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Release of Selected Non-Intentionally Added Substances (NIAS) from PET Food Contact Materials: A New Online SPE-UHPLC-MS/MS Multiresidue Method

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Abstract: Food contact materials (FCMs) are an underestimated source of food chemical contaminants and a potentially relevant route of human exposure to chemicals that are harmful to the endocrine system. Foods and water are the main sources of exposure due to contact with the packaging materials, often of polymeric nature. European Regulation 10/2011 requires migration tests on FCMs and foodstuffs to evaluate the presence of listed substances (authorized monomers and additives) and non-intentionally added substances (NIAS) not listed in the regulation and not subjected to restrictions. The tests are required to ensure the compliance of packaging materials for the contained foods. NIAS are a heterogeneous group of substances classified with a potential estrogenic or androgenic activity. Subsequently, the evaluation of the presence of these molecules in foods and water is significant. Here we present an online SPE/UHPLC-tandem MS method to quantify trace levels of NIAS in food simulants (A: aqueous 3% acetic acid; B: aqueous 20% ethanol) contained in PET preformed bottles. The use of online SPE reduces systemic errors thanks to the automation of the technique. For the developed analytical method, we evaluate the limit of detection (LOD), the limit of quantitation (LOQ), selectivity, RSD% and BIAS% for LLOQ for a total of twelve NIAS, including monomers, antioxidants, UV-filters and additives. LOD ranged between 0.002 µg/L for bisphenol S and 13.6 µg/L for 2,6-di-tert-butyl-4-methylphenol (BHT). LOQs are comprised between 0.01 µg/L for bisphenol S and 42.2 µg/L for BHT. The online-SPE/UHPLC-tandem MS method is applied to the food simulants contained in several types of PET packaging materials to evaluate the migration of the selected NIAS. The results show the presence (µg/L) of NIAS in the tested samples, underlining the need for a new regulation for these potentially toxic molecules.

Keywords: food contact materials; online SPE; UHPLC-MS; non-intentionally added substances; migration

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1. Introduction

Nowadays, the most commonly used food contact materials (FCMs) are plastic materials, usually made from oil or petroleum, composed of monomers and raw materials chemically transformed. Additives are added as a dispersion in the polymeric matrix in small amounts (0.1–1% *w*/*w*) for the following: (i) to improve the physical-chemical properties of the final product; (ii) to prevent thermal oxidation, which is the main responsible for the polymeric chain division and degradation; (iii) avoid reticulation reactions of macromolecules chain [1–3]. Additives are grouped into functional (plasticizers, light and heat stabilizers, lubricants), fillers (kaolin, calcium carbonate), colorants and reinforcers (carbon fiber). They are not chemically linked to the polymer macromolecules except for the reactive organic ones, which are polymerized with the monomer molecules and become an integral part of the polymer chain [2].

The most used additives applied in industries are as follows: (i) plasticizers such as phthalic acid esters (PAEs), diisobutyl phthalate (DBP) and acetyl tributyl citrate (ATBC) [4], commonly used to improve flexibility, elasticity, stretching, deformability and mechanical resistance at low temperature; (ii) antioxidants such as sterically hindered amines (HAS) and sterically hindered phenols (HPS) [5] to delay oxidative degradation acting on the propagation steps of oxidation and finally (iii) light stabilizers, such as alkyl-organophosphates, epoxy compounds and beta-diketones [6]. Many of them are considered endocrine-disrupting chemicals (EDCs). EDCs have been linked to numerous adverse human health outcomes because of their potential to disrupt several hormones. Alteration in sperm quality and fertility, alteration in nervous system function, immune issues, obesity, neurological and learning disabilities are some of the pathologies linked to the presence of EDCs in the human body [7].

Despite these additives being well categorized, described and regulated by law, the non-intentionally added substances, NIAS, became a relevant issue in recent years. NIAS derives from the interactions between components of the packaging, polymer degradation and impurities present in the raw materials used for the production of polymers [8]. This category includes products that spontaneously decompose over time, environmental contaminants, newly formed substances arising from material components reactions, or arising from additives [9]. As previously highlighted NIAS are kept under control because their chronic toxic effects on human health are not yet well studied. Furthermore, nowadays plastic packaging materials have become a burden for society due to environmental pollution from micro- and nano-plastic and the presence of NIAS could represent an additional issue also for environmental compartments.

NIAS could transfer from FCMs to foodstuffs due to the migration process, a specific process regarding plastic material that involves a diffusive mass transfer between food, packaging and the environment.

One of the most used polymers for beverage bottling is PET (polyethylene terephthalate), a polymer characterized by the absence of plasticizers and antioxidants and by a low number of added colorants. Despite starting materials and additives are regulated (European Regulation 10/2011 [10]), different chemicals belonging to the NIAS category can still be characterized. The complexity of the formulation of polymer, the difficulty of the manufacturing process, the storage, the physical stress during daily use, the exposure to sunlight or heat sources, the inorganic composition of the drink, and the presence of bacteria are the main causes of the lack of and complexity of a comprehensive description of NIAS [11].

However, research studies on NIAS are present in the literature [12–17]. For example, Bach et al. proposed research work on the effect of the temperature of exposure on bottling materials in the releasing of both IAS (intentionally added substances) and NIAS [12]. They studied both PET and glass bottles filled with not-carbonated, carbonated and ultrapure water subjected to the worse conditions of temperature (10 days at 60 °C). They found that acetaldehyde, formaldehyde, 2,4-di-tert-butylphenol and bis(2-hydroxyethyl) terephthalate were present in PET bottles. The last two were recognized as NIAS, but were not included in the positive list of European Regulation 10/2011.

Although some substances were not used for polymer formulation, contamination risk does not disappear completely; in recent years, many studies have been proposed for the analytical determination of phthalates and PFCs (per-fluorinated compounds) in food and in FCMs [18–22].

It is clear that FCMs are an opening field of research, with several challenges to overcome. The aim of the present study was the development of a new analytical method based on the use of online SPE before ultra-high-performance liquid chromatography (UHPLC) coupled to a mass spectrometry analyzer for the determination and quantitation of twelve NIAS in food simulants. SPE provides the advantages of an automated sample pre-concentration step before UHPLC-MS analysis and the possibility to analyze a large volume of sample (1 mL) reaching a better sensitivity. After the assessment of the limit of

detection (LOD), quantitation (LOQ), lower LOQ with RSD% and BIAS%, carry over, and selectivity, the developed method was applied to real, commercially available samples of PET bottles.

2. Material and Methods

2.1. Chemicals

The following chemicals were purchased from Sigma Merck (Milano, Italy): acetonitrile, methanol, ethanol (HPLC-MS grade), dichloromethane, ethyl acetate (analytical grade), formic acid, anhydrous Na_2SO_4, hydrochloric acid 37% w/w, anhydrous acetic acid, bisphenol A (BPA), bisphenol A-d16 (BPA-d16), bisphenol S (BPS), toluene-2,4-diisocyanate (2,4-TDI), toluene-2,6-diisocyanate (2,6-TDI), hexamethylene diisocyanate (HDI), 2,6-di-tert-butyl-4-methylphenol (BHT), octocrylene (Eusolex OCR), 2-ethylhexyl salicylate (Eusolex OS), homosalate (Eusolex HMS), 2,4-dicumylphenol, 4-cumylphenol, bis(2-ethylhexyl) terephthalate (BEHT). Samples of PET bottles were kindly provided by several Italian suppliers certifying the polymer of fabrication.

2.2. Online SPE-UHPLC-MS/MS Method

Two stock solutions of analytical standard compounds were prepared in methanol and diluted in aqueous 0.1% formic acid at different concentrations. The analytes were divided into the two solutions as follows: 2,4-TDI, 2,6-TDI, HDI, octocrylene, homosalate, BEHT (mixture 1) and BPA, BPS, 4-cumylphenol, 2,4-cumylphenol, BHT, 2-ethylhexyl salicylate (mixture 2). Structural formulae of the selected analytes are shown in Supplementary Materials Figure S1. Calibration curves were realized in three replicates and each curve was made up of 9 points spanning different concentration ranges depending on the analyzed standard. For 2,4-TDI, 2,6-TDI, HDI, homosalate, octocrylene, 4-cumylphenol, 2,4-dicumylphenol, BPA the concentrations of the calibration curve were the following: 0.5, 1, 10, 25, 50, 100, 250, 300, 500 μg/L. For BHT 25, 50, 100, 250, 300, 500, 800, 1000, 1200 μg/L. For 2-ethylhexyl salicylate 2.5, 10, 50, 100, 250, 300, 500, 800, 1000 μg/L. Additionally, for BPS 0.01, 0.05, 0.1, 0.25, 0.5, 1, 10, 25, 50 μg/L.

The analyses were carried out on a Shimadzu Nexera (Milan, Italy) UHPLC coupled to a Sciex QTRAP 5500 triple quadrupole mass spectrometer through an ESI source. The chromatographic separation was achieved using a Kinetex C18 column (Phenomenex, Torrance, CA, USA), 100 × 2.1 mm, 1.7 μm particle size. Online SPE column was an ISOLUTE ENV+ (Biotage, Uppsala, Sweden), 30 × 2.1 mm, 40 μm particle size.

A mobile phase composed of eluent A (aqueous 0.1% formic acid) and eluent B (acetonitrile:methanol 80:20 v/v) was used. We selected the ternary eluent because it gave better selectivity features to efficiently separate all of the analytes. Furthermore, an eluent C (aqueous 0.1% formic acid) was used for online SPE during the steps of sample loading, column washing and reconditioning.

The chromatographic separation was achieved with a gradient elution as follows: (mixture 1) 0–3 min isocratic step at 5% B, then 3–7 min from 5 to 80% B, 7–11 min from 80 to 100% B, from 11 to 15 min a second isocratic step at 100% of B; then the column went back to the initial condition; (mixture 2) 0–3 min isocratic conditions at 5% B, then 3–7 min from 5 to 100% B, a second isocratic step for one min; then the column went back to the initial condition. Chromatographic and online SPE flow rates were 0.3 and 0.5 mL/min, respectively. Injection volume was 1 mL, and the column compartment was thermostat at 40 °C.

Individual standard compounds at a concentration of 0.5 μg/mL (aqueous 0.1% formic acid: methanol 95:5 v/v) were infused with a syringe at flow rate of 7 μL/min to select precursor and product ions and to set up the proper quadrupoles parameters (DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: collision exit potential) for MRM analysis. The mass spectrometer's best parameters for each analyte were set automatically.

For mixture 1 ESI source was set in positive ion mode as reported in Table 1 and in negative ion mode for mixture 2 (Table 2). We selected two (or three) MRM transitions for each analyte for the qualitative analysis. We used as quantitative MRM transition one of those (the bold ones in Tables 1 and 2) to quantify the analytes in samples and for the evaluation of the following: linearity of calibration curve (DIFF%), selectivity (SEL%), LOD, LOQ, LLOQ precision (BIAS%) and accuracy (RSD%). Fragmentation pathways of the NIAS analytes were studied and reported in Supplementary Materials Schemes S1–S12.

Table 1. Mixture 1 MRM parameters (DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: collision exit potential). Quantifier ions are in bold.

Compounds	$[M + H]^+$ (*m*/*z*)	Product Ions (*m*/*z*)	DP (V)	EP (V)	CE (V)	CXP (V)
2,4-TDI	175.4	**132.2**	170	7	21	7
		147.0			14	8
		104.2			25	6
2,6-TDI	175.4	**132.2**	170	7	21	7
		147.0			14	8
		104.2			25	6
HDI	169.1	**126.2**	122	12	8	6
		98.2			13	7
Octocrylene	362.2	**232.2**	94	14	27	12
		250.0			13	13
Homosalate	263.2	**139.2**	114	6	10	6
		120.9			35	12
BEHT	319.2	**113.1**	125	12	11	13
		166.7			15	12
		279.1			10	9

Table 2. Mixture 2 MRM parameters (DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: collision exit potential). Quantifier ions are in bold.

Compounds	$[M-H]^-$ (*m*/*z*)	Product Ions (*m*/*z*)	DP (V)	EP (V)	CE (V)	CXP (V)
BPA	227.3	**133.0**	−80	−7	−33	−14
		211.1			−21	−20
BPS	249.0	**108.0**	−75	−3	−35	−13
		156.0			−27	−12
		92.0			−38	−13
4-cumylphenol	211.1	**195.2**	−104	−8	−22	−17
		133.1			−32	−11
2,4-dicumylphenol	329.2	**313.2**	−94	−9	−46	−16
		250.9			−37	−14
		237.0			−44	−15
BHT	219.0	**203.0**	−133	−11	−35	−11
		163.0			−35	−13
2-ethylhexyl salicylate	249.2	**93.0**	−120	−13	−28	−10
		137.0			−24	−14
		127.1			−35	−7

As in positive as in negative ion mode experiments, the following parameters were maintained constant: vaporizer temperature 300 °C, curtain gas 20 psi, ion spray voltage ±4.5 kV.

For the developed online SPE-UHPLC-tandem MS method we evaluated the following parameters: linearity of calibration curve (DIFF%), selectivity (SEL%), LOD, LOQ, LLOQ precision (BIAS%) and accuracy (RSD%). LOD and LOQ were determined as three and ten times the signal-to-noise ratio. LLOQ was expressed as the lower experimentally measured analytes concentration. For the values we followed the guidelines proposed by FDA (Food and Drug Administration) [23] and the tested parameters might be the following: SEL% < 30.00; DIFF% < 25.00; $BIAS_{LLOQ}$% < 30.00 and RSD_{LLOQ}% < 25.00.

2.3. PET Samples Treatment

Ten PET bottles made by preforms, and not yet in contact with food (i.e., mineral water, tea, filtered fruit juices) were provided by different Italian suppliers. The identification name and characteristics of samples PET bottles are reported in Table 3.

Table 3. Identification name and characteristic of PET bottles samples.

Identification Name	Sample Characteristics
P1	1.5 L clear PET bottle (32.5 g preform)
P2	0.5 L clear PET bottle (19 g preform)
P3	0.5 L dark blue PET bottle (12 g preform)
P4	0.5 L light blue PET bottle (10.4 g preform)
P5	1.5 L light blue PET bottle (23 g preform)
P6	2 L light blue PET bottle (27.5 g preform)
P7	1.5 L dark blue PET bottle (32 g preform)
P8	0.5 L cleat PET bottle (7.2 g preform)

For each sample overall migration and specific migration of the components studied in this work were assessed accordingly with current directives [10]. Overall migration comprises the total amount of substances that can migrate from packaging to food; the assessment of specific migration concerns the evaluation of the maximum number of specific substances that can migrate from packing to food.

The evaluation of overall and specific migration was carried out using the following two food simulants [10]: 3% acetic acid (w/v) in aqueous solution (simulant A) and 20% ethanol in aqueous solution (simulant B). Each simulant was added to the PET bottles and samples were heated at 60 °C for 10 days. These conditions are indicated for reproducing a packing-food contact for over 30 days [10]. Particular attention was paid to sealing bottles to prevent simulant evaporation.

2.4. Overall and Specific Migration Tests

Overall migration determination was carried out evaporating the simulants solutions to a smaller volume, then the solutions were transferred in a previously calibrated capsule, where a completed evaporation was reached [10]. The capsules were then weighted, and the overall migration was reported as weighted residual amount (mg) per dm^2 of contact surface. The contact surface was a constant parameter depending on the volume of the bottles as follows: 0.5 L = 4.0 dm^2, 1.5 L = 8.5 dm^2, 2 L = 10.5 dm^2 [10].

For the assessment of specific migration, a preliminary manual solid-phase extraction procedure was applied to PET bottle samples. The SPE filter (Empore^{TM} disk, Merck, matrix active group polystyrene-divinylbenzene, 12 μm particle size, 47 mm external diameter) was conditioned with 10 mL of methanol:chloromethane and 5 mL of methanol. After samples loading, the elution was made with 3 aliquots of 10 mL of ethyl acetate:dichloromethane 70:30 (v/v). The extract was dried with 2g of Na_2SO_4 and transferred quantitatively to a test tube. Na_2SO_4 residual was washed once with 2.5 mL of dichloromethane. Solvent was evaporated under gentle stream of nitrogen and reconstituted with 2 mL of aqueous 0.1% formic acid and analyzed with the developed online SPE-UHPLC-tandem MS method. The alcoholic component of simulant B (ethanol 20% aqueous solution) was distilled before the SPE procedure, and the remaining aqueous solution acidified with HCl 6M (2 mL/L). All solutions were added before extraction with BPA-d16 (final concentration 0.5 μg/L) used as internal standard.

3. Results and Discussion

For the twelve tested NIAS, we evaluated the performance of the developed online SPE-UHPLC-tandem MS method, measuring seven parameters as described above. The obtained results are shown in Table 4. BPS showed the best results in terms of LOD

(0.002 µg/L), LOQ (0.01 µg/L) and LLOQ (0.25 µg/L), while BHT the was worst ones (LOD 13.6 µg/L, LOQ 42.2 µg/L and LLOQ 100 µg/L).

Table 4. Parameters for NIAS analytical standards evaluated with the developed online SPE-UHPLC-tandem MS method.

Compounds	SEL%	DIFF%	$BIAS_{LLOQ}$%	RSD_{LLOQ}%	LOD (µg/L) [1]	LOQ (µg/L) [1]	LLOQ (µg/L) [1]
4-cumylphenol	2.10	5.00	20.2	13.4	0.38	1.27	10
BHT	19.5	4.62	15.8	13.0	13.6	42.2	100
2-ethylhexyl salicylate	4.90	10.5	12.9	25.6	2.70	9.01	50
2,4-dicumylphenol	1.13	6.51	8.15	0.18	0.18	0.61	10
BPA	0.01	5.65	14.6	0.11	0.12	0.40	10
BPS	0.27	9.50	10.5	0.1	0.002	0.01	0.25
2,4-TDI	6.40	2.72	1.81	6.05	1.29	4.31	10
2,6-TDI	0.16	3.36	3.73	14.9	0.27	0.91	10
HDI	0.11	11.8	12.8	8.69	0.82	2.74	10
Homosalate	0.83	18.9	10.4	26.4	0.21	0.70	10
Octocrylene	16.7	26.1	12.2	14.7	5.83	19.4	25

Selectivity and LLOQ precision showed good results. All the parameters reached the limits suggested by the FDA [23] with only the following three exceptions: RSD_{LLOQ}% of 2-ethylhexyl salicylate and homosalate, and DIFF% of octocrylene (FDA suggested limits are RSD_{LLOQ}% < 25.00 and DIFF% < 25.00). Since a full validation of the presented method was out of the scope of the work and the obtained measurements were quite close to the suggested limits, we considered the developed analytical method robust enough to perform the analysis of the migration test with bottle samples. Albeit we changed all the connection peek tubes to avoid the presence of plasticizer in the pipelines of the liquid chromatographer, some components of the instrumentation were not replaceable. This caused an accumulation of the analyte BEHT, and for this reason, it was not possible to evaluate the aforementioned parameters for it. For the evaluation of the molecule in PET bottle samples, we always performed a blank analysis after and before the analysis.

Migration from eight different PET-based bottles was evaluated, as previously described, using two different simulants and the newly developed UHPLC-MS/MS method that takes advantage of online SPE cartridges.

The worst using conditions represented by a food-packaging contact time greater than 30 days, accordingly to European Regulation 10/2011 [10], can be exemplified by thermal treatment (10 days at 60 °C). They are accountable for plastic components' migration into food and the consequent damage of food's organoleptic properties.

The overall migration was investigated using simulants A and B. The results (Table 5), expressed by total contact surface, showed an overall migration above acceptable levels of 10 mg/dm^2 or 60 mg/Kg (60 mg/L for water) set by the European Regulation 10/2011 [10] for all PET bottle samples. Analysis was performed in three replicates. Consistent results with those here exposed are presented in the work of Marín-Morocho et al. [24]. The overall migration was, as for us, below the limits proposed by the European Regulation 10/2011; however, some samples gave positive results (not minor of 0.1 mg/dm^2). Together, these findings suggest hypothetical contamination of PET bottles during manufacturing.

Simulant B (ethanol 20% *v*/*v* aqueous solution) is more polar than simulant A (acetic acid 3% *w*/*v* aqueous solution) and is more attractive to polar PET migrants. As Table 5 showed, simulant B had a greater extraction capacity and higher overall migration values for most samples (i.e., sample P2, <0.1 vs. 0.468 mg/dm^2 for simulant A and B, respectively).

Table 5. Overall migration results of real samples in simulant A (acetic acid 3% p/v aqueous solution) and simulant B (ethanol 20% *v*/*v* aqueous solution).

Sample	Migration in Simulant A (mg/dm^2)	Migration in Simulant B (mg/dm^2)
Blank	<<0.1	<<0.1
P1	<0.1	<0.1
P2	<0.1	0.468
P3	<0.1	<0.1
P4	<0.1	0.107
P5	0.153	0.132
P6	<0.1	0.117
P7	<0.1	<0.1
P9	<0.1	0.176

To evaluate the specific migration of the twelve NIAS studied in this work, we selected eight PET bottle samples with different characteristics, and, after extraction, we applied the developed method to quantify analytes in the samples. The results are shown in Table 6. The chromatographic separation of 4-cumylfiphenol and BPS found in sample P3 when treated with simulant B is shown in Figure 1.

Table 6. Quantitation of analytes in PET bottles samples after specific migration test (concentrations units are specified for each analyte). Sim, simulants; nd, not detectable.

Compound	P1 Sim. A/B	P2 Sim. A/B	P3 Sim. A/B	P4 Sim. A/B	P5 Sim. A/B	P6 Sim. A/B	P7 Sim. A/B	P8 Sim. A/B
4-cumylphenol (ng/L)	nd/nd	nd/**4.78**	**7.21/7.00**	**4.77**/nd	nd/nd	nd/nd	nd/nd	nd/nd
2,4-dicumylphenol (µg/L)	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd
2-ethylhexyl salicylate (µg/L)	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd
BPA (µg/L)	nd/nd	nd/**0.03**	0.01/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd
BPS (µg/L)	nd/nd	**0.04**/nd	**0.50/0.05**	nd/nd	**0.59**/nd	nd/nd	**0.02**/nd	nd/nd
BHT (µg/L)	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd
Octocrylene (µg/L)	nd/nd	**0.04**/nd	nd/nd	nd/nd	nd/nd	nd/nd	**0.01**/nd	nd/nd
Homosalate (µg/L)	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd
HDI (µg/L)	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd
2,4-TDI (µg/L)	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd
2,6-TDI(µg/L)	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd

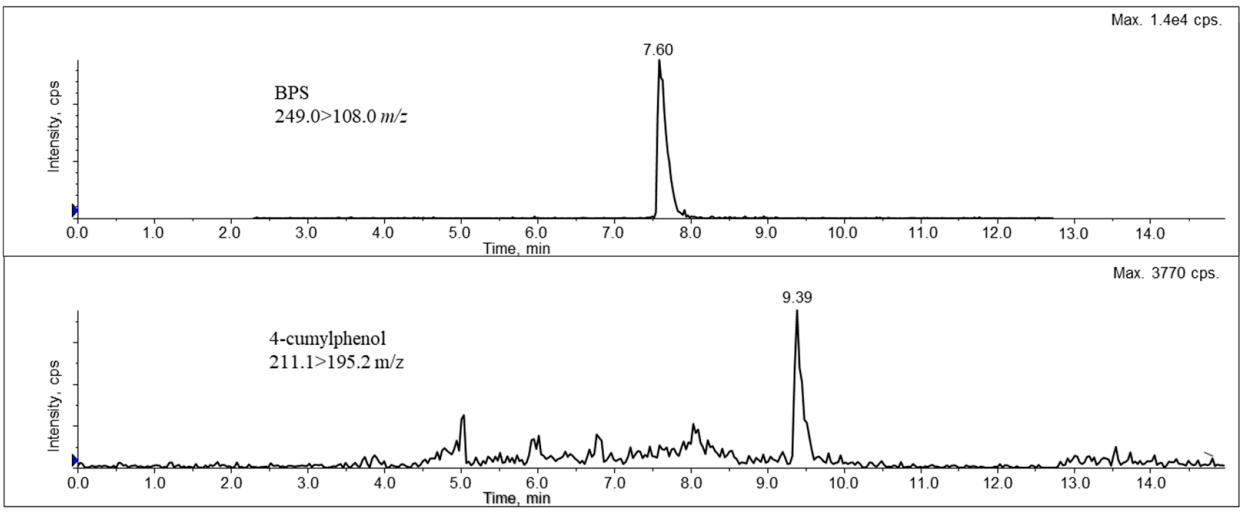

Figure 1. Chromatographic separation of BPS and 4-cumylphenol in sample P3 treated with simulant B (20% ethanol).

Bisphenols were detected in half of the following analyzed samples: in four PET bottles were quantified BPS (P2, P3, P5, P7), and in two of them also BPA was identified (P2, P3). The amounts were in the range between 0.02 and 0.59 µg/L for BPS, and 0.01 and 0.03 µg/L for BPS. In accordance with other research works that detected bisphenols

and bisphenols derivatives in polycarbonate bottles, low-density polyethylene bottles, recycled PET pellets, preforms and bottles, cans of beverages, and baby bottles and sippy cups [25–29] the presence of these Endocrine Disrupters could arise from manufacturing operations or from uncontrolled contamination. However, the detected amounts in the PET bottles here tested were far below the limit of tolerable daily intake (TDI) imposed by the European Regulation 10/2011 [10], that is 4 μg per kg of body weight per day.

Despite the presence of bisphenols being well documented both in packaging materials and in many foodstuffs [26,30–32], the detection of 4-cumylphenol and octocrylene were quite unusual. Only a few studies focused on the identification of these two substances in packaging materials [26,33–35]. We found 4-cumylphenol in three samples (P2, P3, P4) with amounts of ng/L and octocrylene in two samples with a concentration of a few μg/L (0.04 μg/L in P2 and 0.01 μg/L in P7). The specific migration limits [10] for the molecules are 0.05 and 0.04 μg/g for 4-cumylphenol and octocrylene, respectively; moreover, in this case, the quantitation determined by the developed method was far below the limitation.

It is known that 4-cumylphenol is used in the packaging industry as an additive, and in particular, it is a chain terminator for polycarbonate products. It was documented that the molecule affected the accumulation of lipids and the amount of leptin in 3 T3-L1 cells, a model of cell-line suitable for the study of adipocyte differentiation [36]. Its activity is quite similar to BPA and the results raised the need to pay attention to the replacement of bisphenol A with this molecule.

Regarding octocrylene, it is a UV filter normally employed in sunscreen products to enhance emollient properties. As FCM, it is used to reduce the absorption of UV by the polymer matrix and therefore the speed of action of atmospheric agents. Qi-Zhi Su and co-workers [34] classified octocrylene as a substance with toxicity at level IV (level V was the maximum, indicating high toxicity) and other studies underlined its adverse effects on the aquatic environment, suggesting its banning as an ingredient for personal care products [37–40]. Moreover, Downs et al. documented that the known carcinogenic, mutagenic, and endocrine disruptor benzophenone could originate from octocrylene degradation in accelerated-aged conditions [41].

The results obtained by this work revealed the presence of phenols, antioxidants, light and heat stabilizers in the simulants used for migration tests that could arise from different sources, including sealing caps, transport pipelines, disinfection agents and environmental pollution. PET might degrade under certain conditions of daily use, which contribute to the presence of inorganic components or bacteria; degradation might affect not only the polymer but also the additives present, thus determining the formation of new low molecular weight compounds with a high migration coefficient [42]. NIAS might also come from polyurethane adhesives, formed by polyols and diisocyanate monomer polymerization. So, high sensitivity is strongly recommended.

4. Conclusions

In conclusion, the developed online SPE-UHPLC-tandem MS method was a satisfactory tool to quantify twelve NIAS in samples of PET bottles.

The reached sensitivity was high enough to detect, identify and quantify the analytes in samples, and the precision and accuracy of LLOQ guaranteed the quality of the results. Online SPE as an automated extraction procedure reduces systematic and random errors.

Analyzing samples of eight different PET bottles, we found the presence of bisphenol A and S, 4-cumylphenol and octocrylene. All the concentrations were far below the limit indicated by the European Regulation 10/2011 [10]; however, these findings underline that it is mandatory to control the levels of toxic substances that might migrate from packaging to foods. Except for well-known toxic compounds, such as formaldehyde and acetaldehyde, which are thermal degradation products of PET possibly coming from storage conditions [43–45], antimony residuals from polymerization catalysts [46,47], more studies are necessary to claim the direct link between PET use and compounds found in drinking water, including NIAS.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations9080188/s1, Figure S1: structural formulae, name, abbreviation, molecular formulae and molecular weight of the selected analytes. Scheme S1: fragmentation pathway of 2,4-TDI (ESI positive). Scheme S2: fragmentation pathway of 2,6-TDI (ESI positive). Scheme S3: fragmentation pathway of HDI (ESI positive). Scheme S4: fragmentation pathway of octocrylene (ESI positive). Scheme S5: fragmentation pathway of homosalate (ESI positive). Scheme S6: fragmentation pathway of BEHT (ESI positive). Scheme S7: fragmentation pathway of BPA (ESI negative). Scheme S8: fragmentation pathway of BPS (ESI negative). Scheme S9: fragmentation pathway of 4-cumylphenol (ESI negative). Scheme S10: fragmentation pathway of 2,4-dicumylphenol (ESI negative). Scheme S11: fragmentation pathway of BHT (ESI negative). Scheme S12: fragmentation pathway of 2-ethylhexyl salicylate (ESI negative).

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