In fact, dynamic extractions are more efficient than static [16,23]. This can explain the somewhat incoherent result for chloroform.

Due to the unfeasibility of using two different extraction solvents, dibutyl-ether was selected for the extractability tests because it presented the highest enrichment factors for the contaminants for most food simulants.

3.2. Analytical characteristics of SPME-GC–MS, GC–MS, GC–FID and GC–ECD

After the extractability tests on PETc, PETr, and PETp samples, quantification of the surrogates released by each sample (PETc,

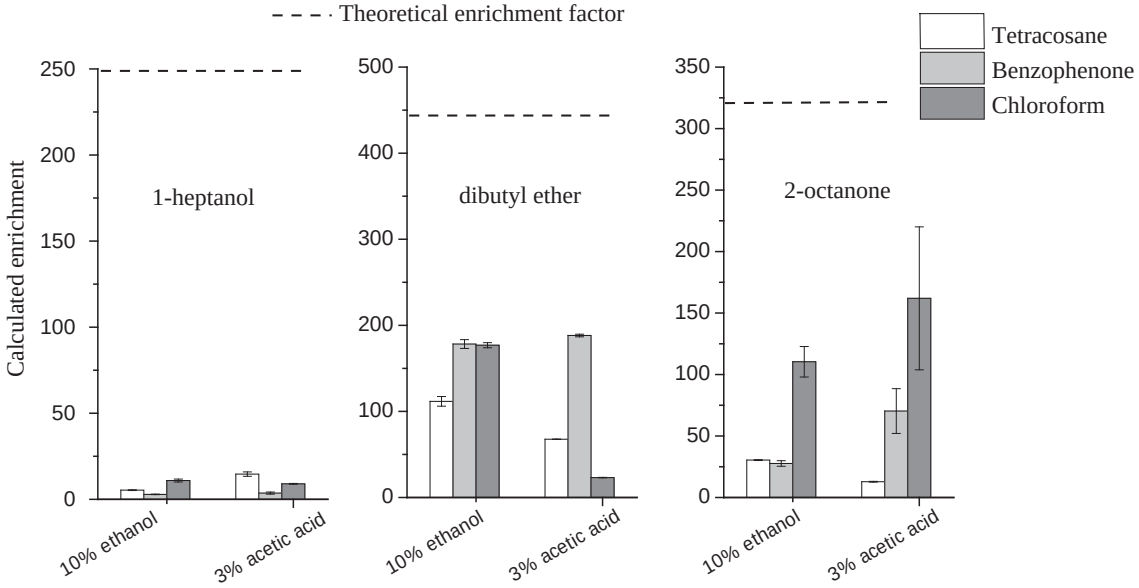

Fig. 2. Enrichment factor for each contaminant using the different organic solvents.

Table 1
Analytical characteristics for the calibration curves of surrogates in food simulants for HF-LPME using dibutyl-ether as preconcentration solvent and SPME methods. Concentrations are expressed in μg of surrogate per kg of food simulant.

Simulants	10% Ethanol				3% Acetic acid			
Surrogates	Toluene	Benzophenone	Tetracosane	Chloroform	Toluene	Benzophenone	Tetracosane	Chloroform
HF-LPME pre-concentration method followed by GC–FID and GC–ECD analysis for aqueous food simulants								
LDR (μg/kg)		5.2–5169.9	20.3–3743.0	1.1–2302.5		1.0–2730.8	37.8–3141.5	1.1–2848.5
R^2		0.9816	0.9403	0.9952		0.9634	0.9397	0.9924
LOQ (μg/kg)		0.6	6.0	2.1		0.5	28	2.8
LOD (μg/kg)		0.2	1.9	0.6		0.1	8.6	0.8
RSD (%) ($n=3$)		13.8	40.0	1.0		15.0	37.8	8.7
Retention time (min)		10.6	14.0	1.5		10.6	14.0	1.5
SPME pre-concentration method followed by GC–MS analysis for aqueous food simulants								
LDR (μg/kg)	1.7–9212.1	2.2–1246.0	11.3–6604.1		1.7–5611.8	5.7–7067.3	13.9–7726.7	
R^2	0.9922	0.9895	0.9816		0.9908	0.9908	0.9922	
LOQ (μg/kg)	104.7	10.8	37.5		64.0	49.0	167.1	
LOD (μg/kg)	31.4	3.2	1.2		9.2	14.7	50.1	
RSD (%) ($n=3$)	5.8	12.7	17.4		15.2	18.4	9.4	
Retention time (min)	4.7	19.3	25.8		4.7	19.3	25.8	

PETr and PETp) into the food simulants was performed. Therefore, it was important to validate the analytical methods established to ensure the reliability of the results. Table 1 shows the results of all methods used.

The results shown in Table 1 demonstrated that the R^2 was greater than 0.9500 for the majority of the methods employed, which represents the capacity of each of them to provide proportional results to the concentration of the contaminant into the LDR. With respect to the LOD, the lowest values were observed for the HF-LPME method. This result shows that liquid phase microextraction technique has better sensitivity for the analyzed compounds when compared to SPME-GC–MS for the aqueous simulants. As shown in the results of selection of the organic solvent (Fig. 2), it was observed that the concentration factors were almost 200 times for benzophenone and chloroform, and therefore, the detection limits were lower.

Comparing the precision parameter (RSD), the results for benzophenone were similar for SPME and HF-LPME, what shows the precise response capacity for both methods to various measurements of the same sample in different preparations. With respect to tetracosane, HF-LPME presented unsatisfactory precision results because the RSD value was 40%. This value was considered unacceptable, although the methods for quantitative analysis of compounds at trace levels are accepted up to 20% RSD values [30]. HF-LPME pre-concentrations were performed according to the set-up described (Fig. 1), where the microsyringes extracted all the samples automatically and simultaneously, but manual difficulties in fixing the membranes between the multi-syringes with each syringe vial containing the organic solvent were found. The procedure inevitably left the membrane exposed to the aqueous simulants while preparing the entire system (six microextractions were carried out at the same time in the experimental set-up) before proceeding with the microextration in automatic mode.

As Table 1 shows, some surrogates could not be quantified by both pre-concentration methods. In the HF-LPME followed by GC–FID analysis, the eluted peak corresponding to toluene presented separation problems with the evaluated organic solvents, as discussed in Section 3.1.With respect to SPME-GC–MS methods, problems were observed for chloroform detection. Usually, in SPME analysis, the type of fiber used plays an important role for the detection and quantification of the compounds [31]. In this technique different kinds of fibers can be employed according to the physico-chemical characteristics of the analyzed compounds. Basically, non-polar fibers are applied for non-polar compounds analysis. Polar fibers are designed for highly volatile polar compounds and medium to high polarity compounds, while bi-polar fibers can be employed to analyze both volatile and non-volatile with low to high polarity [32]. In addition to fiber selection, extraction mode is another parameter that should be considered according to the compounds of interest. The extraction can be done in direct mode where the fiber is immersed in the aqueous solution and is often used for non-volatile compounds or in the case of volatile compounds when the sample is not very complex. In indirect extraction mode or headspace (HS) the fiber is not placed in direct contact with the solution and this mode is widely used for analysis of volatile compounds [33].

In view of the physico-chemical characteristics of the molecules of the contaminants, preliminary studies to investigate suitable parameters for SPME-GC–MS analysis were performed. Thus, different extraction modes and type of fibers were tested. In HS mode, when using a non-polar fiber and a bipolar one, polydimethylsiloxane (PDMS) and DVB/CAR/PDMS, respectively, a peak corresponding to chloroform was observed. However, the sensitivity for the other contaminants was compromised. Tests by immersion mode with both fibers under study improved the results obtained for toluene, tetracosane, and benzophenone being the highest concentration values obtained with the bipolar fiber. However, a poor chloroform peak was observed in this extraction mode. Based on the previous results and taking into account the quality of the analytical results, the immersion mode using a bipolar fiber was adopted when working with SPME, as described in the methodology, and chloroform was evaluated only by HF-LPME.

3.3. *Surrogate levels in the extractability test solutions in the different steps of the PET recycling process*

With the validation of analytical methods, better results were observed for HF-LPME. Even with the experimental difficulties, the technique provided increased sensitivity to benzophenone, tetracosane and chloroform as compared to the SPME method. But the SPME technique was the only possible option to quantify toluene. In addition, it was not possible to analyze chloroform via SPME in immersion mode. For this reason, in the evaluation of the aqueous migration solutions of the different steps of the PET recycling process, toluene was determined by SPME-GC–MS while the rest of the contaminants were pre-concentrated in dibutyl-ether by HF-LPME and analyzed by GC–FID (benzophenone and tetracosane) and by GC–ECD (chloroform).

The recycling process must be able to reduce the contaminants levels in the plastic material to ensure that it is suitable for being used in the manufacture of articles intended to come into contact with foodstuffs. Thus, the FDA recommends that the test conditions of migration must represent the worst scenario possible of real use to slightly overestimate it for the intended applications [6]. This condition was met, as the recycled material was tested in powder form and not as a manufactured PET whole container (bottle, tray). Working with PET powder ensures a greater contact surface area with solutions, and thus a greater possibility of migration into food simulants.

In this work two different conditions of time and temperature were tested, for each food simulant, as mentioned in the experimental section: 10 days at 40 °C simulating the storage of ambient temperature for periods longer than 24 h, and 2 h at 70 °C simulating contact conditions for hot filling [25].

With respect to the food simulants used, 10% ethanol simulates foods with hydrophilic character and therefore is able to extract hydrophilic substances. Three percent acetic acid is used for foodstuffs that have a pH below 4.5. The migration conditions selected will also represent the worst case scenario for further PET food containers.

In this sense, PETc flakes, PETr and PETp pellets were exposed to the conditions of time and temperature in the presence of food simulant solutions, as described above. Tables 2 and 3 show the results for quantitative determination of the surrogates in the respective simulants. It is worth mentioning that the amount of surrogates released by the samples into the food simulants was compared to the amount released by the PETc (not subjected to extrusion and the SSP process). The contaminated sample was always taken as a reference to evaluate the efficiency of each step of the recycling process.

The migration of substances into food simulants depends on several factors such as temperature and time, as well as the molecular characteristics of the polymer. The migrant characteristics such as molecular size, concentration, polarity and solubility in the simulant have also a strong influence [34,35]. Based on these considerations, a discussion of the extractability results from PET samples to food simulants for each test condition is included.

Although food simulants have similar polarities, toluene showed a higher concentration level for 10% ethanol as compared with 3% acetic acid in both test conditions. With respect to time/temperature, toluene showed a similar concentration level for both food simulants, mainly for 10% ethanol. These results appear incoherent considering the diffusion properties of the contaminant between PETc and the simulant. The diffusion processes depend on parameters such as time, temperature and polymer structure. It is considered that the rate of diffusion increases with temperature. Long times also help this process and diffusion even occurs through free spaces of the polymer matrix. Based on these considerations, the diffusion process would be slower at 40 °C and faster at 70 °C. In addition, 70 °C is very close to the glass transition temperature (*Tg*) of PET, where the movements of the amorphous regions of the polymer favor the transport of the compounds to the simulant [34]. Thus, one would expect higher concentrations of toluene in 2 h at 70 °C as seen in 3% acetic acid. The chemical composition of migrating substances is related to the compatibility with the polymer and simulant as well as the diffusion, and this is the reason why different molecules behave differently in the same polymer and simulant system. Additionally, kinetic and thermodynamic factors should be considered in all processes of mass transport. The benzophenone molecule presents a group with oxygen free electrons that can interact with other oxygen present in the aqueous simulants like phenol and hydroxyl groups. Probably because of these interactions the migration process was favorable from PETc to food simulants. The results obtained for 2 h at 70 °C suggest that the molecular interactions were also favored, but these are not determining factors in influencing this extractability assay. Instead, the temperature of 70 °C accelerated the diffusion process through the amorphous part of the polymer chains leading to higher concentrations of benzophenone in 10% ethanol and 3% acetic acid in deionized water.

Tetracosane is an aliphatic chain hydrocarbon with a high molecular weight (328 g/mol) and as such is highly apolar and has hydrophobic character. Due to these characteristics, migration to aqueous simulants is very low. The fact that the condition 2 h at 70 °C favors the migration process reflects that at this temperature

Table 2
Results of extractability tests in the PET samples for 10 days at 40 °C with aqueous simulants. Concentrations are expressed in μg of surrogate per kg of food simulant (μg/kg) ± the standard deviation (* means below LOQ).

Methods	Contaminants	Food simulants					
		10% Ethanol			3% Acetic acid		
		PETc	PETr	PETp	PETc	PETr	PETp
SPME-GC–MS	Toluene	2554.4 ± 636.2	*	*	1712.5 ± 208.2	*	*
HF-LPME-GC–FID	Benzophenone	1741.2 ± 45.4	212.3 ± 9.1	10.9 ± 0.0	1353.8 ± 33.2	86.8 ± 3.9	5.9 ± 0.6
	Tetracosane	41.6 ± 1.3.2	*	*	121.7 ± 0.8	*	*
HF-LPME-GC–ECD	Chloroform	1170.1 ± 22.2	103.0 ± 1.3	*	280.6 ± 27.5	126.8 ± 13.8	*

Table 3
Results of extractability tests in PET samples for 2 h at 70 °C for all food simulants. Concentrations are expressed in μg of surrogate per kg of food simulant (μg/kg) ± the standard deviation (* means below LOQ).

Methods	Contaminants	Food simulants					
		10% Ethanol			3% Acetic acid		
		PETc	PETr	PETp	PETc	PETr	PETp
SPME-GC–MS	Toluene	2688.8 ± 88.9	*	*	1346.4 ± 464.3	614.2 ± 79.0	*
HF-LPME-GC–FID	Benzophenone	3074.4 ± 768.7	246.3 ± 13.8	188.0 ± 0.5	2285.2 ± 335.8	95.3 ± 18.7	71.4 ± 7.0
	Tetracosane	61.5 ± 8.7	*	*	155.3 ± 9.5	124.1 ± 5.1	*
HF-LPME-GC–ECD	Chloroform	959.2 ± 141.3	101.5 ± 9.2	59.0 ± 3.8	757.0 ± 120.5	119.8 ± 17.6	69.2 ± 4.3

the mobility of the tetracosane molecule is higher in the amorphous phase of the polymer, facilitating the diffusion of the surrogate in the polymeric matrix to the simulant.

It is considered that compounds of low molecular weight (less than 300 g/mol) present in the polymeric matrix can migrate more easily compared to those of high molecular weight. This means that these substances have a high diffusion rate and, in the case of PET, can migrate more quickly to food simulants [12,35]. Chloroform has a molar weight of 119 g mol^{-1}, the lowest in comparison with other surrogates used in this study and therefore the diffusion to both aqueous simulants could have been favored.

Figs. 3 and 4 show several chromatograms where the peaks of surrogates present in each PET sample that migrated to the food simulants after the extractability tests are highlighted.

The levels of surrogates (Tables 2 and 3 as Figs. 3 and 4) were reduced considerably after the extrusion and SSP processes. These results indicate that the extrusion followed by the SSP process plays a critical role in the decontamination.

It has been verified that the levels of surrogates present in all simulants after the extractability tests were reduced in approximately 90% after extrusion and SSP processes of the PETc. During the extrusion process of PETc, the high temperature causes fusion of the flakes and the mechanical shear between screw and barrel causes mobility in the melt polymer. In this physical process the surrogates are diluted and their mobility is facilitated into a great mass of molten PET, consequently they are eliminated at the exit of the die. Due to the residence time, temperature and vacuum conditions employed in the SSP process there is a further decrease of the surrogates in PETr. These results indicate that the SSP process promoted a deep cleansing of the material. Besides it can promote condensation of chain segments with carboxylic or hydroxyl groups which increases the length of the polymer chain [13,36,37]. However, the high removal efficiency does not mean that the levels of contaminants in PETr and PETp samples can be accepted by the legislation. According to the European legislation, migration values from a food contact material should not exceed a concentration of 10 μg/kg in food simulant in the studied conditions [38]. However, it was verified by HF-LPME pre-concentration and subsequent analysis by GC–FID migrations that even occurring at very small concentration, the decontamination for tetracosane after extrusion was not enough, only approximately 23% after migration tests using acetic simulant for 2 h at 70 °C. The high molecular weight and especially the boiling point (391 °C) may be the factors that make it difficult the removal of this surrogate during the extrusion process: the temperatures used were below the boiling point of tetracosane.

On the other hand, it was observed that after the SSP process it was not possible to quantify tetracosane in 3% acetic acid showing that this process contributed efficiently to remove this surrogate at concentration below LOQ (28 μg/kg in the simulant). Furthermore, the small peak shown in the chromatogram (Fig. 4) refers to tetracosane migrated from PETp to 3% (w/v) acetic acid in deionized water, which reinforces the SSP process as the step that contributes in a higher extension for the removal of this surrogate.

Benzophenone also showed a lower degree of removal from PETc after migration tests. As highlighted in the literature, benzophenone has a higher residual level because it is a non-volatile compound and has a solubility parameter similar to PET, which makes its removal more difficult. For volatile compounds of low molecular weights such as toluene and chloroform, removal occurred almost immediately after the extrusion step. According to Welle [35,39] low molecular weight substances are efficiently removed during the PET recycling process because of the high rates of diffusion of these small molecules.

For some experiments the surrogate concentrations after extrusion and SSP processes showed values that imply the non-compliance with the law and thus the use of this material for food packaging should be avoided. However it is noteworthy that in real-life contamination levels are expected to be much lower than those applied in the challenge test [12,40]. In fact, it is estimated that 100% of the polymer is contaminated in the challenge test, whereas normally 0.01% (1 in 10,000) of the real contamination levels of misused bottles are estimated. Also, extractability test from a powdered material should give surrogates concentration in the food simulant much higher than migration from a manufactured PET article. So, the results obtained in this study indicate that the quality of recycled PET fulfills the legal requirements given by the legislation.

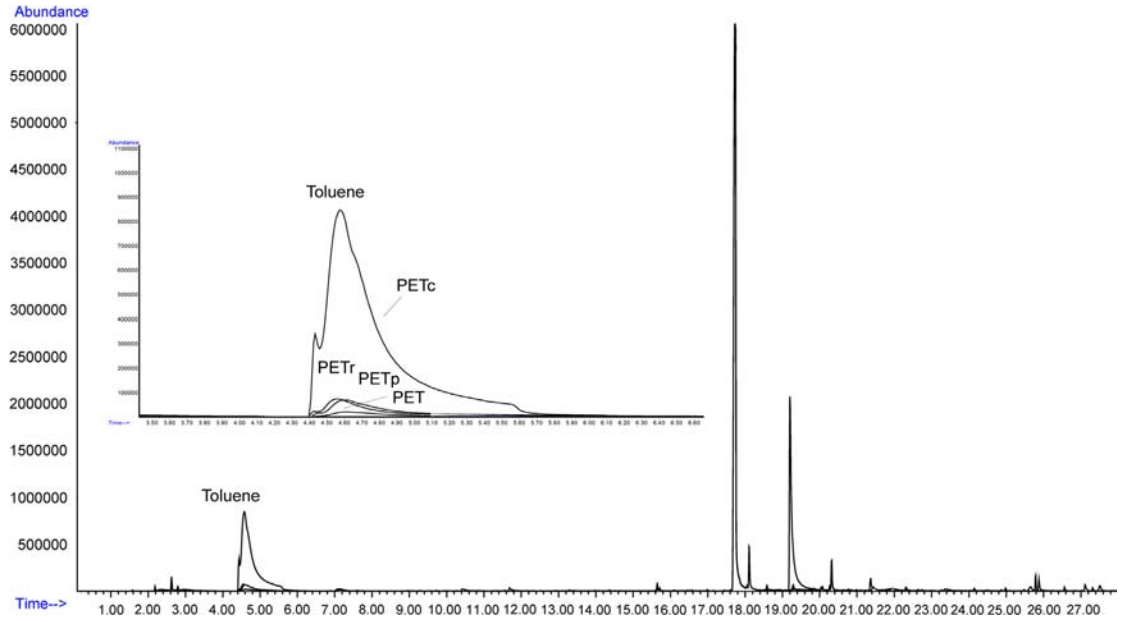

Fig. 3. Chromatograms obtained by SPME-GC–MS of PETc, PETr, PETp and PET not subjected to any type of contamination or recycling process. The highlighted peaks refer to toluene in 10% (w/w) ethanol in deionized water for 10 days at 40 °C.

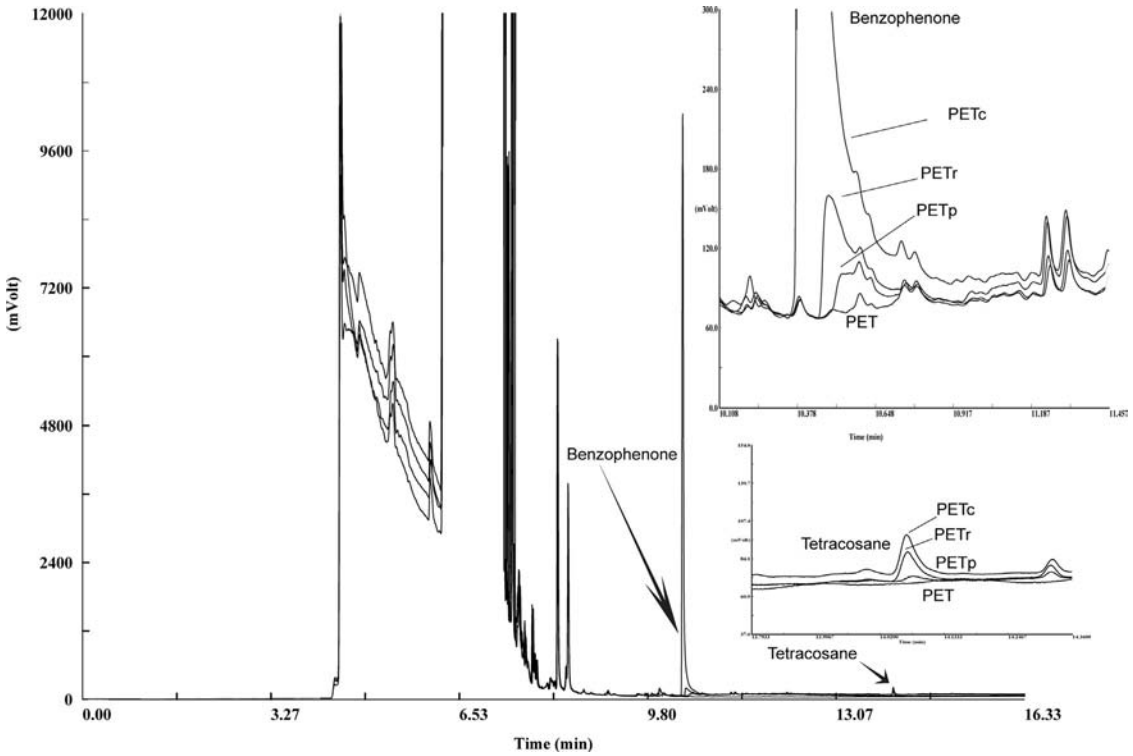

Fig. 4. Chromatogram obtained by HF-LPME-GC-FID of PETc, PETr, PETp and PET not subjected to any type of contamination or recycling process. The highlighted peaks are of tetracosane and benzophenone in 3% (w/v) acetic acid in deionized water in test conditions of 2 h at 70 °C.

4. Conclusions

In this paper the comparison of two analytical methods for studying the compliance of recycled PET for food contact has been investigated. To detect toxic substances at trace levels, at 10 μg/kg level, different pre-concentration methods, that could be used to properly quantify the surrogates and thus comply the requirements of legislation, were compared. Accordingly, SPME and HF-LPME pre-concentration methods were evaluated. The results showed advantages to HF-LPME due to the lower detection limits. In contrast, SPME coupled to GC–MS can be recommended for some of the surrogates, but the saturation of the microfiber or the competition of some compounds in the sorption process makes it also unsuitable for all kind of analytes. The work here shown emphasizes the importance of a right optimization for all surrogates and recommends the best analytical technique for each particular case. To select only one technique for everything can be a solution of compromise, but it does not mean that the surrogates can be detected at the required level of sensitivity to guaranty the decontamination efficiency of the recycled polymer.

After preliminary studies for selection of methods and analytical techniques, the PET recycling process was evaluated, studying the decontamination of the polymer in each step. First, the resin was contaminated according to FDA recommendations subjecting the polymer to a challenge test, which simulated the worst case for the consumer and extractability tests with food simulants under different conditions of time and temperature were carried out. It was possible to verify that decontamination efficiency was approximately 90% for most contaminants immediately after the extrusion step. The SSP process further promoted to reduce the levels of contaminants contributing to the cleaning of the polymer close to 100%. However, residual levels of some surrogates above those permitted by the legislation were still detected in the resin even after the SSP process.

Acknowledgments

The authors acknowledge the National Council for Scientific and Technological Development (CNPq, grant number 202482/2011-1), Rhodia and Federal University of ABC (Brazil). Thanks are given to Aragón Government for the financial help provided to GUIA research group T-10 at the University of Zaragoza (Spain). Yolanda Echegoyen thanks the Spanish Ministry of Science and Innovation for the concession of a Juan de la Cierva contract.

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