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Identification of oligomers in polyethyleneterephthalate bottles for mineral water and fruit juice Development and validation of a high-performance liquid chromatographic method for the determination of first series cyclic trimer

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Abstract

Cyclic oligomers were identified in PET bottles used for mineral water and fruit juice using MS and ^{1}H and ^{13}C NMR: a first series cyclic trimer, a first series cyclic tetramer, a first series cyclic dimmer and a second series cyclic trimer. An analytical method to determine first series cyclic trimer in these bottles was developed and validated, using HPLC. The first series cyclic trimer levels were 316–462 mg/100 g of PET bottle.

Keywords: Oligomers; PET bottles; HPLC; Validation; MS; ^{1}H and ^{13}C NMR

1. Introduction

Polyethyleneterephthalate (PET) polymer is increasingly being used for food packaging. Typical applications are in film, foil, and bottle forms. Bottles are nowadays one of the main uses of PET in direct contact with foods. They are widely used for carbonated soft drinks, mineral water, fruit juices, edible oils, wines and spirits [1].

The PET packaging materials may contain low levels of residual monomer and low molecular weight oligomers which are formed during the resin polymerization and melting process, besides additives, reaction by-products and polymer degradation products. All of them have potential to migrate into foods [2–5].

The main oligomers of PET are the cyclic oligomers from the first and second series (dimer to nonamer) besides the linear ones. Because of ring tension, the first series cyclic trimer is predominant (60–80% of the total amount of cyclic oligomers). It has been shown that the cyclic trimer migrates and represents 98% of all surface oligomers [6].

A variety of chromatographic methods has been used to identify oligomers in PET: gel permeation chromatography (GPC) [7]; high-performance liquid chromatography (HPLC) [8–11]; gas chromatography–mass spectrometry (GC/MS) [4,12,13] and liquid chromatography–mass spectrometry (LC/MS) [14–17]. The widely used are LC and LC/MS which permit the analysis of a large range of molecular weight oligomers. Extraction of oligomers from the plastic matrix followed by MS analysis has also been described [17–19]. However, MS technique does not allow establish the substitution pattern of the aromatic rings. So, when different isomers are present, MS is not able to detect the difference between them.

The aim of this work was to identify the oligomers in PET bottles used for mineral water and fruit juice and to develop and validate an analytical method to determine first series cyclic trimer in these PET bottles.

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2. Experimental

2.1. Samples, chemicals and reagents

PET bottles used for mineral water and fruit juice were supplied by Brazilian beverage companies. Virgin bottles (empty) from four commercial brands of mineral water (500 and 1500 mL) and two brands of fruit juice (500 mL), named brands 1–5 and 6 were studied.

Silica gel 60, Merck (Darmstadt, Germany) for thin layer chromatography (TLC) and silica gel 60 PF254, Merck (Rio de Janeiro, Brazil) for preparative thin layer chromatography were used.

Acetonitrile (ACN), methanol (MeOH) and dichloromethane (DCM) HPLC grade were purchased from Mallinckrodt Chemical (Phillipsburg, USA) and tetrahidrofuran (THF), hexane and ethylacetate P.A. grade were purchased from Merck (Darmstadt, Germany). Chloroform-d ($CDCl_3$) from Merck (Darmstadt, Germany) was also used. Highly purified water from a Milli-Q RG water purification system from Millipore (Bedford, USA) was used in all procedures.

The first series cyclic trimer of PET, whose aromatic rings are 1,4-dissubstituted, was isolated and purified in our laboratory and used as standard (Fig. 1).

2.2. Instrumentation

The chromatographic system consisted of a Shimadzu Liquid Chromatograph (Kyoto, Japan) with a SCL-10A controller, a LC-10AD pump and a SPD-10A UV-visible detector. The NMR spectrometer was a Varian INOVA-500 (Palo Alto, USA) with 11.7T at 500 MHz (^{1}H) and 126 MHz (^{13}C), using the solvents as an internal standard. A Fisons Platform II mass spectrometer (Manchester, UK) with flow injection into the electrospray source (ESI-MS) was also used. The instrument was operated in the positive ion mode.

2.3. Chromatographic conditions

A reverse phase OmniSpher RP-C_{18} column (20 mm × 4.6 mm), with a 5 μm particle size from Varian (Palo Alto, USA) and mobile phase of MeOH–H_2O (85:15, v/v) were used as well as a normal phase New-Pak Silica 60°A column (150 mm × 3.9 mm), with a 4 μm particle size from Merck (Massachusetts, USA) and mobile phase DCM–THF–CH_3COOH (99:0.5:0.5, v/v). The mobile phases were degassed. The flow-rate was 1.0 mL/min and the injection volume was 5 μL for both columns. The experiments were performed at room temperature. Absorption was measured at 254 nm. The total elution time was less than 30 min.

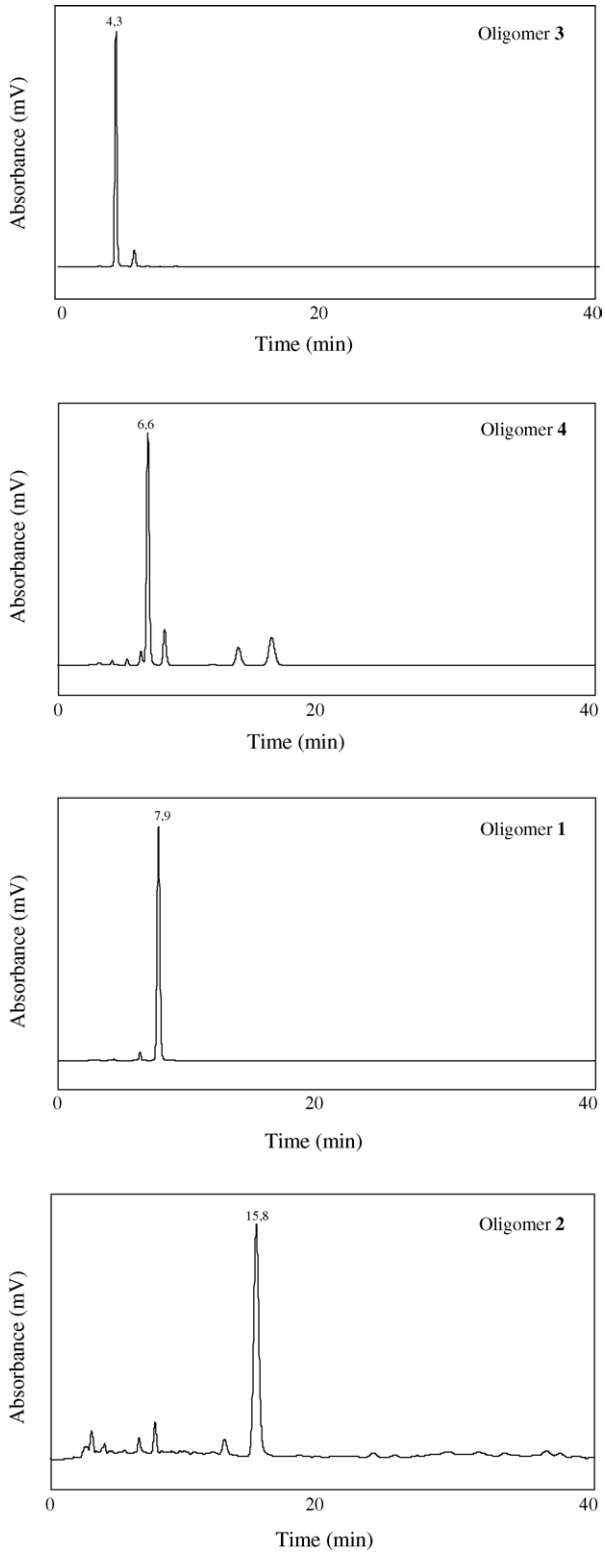


Fig. 1. Chromatograms of oligomers **1**–**4** obtained using HPLC.

2.4. Sample preparation

PET bottles ($n = 6$) from all brands were manually cut and samples (0.500 g) from each brand were extracted with 10.0 mL of DCM, using maceration for 24 h followed by ultrasonic bath for 1 h. The extracts were filtered (1PS Whatman paper) from the polymeric material and then filtered through PTFE (0.45 μm) filters. PET extracts were injected into the HPLC after the HPLC system had been conditioned with the mobile phase for 1 h at a flow-rate of 1.0 mL/min.

2.5. Isolation and purification of the oligomers in PET bottles

To isolate and purify the oligomers, the extract of brand-4 was evaporated to dryness and submitted to a comparative TLC using silica gel as stationary phase and DCM–THF (99.5:0.5, v/v) as mobile phase. The dry extract (100.0 mg) were dissolved in DCM and submitted to a preparative TLC, using the same stationary phase and mobile phase as the comparative TLC to give six fractions. These fractions were resubmitted to the preperative TLC and eluted with DCM to give eight sub-fractions, from which four substances were obtained.

2.6. Identification of the oligomers

The substances were submitted to instrumental analysis using MS and NMR ^{1}H and ^{13}C and were identified as oligomers **1**–**4** [oligomer **1** (40 mg), oligomer **2** (2 mg), oligomer **3** (3 mg) and oligomer **4** (7 mg)].

The identification of the oligomers from the other five brands was carried out, using HPLC and the oligomers **1**–**4** as standards, comparing the t_R of the peaks obtained from the oligomers of each brand of PET bottle with those of the oligomers whose chemical structures were previously identified (brand-4).

2.7. Standard solutions

Stock standard solutions (1000 μg/mL) of the first series cyclic trimer were prepared weekly by dissolution in DCM and stored at 5 °C. Calibration was performed with working solutions diluted in DCM.

2.8. Recovery

Standard solutions containg 41, 410 and 2048 μg/g of first series cyclic trimer in DCM were placed in contact with PET samples (0.500 g) of brand-4 for 24 h and then the extraction procedure described was undertaken.

2.9. Quantification

For quantification, the peak area was measured and external standard procedure was used for both calibration and real sample analysis. The first series cyclic trimer levels obtained were submitted to the ANOVA. Tukey test was used to compare the difference among means at $p \leq 0.05$.

3. Results and discussion

3.1. Chromatographic conditions

To obtain the best chromatographic conditions, the column and the mobile phase composition were adequately selected, working in isocratic mode, in order to determine the content of the first series cyclic trimer and to identify without interference other oligomers in PET bottles.

Regarding the chromatographic procedure, it was first assayed the silica column with a mobile phase DCM–THF–CH_3COOH, varying the proportion of THF to achieve a good separation of all compounds. The best conditions were DCM–THF–CH_3COOH (99:0.5:0.5, v/v) and a flow rate of 1 mL/min. The retention time for the first series cyclic trimer peak was 6.3 min. The same study was carried out using a C_{18} column, where the initial mobile phase was MeOH–H_2O, varying the methanol proportion. The best separation was achieved using MeOH–H_2O (85:15, v/v) and a flow rate of 1 mL/min. The t_R for the first series cyclic trimer peak was 7.9 min.

The C_{18} column presented advantages over the silica column as it gave better resolution, more symmetric peaks and shorter variation in the t_R of the substances present in the PET bottle extracts. The C_{18} column was then chosen for the analysis performed in this work. The silica column was used to evaluate the selectivity during the validation step.

3.2. Structural identification of the oligomers

The ^{1}H NMR data (Table 1) from oligomer **1** show the presence of four aromatic hydrogens at δ 8.10 (*s*), suggesting that the aromatic ring is 1,4-dissubstituted. The coincident chemical shift, as well as the multiplicity of carbinolic hydrogens at δ 4.69 (*s*) indicated that H-1′, H-2′ were magnetically equivalent. The proportion of carbinolic and aromatic hydrogens (1:1) suggests that each polymeric unit contained an oxy-1,2-ethanediyl group.

The ^{13}C NMR and DEPT 135° data for oligomer **1** (Table 2) indicate the presence of four signals corresponding to carbinolic carbons at δ 62.7 (*t*), aromatic carbons at δ 129.7 (*d*), 133.8 (*s*) and carbonylic carbons at δ 165.3 (*s*). The coincident chemical shift, as well as the multiplicity of aromatic carbons, suggest that carbons C-3, C-4, C-6 and C-7 were magnetically equivalent as

Table 1
^{1}H NMR spectra data for oligomers **1**, **2** and **3**

H	**1**	**2**	**3**
1′	4.69 (*s*)	4.63 (*s*)	4.63 (*s*)
2′	4.69 (*s*)	4.63 (*s*)	4.63 (*s*)
3	8.10 (*s*)	8.02 (*s*)	8.25(*dd*) *J* = 2.0 and 8.0
4	8.10 (*s*)	8.02 (*s*)	
5			7.55 (*t*) *J* = 8.0
6	8.10 (*s*)	8.02 (*s*)	8.25(*dd*) *J* = 2.0 and 8.0
7	8.10 (*s*)	8.02 (*s*)	8.84 (*t*) *J* = 2.0

Table 2
^{13}C NMR, *g*HMQC and *g*HMBC data for oligomer **1**

C	^{13}C (δ)[a]	*g*HMQC	*g*HMBC
1′, 2′	62.7 (*t*)	H-1′, H-2′	H-1′
2, 5	133.8		H-3, H-4, H-6, H-7
3, 4, 6, 7	129.7 (*d*)	H-3, H-4, H-6, H-7	H-3, H-4, H-6, H-7
1, 8	165.3		H-3, H-4, H-6, H-7, H-1′, H-2′

[a] Multiplicity was established by DEPT pulse sequence.

well as carbons C-1 and C-8, confirming which aromatic rings were 1,4-dissubstituted.

The ESI-MS of oligomer **1** displayed a quasi-molecular ion $[M + H]^+$ at *m/z* 577 and the base peak at *m/z* 149, which represents a phthalate unit. Based on the ^1H and ^{13}C NMR data and the *g*HMQC, *g*HMBC and MS data, oligomer **1** was determined as being the first series cyclic trimer of PET, whose aromatic rings were 1,4-dissubstituted. The ESI-MS of oligomer **2** displayed a quasi-molecular ion $[M + H]^+$ at *m/z* 791 and the base peak at *m/z* 407, which represents two PET monomers + Na^+. Based on the ^1H NMR and MS data, oligomer **2** was determined as being the first series cyclic tetramer, whose aromatic rings were 1,4-dissubstituted.

The ^1H NMR spectra of oligomer **3** (Table 1) gave evidence of characteristic chemical shifts of four aromatic hydrogens being observed at δ 7.55 (1H, *t*, $J = 8.0$ Hz), 8.25 (2H, *dd*, $J = 2.0$ and 8.0 Hz) and 8.84 (1H, *t*, $J = 2.0$ Hz). The multiplicity of these hydrogens indicated that the aromatic rings were 1,3-dissubstituted. The coincident chemical shifts, as well as the multiplicity of carbinolic hydrogens, at δ 4.63 (*s*) indicated that the H-1′, H-2′ were magnetically equivalent. The ratio of carbinolic and aromatic hydrogens (1:1) indicated that for each polymeric unit, there was an oxy-1,2-ethanediyl group.

The ESI-MS of oligomer **3** displayed a quasi-molecular ion $[M + Na]^+$ at *m/z* 407 and $[M + H]^+$ at *m/z* 385 and the base peak at *m/z* 193, which represents a polymeric unit of this oligomer. Based on the ^1H NMR and ESI-MS data, oligomer **3** was in accordance with the first series cyclic dimer whose aromatic rings were 1,3-dissubstituted.

The ^1H and ^{13}C NMR, *g*HMQC and *g*HMBC data from oligomer **4** identified the presence of carbinolic carbons at δ 63.1, 65.0 and 69.8, aromatic carbons at δ 130.0 and 134.0, and carbonylic carbons at δ 166.0 (Table 3). The δ of the carbinolic carbons present in units II and III were similar to the carbinolic carbons of oligomers **1**, **2** and **3** (δ 63.1). To attribute the respective δ values to the carbinolic carbons of unit I, δ values of carbinolic carbons of oligomer **4** were compared with δ values of diethylene glycol (DEG) [20]. The esterification of an alcohol has an unprotection effect on the Cα and a protection effect on the Cβ [21]. In the case of oligomer **4**, there were also esterifications from the alcohol function. Considering the α and β esterification effects on the δ of DEG and the structure symmetry, δ 65.0 can be attributed to the Cα (C-1′ and C-2″) and 69.8 to the Cβ (C-1″ and C-2′) of unit I. Comparing the data of oligomer **4** with those from oligomer **1**, it can be seen that the aromatic rings of this oligomer were also 1,4-dissubstituted. Oligomer **4** presented two extra signals for the carbinolic carbons at different δ values from those observed for oligomers **1**, **2** and **3**, indicating that oligomer **4** was a second series PET oligomer.

The ^1H NMR spectrum of oligomer **4** showed aromatic hydrogen chemical shift characteristics at δ 8.01 (*s*), at 8.04 (*s*) and at 8.05 (*s*). The first indicated that the hydrogens of one of the aromatic rings were magnetically equivalent (4H). The second and third chemical shifts referred to the hydrogens from other aromatic rings and indicated that two hydrogens of the ring were magnetically equivalent and that the other two hydrogens of the ring were equivalent too, however, the groups themselves are different from each other, confirming that these aromatic rings were also 1,4-dissubstituted (Table 3). The characteristic chemical shift of the carbinolic hydrogens were observed at δ 4.63 (*m*), at 4.45 (*t*) and at 3.80 (*t*). The first chemical shift refered to the H-1′ and H-2′ hydrogens of units II and III, the second referred to H-1′ and H-2″ hydrogens of unit I and the third referred to hydrogens H-1″ and H-2′ of unit I. From the *g*HMQC experiments, carbon correlations at δ 134.0 and at 166.0 with hydrogens were not observed, indicating that these carbons were quaternary, or in other words, aromatic carbons (C-2 and C-5) of units I, II and III and carbinolic carbons (C-1 and C-8) of units I, II and III, respectively (Table 3). In the *g*HMBC experiments, C-H correlations to two bonds were observed. These *g*HMQC and *g*HMBC experiments confirmed the identity of oligomer 4 (Table 3).

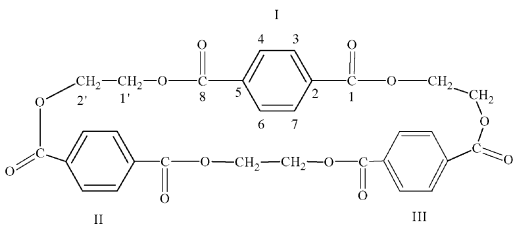
The MS data of oligomer **4** indicated the presence of the pseudo-molecular ion at *m/z* 643 $[M + Na]^+$, which was ion base itself. The fragmentary ions *m/z* 619, 575, 324 and 207, were also in agreement with the proposed structure for oligomer **4**. Based on this ^1H and ^{13}C NMR, *g*HMQC, *g*HMBC and EIS-MS data, oligomer **4** was determined as being the second series cyclic trimer, whose aromatic rings were 1,4-dissubstituted. The structures of oligomers **1**-**4** herein identified are in Fig. 2.

According to the literature, only ^1H and ^{13}C NMR data for the PET polymer [22,23], ^1H NMR data for the first series cyclic PET trimer [24] and ^1H and ^{13}C NMR data for EG, DEG, terephthatic acid and first series cyclic hexamer [20], are available. The ^1H NMR data obtained for oligomer **2** (first series PET cyclic tetramer, whose aromatic rings were 1,4-dissubstituted), oligomer **3** (first series PET cyclic dimer, whose aromatic rings were 1,3-dissubstituted) and the ^1H and ^{13}C NMR data obtained

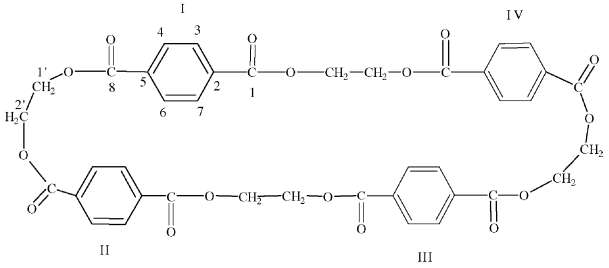
Table 3
^1H NMR, *g*HMQC and *g*HMBC data for oligomer **4**

Unit	C	^1H	^{13}C	*g*HMQC	*g*HMBC
II, III	1′, 2′	4.63 (*m*)	63.1	H-1′, H-2′	
I	1″, 2′	3.80 (*t*)	69.8	H-1″, H-2′	
I	1′, 2″	4.45 (*t*)	65.0	H-1′, H-2″	
III	3, 4, 6, 7	8.01 (*s*)	130.0	H-3, H-4, H-6, H-7	
I/II	4, 6/3, 7	8.03 (*s*)[a]	130.0	H-3, H-4, H-6, H-7	
I/II	3, 7/4, 6	8.04 (*s*)[a]	130.0	H-3, H-4, H-6, H-7	
I, II, III	2, 5		134.0		H-3, H-4, H-6, H-7
I, II, III	1, 8		166.0		H-3, H-4, H-6, H-7

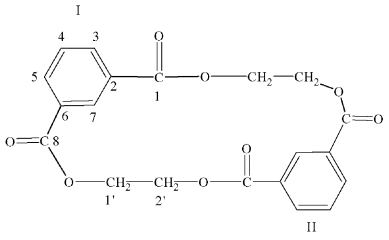
[a] Assignments may be interchangeable within the same column.



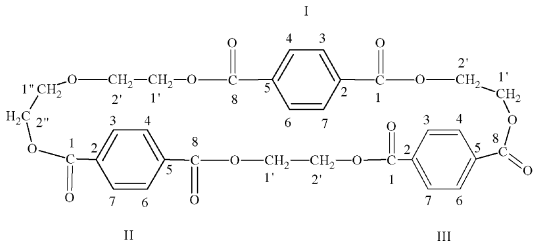
First series cyclic trimer
(oligomer **1**)



First series cyclic tetramer
(oligomer **2**)



First series cyclic dimer
(oligomer **3**)



Second series cyclic trimer
(oligomer **4**)

Fig. 2. The structures of the identified oligomers (oligomers **1**–**4**).

for oligomer **4** (second series PET cyclic trimer, whose rings were 1–4, dissubstituted) are being reported for the first time in this work.

It should be noticed that the ^{1}H NMR data of oligomer **3** (first series PET cyclic dimer, whose aromatic rings were 1,3-dissubstituted) were of fundamental importance to elucidate the structure of this oligomer, since such data permit the position of the substituents within the aromatic rings of this oligomers to be identified. Most of works that identify PET oligomers use only mass spectrometry, which does not permit such an observation to be made.

It is also worth mentioning that oligomers **1**, **2** and **4** presented 1,4-dissubstituted aromatic rings, while oligomer **3** presented 1,3-dissubstituted aromatic rings, which may be related to the presence of isophthalic acid used to obtain PET.

3.3. Identification of oligomers present in PET mineral water and fruit juice bottles

Comparing the t_R peaks obtained from the extracts of each brand of bottle tested with those referring to oligomers **1** to **4**, it was verified that the chromatographic profiles of the various brands of PET bottle were very similar. Hence, the peaks present in the chromatograms of the extracts of all the PET bottle brands analyzed were identified as being the same oligomers of the brand-4 PET bottle.

3.4. Validation of the chromatographic method

The validation has been carried out following the protocols reported in the literature [25–31]. The selectivity, calibration and linearity, limit of detection (LOD) and quantification, precision and accuracy were evaluated.

3.4.1. Selectivity

The selectivity was evaluated by carrying out six analyses of the extract of brand-4 in a silica column and in a C_{18} column. In agreement with the chromatograms obtained, it was verified that the analyte of interest (first series cyclic trimer) was free from interfering compounds or those that matched its signal.

3.4.2. Calibration and linearity

The calibration curve for the first series cyclic trimer was linear over the concentration range of 1.00–500 μg/mL. The linear regression equation was $y = 17124.15 + 17908.97x$, where y is the peak area; x, the first series cyclic trimer concentration (μg/mL), and the correlation coefficient (r) was 0.9999. Seven replicates of each first series cyclic trimer calibration curve concentration (1.00, 10.0, 100, 200, and 500 μg/mL) were performed and the relative standard deviations obtained were less than 3.5% for all concentrations.

The linearity was also studied utilizing the area/concentration ratio of the first series cyclic trimer versus the concentration of the first series cyclic trimer used in the calibration curve [25,26]. It was verified that the solution concentrations used in the calibration curve were within the confidence interval of 95% (Fig. 3).

3.4.3. Precision and accuracy

The precision is an important criteria for evaluating an analytical method or equipment system performance [27,28]. The precision of the chromatographic system was evaluated using repeatability and reproducibility. The repeatability was carried

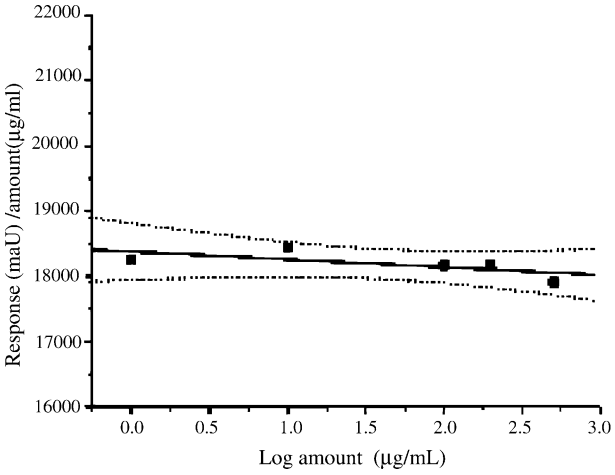


Fig. 3. Response (mAU)/amount (μg/mL) ratio curve of the first series cyclic trimer vs. the concentration of standard solutions of the first series cyclic trimer used in the calibration curve expressed in logarithmic scale. Values inside dash lines are between confidence interval of 95%.

out using intra-day precision, which were analyzed at three concentration levels in triplicate. The reproducibility was carried out using inter-day precision, which were analyzed at three concentrations in septuplicate. The RSD values obtained from the intra-day and inter-day precision were less than 1.1 and 3.5%, respectively (Table 4). The results were fairly good for the concentration levels investigated.

The accuracy of the chromatographic response was studied during the reproducibility evaluation and was expressed as a percentage between the true value of analyte in the sample and the value obtained by analysis. Table 4 shows that the values obtained were near 100%, indicating good accuracy from the chromatographic system.

The precision of the method and accuracy were evaluated via the recovery using different spiked levels. Recovery was measured as the response of a processed spiked matrix standard, expressed as a percentage of the response of a pure standard, which had not been subjected to sample pretreatment. It indicated whether the method provides a response for the entire amount of analyte that is present in the sample [27,28]. The recovery was calculated as the ratio between response after extraction and response of pure standard and was expressed as a percentage (%). For the recovery, three concentration levels of standard solution were added to the matrix. For each concentration added, three extractions were carried out where two injections were made in reference to each extraction. Recovery

Table 5
Recovery (%) of first series cyclic trimer in PET bottles

Spiked level (μg/g)	41	410	2048
Recovery[a]	96 ± 1.7	96 ± 1.5	95 ± 0.8
RSD[b]	1.7	1.6	0.9

[a] Mean of recovery (%), $n = 6$.
[b] RSD, relative standard deviation (%).

values for the three spiked levels studied were from 95 to 96% having a maximum RSD of 1.7% (Table 5), indicating good accuracy and precision of the method.

3.4.4. Limit of detection and limit of quantification

The limit of detection of the chromatographic system was determined experimentally using successive dilutions of a stock solution whose concentration was 1.0 μg/mL, which were injected into the chromatographic system ($n = 6$). The LOD (S/N = 3) value obtained was 0.05 μg/mL.

The limit of quantification (LOQ) of the chromatographic system corresponded to two times LOD, with a RSD ≤ 10% [27,29,31]. The LOQ of the chromatographic system was 0.10 μg/mL (RSD = 3.8).

The LOQ of the method was determined using the recovery study. The LOQ corresponded to the lowest quantity of first series cyclic trimer added, determined with accuracy and precision, in the linear response interval of the detector and within the confidence interval of 95%. The LOQ of the method was 41 μg/g (RSD = 1.7).

3.4.5. Quantification

The method was utilized to quantify first series cyclic trimer in PET bottles from four commercial brands used for mineral water and two brands used for fruit juice. The extractions from the bottles of each commercial brand were carried out in triplicate and two injections of each extraction were made into the chromatographic system. The chromatogram of the blank did not present any interference in the t_R band of the analyte of interest, indicating the quality of the solvent and the efficiency of the procedure utilized to clean the glassware. The blank test was constituted of submitting the solvent utilized to the same procedure used for the sample, for all analytical steps.

According to the chromatograms obtained for the extracts from all the PET bottles of the different commercial brands, the peak of the first series cyclic trimer (t_R = 7.9 min) was the predominant one.

Table 4
Precision and accuracy of the chromatographic system

	Repeatability (intra-day precision) ($n = 3$)			Reproducibility (inter-day precision) ($n = 7$)		
Spiked level (μg/mL)	100	200	500	100	200	500
Found[a] (mean ± SD)(μg/mL)	99 ± 0.4	201 ± 1.3	499 ± 5.7	100 ± 1.4	204 ± 6.1	508 ± 18.0
RSD[b]	0.4	0.6	1.1	1.4	3.0	3.5
Accuracy[c]				100	102	102

[a] Mean of six replicates of the found value ± standard deviation (SD).
[b] RSD, relative standard deviation (%).
[c] Accuracy (%).

Table 6
Quantities of first series cyclic trimer found in the PET bottles

	First series cyclic trimer (mg/100 g)					
	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Brand 6
Replicates	312	356	388	300	425	449
	317	356	393	305	464	483
	318	361	395	307	455	455
Mean	316 d	358 c	392 b	304 d	448 a	462 a
RSD[a]	1.0	0.8	0.9	1.2	4.6	3.9

Means scores within a row followed by same letters (a, b, c, d) are not significantly different ($p < 0.05$).
[a] RSD, relative standard deviation (%).

The ANOVA and Tukey test results demonstrated that there was a significant difference ($p < 0.05$) between the levels of first series cyclic trimer present in the four commercial brands of PET bottles. Brands 5 and 6 presented the highest levels of first series cyclic trimer, 448 and 462 mg/100 g of PET bottle, respectively, not having differed between themselves ($p > 0.05$). However, they differed significantly ($p < 0.005$) from the other brands (Table 6).

Some works have described the quantification of oligomers present in PET packaging [14,8,10]. HPLC has been used to determine the various oligomers, amongst which the first series cyclic trimer, whose levels were in the range of 900–1200 mg/100 g of bottle [14]. The first series cyclic trimer has been quantified in samples of PET film using HPLC, thus obtaining 57.2% (m/m) [8]. Terephthalic acid, bis(2-hydroxyethyl) terephthalate, first series cyclic trimer and other compounds in PET bottles have been also determined and the contents obtained were 0.7; 4.9 and 959 mg/100 g of bottle, respectively [10]. The levels of first series cyclic trimer obtained in this work were much lower than those described in previous works [14,8,10].

It should be mentioned that the results obtained in this work were the first reported for Brazilian PET.

4. Conclusions

Cyclic oligomers were identified in PET bottles used for mineral water and fruit juice using HPLC, MS and ^{1}H and ^{13}C NMR data: a first series cyclic trimer of PET, a first series cyclic tetramer, a first series cyclic dimmer and a second series cyclic trimer.

The use of MS and ^{1}H and ^{13}C NMR techniques was essential to identify the oligomers present in the PET bottles. HPLC provided good separation of the PET bottle oligomers, allowing the identification of the oligomers present in the brands of PET bottles by comparing their retention times.

The method developed and validated was considered effective for determining the first series cyclic trimer. The levels of first series cyclic trimer in the PET bottles varied from 300 to 462 mg/100 g.

The levels of first series cyclic trimer were the first reported for Brazilian PET bottles used for mineral water and fruit juice. One of the structures identified, the first series cyclic dimer, whose aromatic rings are 1,3-dissubstituted and the ^{1}H and ^{13}C NMR data of the first series cyclic tetramer, the first series cyclic dimmer and the second series cyclic trimer are being described for the first time.

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