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RESEARCH PAPER

Liquid chromatography-mass spectrometry method for the determination of polyethylene terephthalate and polybutylene terephthalate cyclic oligomers in blood samples

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Abstract

Food contact materials (FCM) polyethylene terephthalate (PET) and polybutylene terephthalate (PBT) used extensively in food packaging may contain cyclic oligomers which may migrate into food and thus cause toxic effects on human health. A simple, fast, and sensitive ultra-high-performance liquid chromatography method quadrupole time-of-flight mass spectrometer was developed for the analysis of 7 cyclic oligomers in post-mortem blood samples. The targeted analytes were separated on a Waters BEH C18 (150 × 2.1 mm, 1.7 µm) analytical column by gradient elution. Calibration curves were constructed based on standard solutions and blood samples and Student's *t*-test was applied to evaluate the matrix effect. The LODs ranged from 1.7 to 16.7 µg mL^{-1}, while the method accuracy was assessed by recovery experiments and resulting within the range 84.2–114.6%. Such an analytical method for the determination of PET and PBT cyclic oligomers in biological samples is reported for the first time. The developed methodology allows the determination of these oligomers in blood providing a useful analytical tool to assess the exposure and thus the potential hazard and health risks associated with these non-intentionally added substances (NIAS) from PET and PBT FCM through food consumption. The method was validated and successfully applied to the analysis of 34 post-mortem whole blood samples. Polyethylene terephthalate trimer was detected in four of them, for the first time in literature.

Keywords Ultra high performance liquid chromatography (UHPLC) · Quadrupole time-of-flight mass spectrometry (qTOF-MS) · Polyethylene terephthalate (PET) · Polybutylene terephthalate (PBT)

Introduction

Polyesters (PES) are a group of polymers used extensively in many fields of applications. They are among the plastics of preference used in the production of food contact materials (FCMs), due to properties like impact resistance, strength, flexibility, and resistance to high temperatures [1, 2]. Among the most relevant FCMs of PES, polyethylene terephthalate (PET) and polybutylene terephthalate (PBT) are prevailing [3]. PET is the most used PES in beverage packaging applications due to its excellent properties like light-weight and low carbon dioxide permeability [1, 4–6].

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However, during the synthesis of almost all polymers, low molecular weight circular oligomers are formed as a result of uncontrolled polymerization reactions that take place. These cyclic oligomers are characterized as "non-intentionally added substances" (NIAS). Undesired and unregulated, NIAS apart from cyclic oligomers encompass several different types of chemical families, and their presence in the FCMs may originate from impurities in the raw materials, by-products from reaction processes, and even degradation products of the used additives or the polymers [7].

Among the most well-known NIAS in plastics are a group of substances called oligomers. Normally formed during polymerization, oligomers are side-reaction products ranging from simple dimers up to decamers, in either cyclic or linear forms. Their formation seems to be unavoidable and even thermodynamically favored, with some works also pointing at their potential formation due to degradation of the plastic packaging itself [1, 5, 8]. Oligomers have been shown to migrate from the plastic material into the food, which has led in recent years to an increase of published works on this topic [1, 8–10]. There is also a growing interest on oligomers at EU level, with the European Union Reference Laboratory for Food Contact Materials (EURL-FCM) having organized a couple of proficiency tests (PTs) on the topic [5, 11, 12]. These PTs evaluated the capacity of EU National Reference Laboratories and Official Control Laboratories in the determination and quantification of both PET and PBT cyclic oligomers in food simulants, substances which have not been analyzed before by any of the FCM network laboratories.

It has been long known that FCMs can be a medium of human exposure to hazardous substances present in these materials. There is a wide range of toxicologically uncharacterized chemicals, both intentionally added substances (IAS) and NIAS, that can end up in food [13]. That is also the case of the PES plastic packaging oligomers, which unavailable toxicological data leaves their risk for human health largely unclear [5]. However, in a recent study, some PET cyclic oligomers have been classified via an in silico assessment as having a Cramer III toxicity, the highest level apart from genotoxicity [11, 14, 15]. This toxicity is expected only for the cyclic oligomers, as their linear counterparts are Cramer I, thus having a reduced toxicity when consumed. In another work, the endocrine activity of cyclic PES oligomers has been studied with in vitro assays, where it was indicated a weak androgen receptor antagonism for these substances, with no arylhydrocarbon receptor activity or binding to the thyroid hormone transport protein being reported [16].

Despite the potential toxicity of the cyclic PES oligomers, existing works claim that upon ingestion their amounts are considerably decreased via hydrolysis reactions that occur during digestion [16]. These reactions originate from their corresponding non-toxic linear counterparts, thus decreasing the overall toxicity concerns. In another work, Eckardt et al. studied the in vitro intestinal digestion of individual cyclic PET and PBT oligomers. The results showed a cleavage of the ester group of these cyclic oligomers after 4 h of simulated intestinal incubation, pointing to an expected similar occurrence in real human intestinal conditions [17]. However, in order to evaluate human exposure and hazard assessment, accurate analytical tools are required.

In the literature, few analytical methodologies have been described for oligomers' determination probably due to the difficulties that may occur from the lack of the available analytical standards [15]. Most studies use semi-quantitative approaches, quantifying all PET linear and cyclic oligomers using the PET cyclic trimer as an analytical standard [4, 18–21]. However, these semi-quantitative methods may suffer from limitations related to the accurate quantification of oligomers with longer chain lengths and higher molecular masses. Recently, some studies [15, 16, 22–25] reported their synthesis and the development and optimization of analytical methods used for the quantification of PET and PBT oligomers presenting satisfactory results. However, none of these methodologies has been applied in biological samples.

Here, an analytical methodology was developed and validated for the quantification of seven PET and PBT cyclic oligomers in blood plasma with the aim to investigate the potential exposure through the detection and quantification of these substances in blood. The method included a liquid–liquid (LLE) extraction step followed by a targeted UHPLC-qTOF-MS analysis achieving low detection limits. The optimized method was successfully applied in post-mortem blood samples, where the identification and quantification of one of the oligomers were found for the first time. The obtained results indicate that these compounds are not fully hydrolyzed during digestion as previously expected, giving rise to the possibility of accumulation in the human body in their cyclic and toxic conformation. This is the first method reported for determining cyclic PES oligomers in biological samples that can provide critical data enabling the assessment of exposure to these NIAS.

Materials and methods

Reagents and materials

Acetonitrile, methanol, dichloromethane, n-hexane, and formic acid (≥ 99%) were all LC–MS analytical grade (HiPerSolv CHROMANORM) and were supplied from VWR Chemicals (Darmstadt, Germany). Butyl acetate (LC–MS grade) was purchased by Sigma-Aldrich (Merck Darmstadt, Germany), while glacial acetic acid (99–100%) and ethyl acetate (LC–MS grade) were acquired from Chem-Lab (Zedelgem, Belgium). 1,1,1,3,3,3-Hexafluoro-2-propanol

(HFIP) was supplied from Honeywell Fluka™ (Buchs, Switzerland). Ultrapure water by a Milli-Q purification system (Merck Darmstadt, Germany) at 18 MΩ-cm (at 25 °C). The first series PET cyclic oligomer standards up to pentamer and the internal standard PET cyclic trimer-d_{12} were obtained from TRC chemicals (Toronto, Canada), with stated purities ranging from 95 to 97%. PBT cyclic dimer, trimer, and tetramer were obtained as a standard mixture (20 μg mL^{-1}) from the European Union Reference Laboratory for Food Contact Materials (EURL-FCM).

Calibration

Calibration and preparation of solutions in standard mixtures

Stock solutions of the PET dimer and the isotope internal standard were prepared at a concentration of 5000 mg L^{-1} in HFIP whereas PET trimer, tetramer, and pentamer stock solutions were at 2500 mg L^{-1}. PBT mix solution in HFIP was at a concentration of 20 mg L^{-1}, in HFIP as solvent. A multi-component solution (2.5 mg L^{-1}) containing equal concentrations of every analyte was prepared weekly by mixing the appropriate volumes of the stock solutions. Serial dilutions of this mix solution followed in amber vials with ethanol:H_2O 50:50 (v/v), to prepare working standard mixtures at ten concentrations (5, 10, 25, 50 100, 250, 500, 750, 1000, 2500 μg L^{-1}). Stock solutions and working standards were both kept at − 20 °C. Calibration curves were constructed by plotting the means of ratios of the compound peak areas to the internal standard peak areas (PET cyclic trimer-d_{12}) against concentrations of the analytes.

Preparation of solutions for calibration

A post-mortem blood sample in which the analytes were not detected was selected to constitute the "blank blood sample" providing the matrix background. For the construction of the calibration curves in blood, the blank blood sample was fortified with known standard mixtures of the 7 cyclic oligomers to a final concentration of 12.5, 25, 50, 125, 250, 500, 750, 1000, and 1500 μg L^{-1}. A blank sample was also prepared with the same procedure. The calibration curves were constructed as mentioned above, in the "Calibration and preparation of solutions in standard mixtures" section.

Sample preparation

A total of 34 post-mortem blood samples were collected at the Forensic Service of Greek Ministry of Justice in Thessaloniki during autopsy and stored in glass tubes containing NaF from cases aged 65 years old or older and kept at − 20 °C. A 250 μL aliquot of blood sample was fortified with 12.5 μL of IS and 600 μL of ethyl acetate was added as the extraction solvent. The mixture was vortexed for 10 min and then centrifuged at 12,000 rpm for 10 min. The upper organic phase was transferred into a 1.5 mL Eppendorf tube and the extract was evaporated to dryness, using an eppendorf concentrator. Finally, the samples were reconstituted with 100 μL ethanol:H_2O 50:50 (v/v) and were transferred in LC vials and 5 μL was injected into the chromatographic system. Prior to the main analytical run, 5 injections (5 μL) of blank blood samples were performed to ensure the absence of the cyclic oligomers' peaks in the chromatographic run and adequate system conditioning. In order to avoid any potential source of contamination, the samples came in contact only with containers (e.g., Eppendorf tubes) and syringes made of other plastic materials, such as polypropylene and polyethylene with metal needles.

UHPLC-TOF–MS analysis

An UHPLC system (Bruker, Germany) was used for chromatographic separations using a Waters BEH C18 (150 × 2.1 mm, 1.7 μm) analytical column, protected by a UPLC BEH C18 (5 × 2.1 mm, 1.8 μm) VanGuard pre-column. The mobile phase consisted of solvent A: H_2O with 0.1% formic acid and solvent B: ACN with 0.1% formic acid at a flow rate of 0.3 mL/min. The elution was performed in a 12 min gradient as follows: 0–1 min: 50–70% B; 1–12 min: held to 90% B. The composition was returned to the initial (50% B) in 0.1 min. An equilibration time of 4 min was set for the column before the next injection. The analytical column was temperature-controlled at 60 °C and the injection volume was 5 μL. The system was operated by Compass HyStar 5.1 software (Bruker, Germany) and was hyphenated to a timsTOF mass spectrometer (Bruker, Germany) operating in positive ionization mode (ESI) at a 3.5 kV capillary voltage. The source operated at 300 °C and nitrogen was used as drying (12.0 L/min) and nebulizing gas (2.0 Bar). The Funnel 1 RF, Multipole RF, Deflection Delta, and Collision RF were set at 200 V, 200 Vpp, 50 V, and 700 Vpp, respectively. The acquisition mode was set at full scan MS acquiring data over the range of 300 to 1000 m/*z* at a rate of 3 spectra/s. For individual recalibration of the chromatograms, sodium formate solution was injected before every analysis from 0.1 to 0.3 min. Data Analysis 5.3 software was used for data handling (Bruker, Germany).

Extraction recovery

The extraction recovery of the method was determined by adding different concentrations of the analytes to the blank blood sample in two replicates. Solvents tested for the extraction of the seven oligomers included (a) ethyl acetate, (b) butyl acetate, (c) hexane, and (d) dichloromethane. The

samples were fortified to obtain a nominal concentration of 1000 μg L^{-1} for each analyte and were extracted and analyzed according to the method described in the "Sample preparation" section. Finally, the extract was transferred into a LC–MS vial prior to LC–MS analysis.

The recovery was expressed as a percentage and calculated by using the following equation:

$$R\% = 100 \times \left(\frac{\text{spiked}(b)}{\text{spiked}(a)} \right) \quad (1)$$

where spiked (b) is the calculated concentration in the blood sample before the extraction process and spiked (a) is the nominal concentration of the extracts.

Matrix effect

The effect of the matrix can be variable and unpredictable in the occurrence of coeluting endogenous components [26] and it is important to evaluate the matrix-induced signal suppression or enhancement of the analytes. Thus, the matrix effect (ME) was determined by comparing the slopes of the solvent and SAM calibration curves, using Student's t-test [27], according to the equation:

$$t = \frac{b_1 - b_2}{s_{b1-b2}} \quad (2)$$

where b_1 and b_2 are the slopes of the regression lines. The standard error of the difference between the regression slopes is calculated as:

$$s_{b_1-b_2} = \left[\frac{(s^2_{y\times x})_p}{\sum x_1^2} + \frac{(s^2_{y\times x})_p}{\sum x_2^2} \right]^{\frac{1}{2}} \quad (3)$$

where $(s^2_{y\cdot x})_p$ is the pooled residual mean square and the subscripts 1 and 2 refer to the two regression lines (SAM and solvent) when compared. The critical values of t-test were calculated taking into account $(n_1-2)+(n_2-2)$ degrees of freedom.

Method validation

Linearity and limits of detection and quantification

The sensitivity and linearity of the proposed method were assessed by analyzing both standard solution mixtures and spiked samples over the range of 5–2500 μg L^{-1} and 12.5–2500 μg L^{-1}, respectively. The slopes, intercepts, and correlation coefficients were calculated using linear least squares regression. The limits of detection (LOD) were calculated as the lowest concentrations of standard solutions or fortified samples with a 3 times signal to noise (S/N) ratio, while the limits of quantification (LOQ) were defined as three times the LODs.

Precision and accuracy in standard solutions

Precision and accuracy were assessed with standard solutions in short term (repeatability and intra-day accuracy) and for a longer period (intermediate precision and inter-day accuracy), by evaluating relative standard deviation at four concentration levels. Both short-term repeatability and accuracy were calculated by six replicate injections of each of the four multicomponent standards on the same day while inter-day precision and accuracy were determined by performing triplicate measurements for three consecutive days. The measurement accuracy of the analytical method was expressed as relative error and precision as relative standard deviation S_r (%).

Results and discussion

Method development

Chromatographic parameters

PET and PBT cyclic oligomers exhibit limited solubility in most common organic solvents; hence, HFIP was selected as the solvent of choice for the solubilization of the 1st series of the target compounds based on previous studies [2, 18]. For further dilutions, a mixture of ethanol:H_2O 50:50 (v/v) was used, in order to facilitate their chromatographic analysis.

Chromatographic conditions, such as the mobile phase composition, affect apart from the retention times also the ionization efficiency of the compounds and their respective analysis sensitivity. In ESI mode in particular, volatility and solvent's ability to donate a proton are of high importance. According to previous reports, a mobile phase solvent composed of ACN and H_2O was the most effective [5, 18]. Hence, a mobile phase consisting of solvent A: H_2O with either 0.1% acetic acid or formic acid and solvent B: ACN with 0.1% acetic acid or formic acid was tested in gradient mode. The formic acid was finally chosen as it gave better chromatographic peak shapes and ion intensities. The gradient program was optimized to obtain the elution of the 7 analytes within an analytical run of 12 min, and with satisfactory separation of the oligomers. Figure 1 illustrates the extracted ion chromatograms of the analytical standard mixture and the representative spiked sample at 1000 μg L^{-1}.

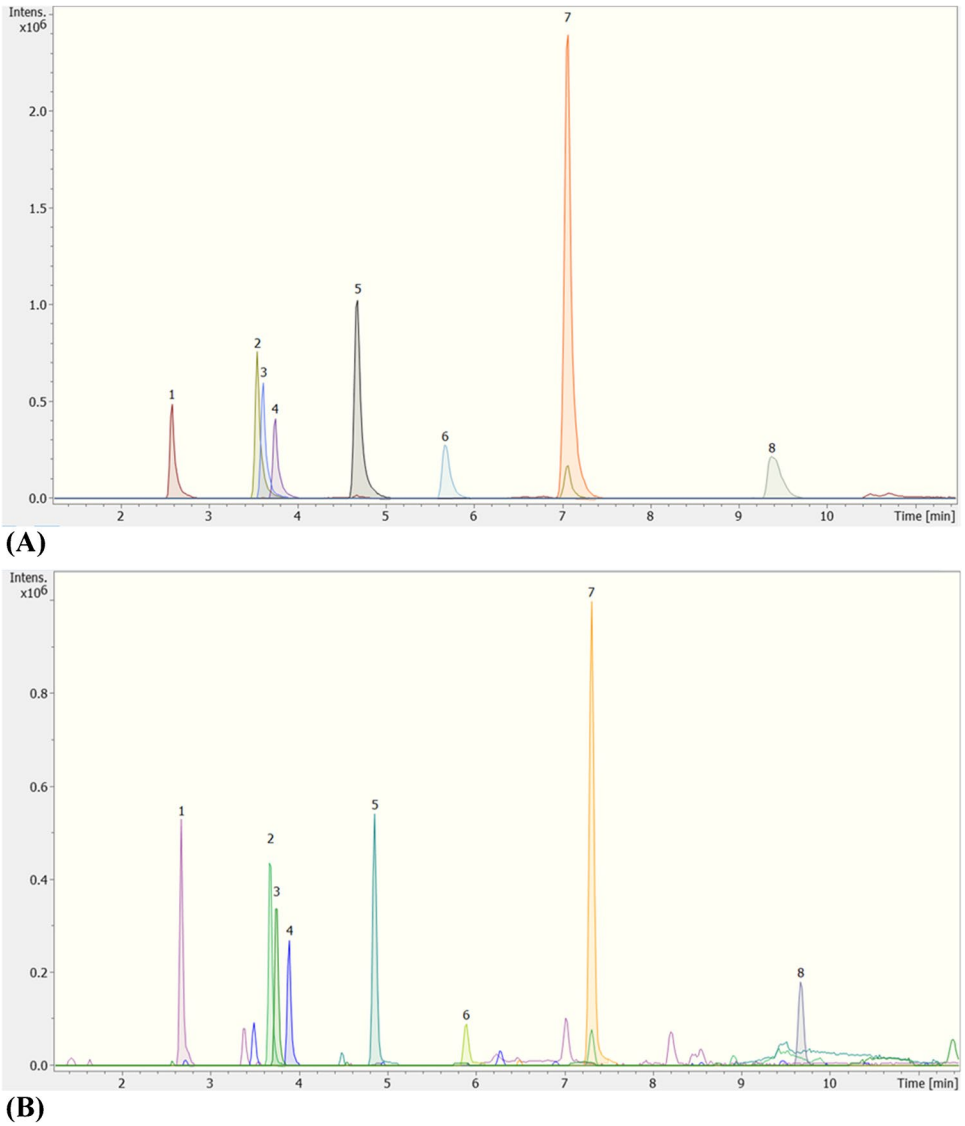

Fig. 1 UHPLC-QTOF-MS extracted ion chromatogram (XIC) of the analytes (1) PET dimer, (2) PET trimer d12, (3) PET trimer, (4) PBT dimer, (5) PET tetramer, (6) PET pentamer, (7) PBT trimer, (8) PBT tetramer, at the optimal chromatographic conditions. (A) Standard multi-component mixture (1000 μg L^{-1}) in ethanol:H_2O 50:50 (v/v) solution. (B) Example chromatogram from a representative blood sample spiked with a standard multi-component mixture (1000 μg L^{-1}) of the analytes

TOF–MS parameters

In full scan MS mode, the source (capillary voltage, temperature) and tune general parameters (Funnel 1 RF, Funnel 2 RF, Multipole RF, Collision RF, and transfer time) were optimized based on the target compounds' precursors, in order to maximize the ion signals. Full scan data acquisition from 300 to 1000 m/z was performed for all oligomers, after direct injection of a multi-component standard mix solution at a concentration of 1000 μg L^{-1}. All target compounds showed higher response in positive ESI mode, and these findings were in agreement with those previously reported [5, 18]. Every analyte was identified based on its precursor ion and its retention time.

Method validation

Linearity and sensitivity

The method was validated in terms of linearity, LODs, and LOQs and the results along with molecular formulas, molecular ions, and retention times are presented in Table 1. In all cases, the analytes displayed excellent linearity with correlation coefficients (r^2) > 0.99. For solvent calibration, the LODs ranged from 1.7 to 6.7 μg L^{-1}, while LOQs from 5 to 20 μg L^{-1}. For the SAM calibration, the LODs ranged between 4.2 and 16.7 μg L^{-1} and the LOQs between 12.5 and 50 μg L^{-1}. To our knowledge, this is the first method that determines cyclic oligomers in blood samples. Hence, no relevant previous analytical work exists for these groups of analytes in this matrix and a comparison of the figures of

Table 1 Analytical features of the proposed method

Compound	Molecular formula	Molecular ion (*m/z*)	Retention time in standard solution (min)	Retention time in matrix (min)	Linear range (μg L^{-1})	Linear regression coefficient (R^2)	LOD (μg L^{-1})	LOQ (μg L^{-1})
PET dimer	$C_{20}H_{16}O_8$	385.09	2.5	2.4	20–750[a]	0.998[a]	6.7[a]	20[a]
					25–750[b]	0.995[b]	8.3[b]	25[b]
PET trimer	$C_{30}H_{24}O_{12}$	577.13	3.4	3.1	20–750[a]	0.997[a]	6.7[a]	20[a]
					12.5–750[b]	0.999[b]	4.2[b]	12.5[b]
PET tetramer	$C_{40}H_{32}O_{16}$	769.18	4.3	3.9	20–2500[a]	0.998[a]	6.7[a]	20[a]
					25–500[b]	0.992[b]	8.3[b]	25[b]
PET pentamer	$C_{50}H_{40}O_{20}$	961.22	5.1	4.5	20–750[a]	0.996[a]	6.7[a]	20[a]
					50–500[b]	0.996[b]	16.7[b]	50[b]
PBT dimer	$C_{24}H_{24}O_8$	441.15	3.5	3.2	20–500[a]	0.991[a]	6.7[a]	20[a]
					25–750[b]	0.999[b]	8.3[b]	25[b]
PBT trimer	$C_{36}H_{36}O_{12}$	661.23	6.3	5.5	5–750[a]	0.998[a]	1.7[a]	5[a]
					25–1000[b]	0.994[b]	8.3[b]	25[b]
PBT tetramer	$C_{50}H_{40}O_{20}$	881.30	8.3	7.3	20–750[a]	0.998[a]	6.7[a]	20[a]
					50–750[b]	0.999[b]	16.7[b]	50[b]

[a]Solvent calibration; [b]SAM calibration

merit of the method presented here can only be evaluated per se (Table 2).

Extraction recovery and matrix effect

With the aim to investigate the optimum extraction recovery, different extraction solvents were tested, including (a) ethyl acetate, (b) hexane, (c) butyl acetate, and (d) dichloromethane. The blank blood sample was fortified with the compounds of interest to obtain a nominal concentration of 1000 μg L^{-1} for each analyte and the results obtained from the 4 different extractions solvents are illustrated in Fig. 2. Ethyl acetate was able to extract all seven analytes and showed the most satisfactory results, with good extraction efficiency and acceptable extraction repeatability. Among all four solvents, hexane provided the least satisfactory results with the lowest peak areas, while butyl acetate was not able to extract the PBT tetramer and PET pentamer compounds. Dichloromethane showed high extraction efficiency in five out of the seven compounds of interest; however, PBT dimer was not extracted when the specific solvent was used. Thus, ethyl acetate was selected for the extraction of the blood samples. Extraction recovery was calculated for the selected protocol based on Eq. 1 for all the analytes, which showed *R*% ranging from 84.2 to 114.6% at the studied concentrations (50, 250, and 500 μg L^{-1}) levels as it is shown in Table 3.

Student's *t*-test was performed in order to evaluate the matrix effect, as described in the "Matrix effect" section. The results are summarized in Table 4. Clearly, a significant difference (at 95% confidence level) existed between the calibration curves in blood and solvent calibration curves for almost all oligomers ($t_{experimental} = 3.292–27.849 > t_{critical} = 2.262–2.447$), except for PBT tetramer ($t_{experimental} = 2.229 < t_{critical} = 2.306$); therefore, the SAM calibration curves were used for quantification to compensate for the bias due to matrix effect.

Precision and accuracy in standard solutions

Intra-day precision of the method, calculated as relative standard deviation (S_r), ranged between 0.6 and 21.6%. Similarly, intermediate precision of all studied compounds was expressed with S_r values below 17.4% at low concentration standards. The method accuracy was expressed as relative error (percentage difference of the nominal value, *Er*%) and ranged for intra-day from − 12.5 to 16.9% and for inter-day between − 11.2 and 20.5%. Therefore, the results ranged within the acceptable values of ± 0.1 to ± 20.5% for the low concentrations, while higher concentrations exhibited lower relative error values. The data of the precision and accuracy study are provided in Table 2.

Analysis of blood samples

The blood samples were analyzed under the optimal conditions, and as it is shown in Table 5, PET cyclic trimer was detected in four out of the 34 examined samples. Furthermore, in sample 6, apart from the ion 577.1341, which derives from the cyclic PET trimer, a molecular ion with *m/z* 576.7249 was detected. A comparison of the different proposed structures using the ACD Lab MS workbook (Toronto, Canada) revealed a match of 78% for an open trimer structure, which indicates the possibility of the presence of the PET linear compounds in human body.

Table 2 Intra-day and inter-day precision of assay in standards solutions

Compound	Intra-day ($n=6$)				Inter-day ($n=3$)			
	Added (μg L^{-1})	Found ± s (μg L^{-1})	S_r (%)	Er (%)	Added (μg L^{-1})	Found ± s (μg L^{-1})	S_r (%)	Er (%)
PET dimer	20	23.39 ± 2.03	8.7	16.9	20	24.10 ± 0.72	3.0	20.5
	40	40.89 ± 1.82	4.4	2.2	40	41.97 ± 2.67	6.4	4.9
	400	410.93 ± 25.07	6.1	2.7	400	390.47 ± 59.45	15.2	− 2.4
	750	749.33 ± 18.40	2.5	− 0.8	750	764.41 ± 7.89	1.0	1.9
PET trimer	20	22.59 ± 2.26	12.0	13.0	20	22.74 ± 2.05	9.0	13.7
	40	35.87 ± 0.21	0.6	− 10.3	40	37.11 ± 4.36	11.7	− 7.2
	400	383.44 ± 29.11	7.6	− 4.1	400	357.66 ± 41.78	11.7	− 10.6
	750	744.75 ± 13.17	1.8	− 0.7	750	739.97 ± 52.83	7.1	− 1.3
PET tetramer	20	19.49 ± 4.22	21.6	− 2.5	20	19.77 ± 3.43	17.4	− 1.2
	40	37.56 ± 5.00	13.3	− 6.1	40	38.83 ± 4.15	10.7	− 2.9
	750	781.23 ± 35.45	4.5	4.2	750	791.89 ± 47.09	5.9	5.6
	2000	1863.52 ± 19.92	1.1	− 6.8	2000	1775.00 ± 109.10	0.1	− 11.2
PET pentamer	20	22.54 ± 3.39	15.0	12.7	20	23.21 ± 2.96	12.8	16.0
	40	44.29 ± 2.30	5.2	10.7	40	42.32 ± 3.27	7.7	5.8
	400	355.80 ± 11.03	3.1	− 11.0	400	376.08 ± 34.94	9.3	− 5.9
	750	767.82 ± 35.61	4.6	2.4	750	774.71 ± 28.23	3.6	3.3
PBT dimer	20	19.97 ± 0.59	2.9	− 0.1	20	20.92 ± 0.94	4.5	4.6
	40	35.00 ± 1.32	3.8	− 12.5	40	36.41 ± 5.63	15.5	− 9.0
	200	192.06 ± 7.42	3.9	− 4.0	200	197.40 ± 13.04	6.6	− 1.3
	400	418.01 ± 17.72	4.2	4.5	400	368.86 ± 31.78	8.6	− 7.8
PBT trimer	20	22.45 ± 0.73	3.2	12.2	20	21.48 ± 2.23	10.4	7.4
	40	36.47 ± 0.51	1.4	− 8.8	40	37.61 ± 3.01	8.01	− 6.0
	400	358.90 ± 13.42	3.7	− 10.3	400	366.35 ± 34.11	9.3	− 8.4
	750	747.86 ± 23.22	3.1	− 0.3	750	791.88 ± 52.16	6.6	5.6
PBT tetramer	20	19.60 ± 3.33	17.0	− 2.0	20	19.61 ± 3.02	15.4	− 2.0
	40	39.03 ± 1.80	4.6	− 2.4	40	37.76 ± 4.44	11.8	− 5.6
	400	381.11 ± 28.04	7.4	− 4.7	400	400.59 ± 38.22	9.5	0.1
	750	758.82 ± 5.17	0.7	1.2	750	760.13 ± 4.79	0.6	1.4

s, standard deviation; S_r, relative standard deviation; Er, relative error

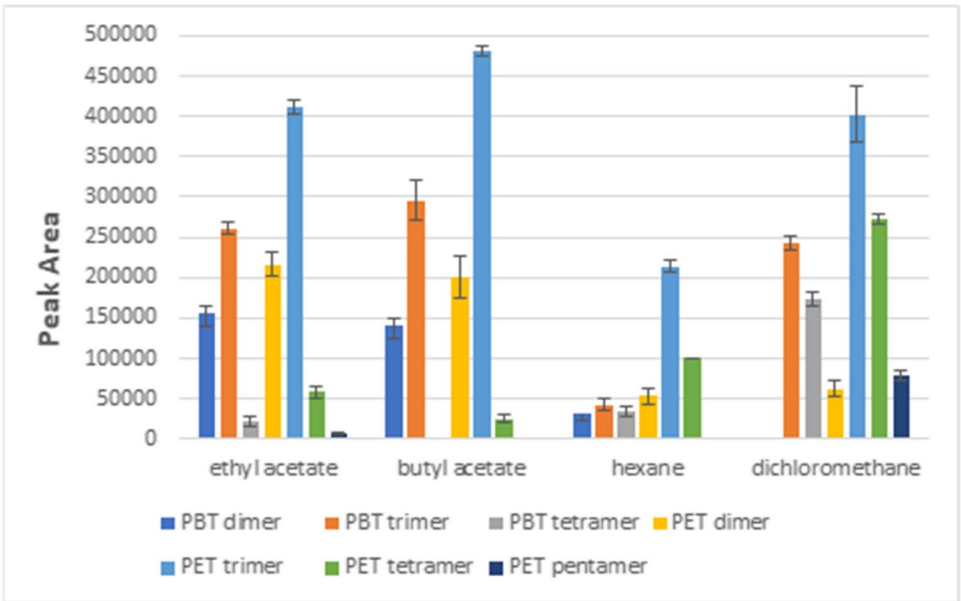

Fig. 2 Peak areas for each of the analytes with the four extraction solvents applied. Peak areas of more abundant oligomers PBT dimer, PBT trimer, and PET dimer were divided by ten in order to fit the scale of the figure

Table 3 Spike recovery (%) of target compounds at three concentration levels

Compound	Concentration level (μg L^{-1})	Spike recovery (%)
PET dimer	50	103.6
	250	103.4
	500	96.1
PET trimer	50	108.4
	250	103.2
	500	92.0
PET tetramer	50	98.1
	250	88.7
	500	104.4
PET pentamer	50	101.6
	250	100.8
	500	87.2
PBT dimer	50	99.3
	250	92.0
	500	99.3
PBT trimer	50	84.2
	250	87.8
	500	87.0
PBT tetramer	50	114.6
	250	95.2
	500	96.3

Table 4 Student's *t*-test

Compound	$t_{experimental}$	$t^{a}_{critical}$
PET dimer	17.226	2.365
PET trimer	27.849	2.262
PET tetramer	3.325	2.306
PET pentamer	7.655	2.365
PBT dimer	15.305	2.447
PBT trimer	3.292	2.262
PBT tetramer	2.229	2.306

[a]At the 95% confidence level and $(n_1-2)+(n_2-2)$ degrees of freedom

Table 5 Concentration of PET trimer in the analyzed blood samples

Samples (no.)	PET trimer (μg L^{-1}) ± *s*
3	13.18 ± 2.51
5	6.50 ± 1.81
6	9.94 ± 0.90
21	23.29 ± 0.94

In the past, in vitro experiments provided evidence for the cleavage of cyclic polyester oligomers under human intestinal conditions [20]. Thus, the relatively more toxic cyclic oligomers (Cramer III) are transformed to the respective linear molecules (Cramer I toxicity). However, in this paper, the presence of cyclic PET trimer is reported for the first time in human biological samples indicating that a significant portion escapes hydrolysis at intestinal conditions during digestion and may result in the human blood at concentration levels of ca. 6–25 μg L^{-1}. Since the fate of the more potent cyclic PET trimer in the human body has not been investigated yet, nor experimental toxicological studies have been performed, it is unknown whether this concentration is limited by hydrolysis or other metabolic activities or if cyclic PET trimer accumulates in adipose, liver, or other fatty tissues. The latter could certainly constitute significant risk that needs to be assessed with regard to public safety and exposure.

The developed methodology allows us for the determination of these oligomers in blood and thus provides a useful analytical tool to assess the exposure to these NIAS from PET and PBT FCM through food consumption.

Conclusions

The presence of oligomers in real human samples is reported for the first time using an efficient liquid–liquid extraction protocol followed by UHPLC-TOF–MS analysis. The proposed analytical method reports the quantification of 7 of the most common cyclic oligomers of PET and PBT in less than 12 min. The acquisition mode offered high sensitivity with sufficient low LOQs, capable of making quantitative statements at target levels of interest. Precision and accuracy were within the acceptable ranges for all target analytes. Finally, the method was applied for the quantification of the substances in postmortem blood samples where only one of the examined oligomers was detected and highlighted the need for toxicological data, in order to evaluate the human health risk assessment.

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Declarations

Ethics approval The collection and analysis of post-mortem blood samples used in this study were approved by Forensic Service of Thessaloniki.

Conflict of interest The authors declare no competing interests.

Disclaimer This manuscript does not represent European Food Safety Authority (EFSA) position.

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