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Determination of cyclic PET oligomers migrated from new and recycled polymeric material in olive oil, water samples and food simulants

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Introduction

Polyethylene terephthalate (PET) is among the most frequently used Food Contact Materials (FCMs) and the main plastic currently recycled for FCM applications. Over the last years there is an increased concern on the fate of non-intentionally added substances (NIAS) present in FCMs, which can be formed during polymerization [1].
The EU legislation has not defined specific migration limits for PET cyclic oligomers as yet. However, their risk for human health still remains vague due to the lack of toxicological data. Moreover, according to in silico calculations and taking into consideration the European Food Safety Authority's (EFSA) Toxicological Threshold of Concern (TTC), PET cyclic oligomers are considered as Cramer III toxicity substances, indicating a potential public health risk [2].

Here, an analytical methodology was developed and validated for the quantification of four PET cyclic oligomers in virgin olive oil with the aim to investigate the potential exposure of these substances in this type of food.

Methods

Samples: Virgin olive oil; stored in new and recycled PET containers for 12 months.

Preparation protocol: Sub-samples (0.3 g); 100 mg of anhydrous $MgSO_4$ was added; extraction with 600 µL acetonitrile (ACN), 2% HFIP; vortexed and centrifuged; frozen for 1.5h at -20°C; QuEChERS clean-up step (with 25 mg silica); vortexed; evaporation until dryness; reconstitution with 200 µL ethanol, 5% HFIP; filtered (0.22µm); LC-MS analysis.

LC: Elute UHPLC, Waters BEH C18 column (150 x 2.1 mm, 1.8 µm).

- LC gradient according to Diamantidou et. Al [3] as follows:.

Table 1. Gradient elution program. Solvents: (A) H_2O, 0.1% formic acid, (B) acetonitrile, 0.1% formic acid.

Time (min)	Flow (mL/min)	%B
0	0.3	50
1	0.3	70
12	0.3	90
12.1	0.3	50
16	0.3	50

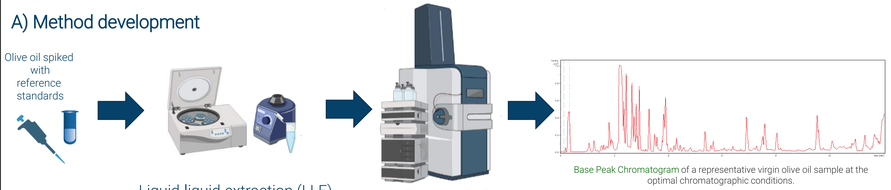
MS: timsTOF (Bruker) equipped with ESI source.

Acquisition: Optimized broad range full scan MS acquisition.

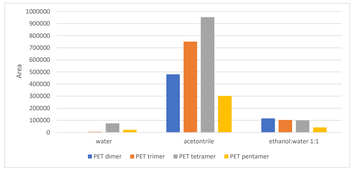
Software: Compass HyStar Version 6.0 and Compass Data Analysis Version 5.3 (Bruker).

Results

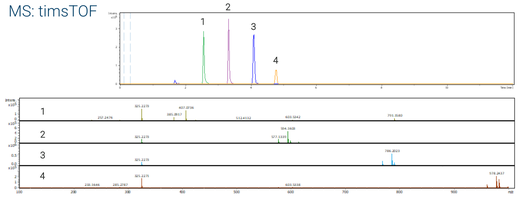
A) Method development



Base Peak Chromatogram of a representative virgin olive oil sample at the optimal chromatographic conditions.



Peak areas for each of the analytes with the tested extraction solvents.



Extracted Chromatograms and ms spectra for spiked targets (1500 µg L^{-1}) (1) PET dimer (2) PET trimer (3) PET tetramer (4) PET pentamer.

Optimization of LLE and QuECheRS clean-up

Extraction of the analytes by using a QuEChERS protocol facilitated both their proper extraction and sample clean-up. $MgSO_4$ was added to the extraction process to minimize interactions and potential hydrolysis during the analysis. Different LLE solvents were tested including (a) water (b) acetonitrile and (c) ethanol:water 1:1.Acetonitrile showed the most satisfactory results and HFIP solvent ratio was also assessed (0%, 2%, 5% and 15%). Three different dilution solvents were tested to evaluate the extraction recovery; (a) dichloromethane (b) hexane and (c) ethyl acetate. However, none of them was chosen for further experiments. Multiple extractions showed no significance difference in extraction recoveries.

After centrifugation, the supernatant was subjected to different clean-up steps to optimize extraction efficiency. The selected sorbents were silica (SiO_2), PSA and their combination (silica:PSA 1:1).

All the aforementioned combinations of solvents and sorbents were tested in the treatment of the spiked olive oil samples to evaluate the extraction efficiency, based on the recoveries of the known-spiking levels. According to the results, using 600 µL of acetonitrile, 2% HFIP as extraction solvent together with 100 mg of anhydrous $MgSO_4$ and 25mg PSA for the sample clean-up finally selected as the sample preparation protocol.

B) Method validation

1. Linearity, LODs and LOQs:

Compound	Molecular formula	Molecular ion (m/z)	Retention time (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	100 - 2500	0.998	33.3	100
PET trimer	$C_{30}H_{24}O_{12}$	577.13	3.3	10 - 1000	0.998	3.3	10
PET tetramer	$C_{40}H_{32}O_{16}$	769.18	4.1	10 - 2500	0.996	3.3	10
PET pentamer	$C_{50}H_{40}O_{20}$	961.22	4.8	10 - 1500	0.992	3.3	10

The method validated in terms of **linearity**, **LODs** and **LOQs**. **Precision** and **accuracy** were also assessed in short term (repeatability & intra-day accuracy) and for a longer period (intermediate precision & inter-day accuracy) and calculated as relative standard deviation (S_r) and relative error (E_r%), respectively.

2. Precision and accuracy:

Compound	Intra-day (n = 3)				Inter-day (n = 3)			
	Added (µg L^{-1})	Found ± s (µg L^{-1})	Sr (%)	Er (%)	Added (µg L^{-1})	Found ± s (µg L^{-1})	Sr (%)	Er (%)
PET dimer	100	115.87 ± 8.27	7.1	15.9	100	118.36 ± 7.09	6.0	18.4
	500	497.02 ± 18.39	3.7	-0.6	500	457.06 ± 43.72	9.6	-8.6
	1500	1600.11 ± 161.29	10.1	6.7	1500	1579.74 ± 152.09	9.6	5.3
PET trimer	10	8.45 ± 1.14	13.5	-15.5	10	8.99 ± 0.76	8.5	-10.1
	100	115.15 ± 2.77	2.4	15.1	100	111.66 ± 12.23	10.9	11.7
	500	511.23 ± 15.99	3.1	2.2	500	468.97 ± 39.65	8.5	-6.2
PET tetramer	10	10.64 ± 0.85	8.01	6.3	10	10.58 ± 1.15	10.9	5.9
	500	504.92 ± 75.40	14.9	1.0	500	459.64 ± 58.87	12.8	-8.1
	1500	1519.88 ± 110.13	7.2	1.3	1500	1565.38 ± 46.95	3.0	4.4
PET pentamer	10	11.74 ± 0.24	2.0	17.4	10	10.61 ± 1.41	13.3	6.1
	100	93.84 ± 4.13	4.4	-6.2	100	90.94 ± 6.18	6.8	-9.1
	750	765.52 ± 36.57	4.8	2.1	750	734.35 ± 50.09	6.8	-2.1

C) Analysis of real olive oil samples

The developed method has been used for the analysis of the target cyclic PET oligomers that could potentially migrate from new and recycled FCMs. For this purpose, virgin olive oil samples were stored in PET containers for over a year under household conditions. All food samples were analyzed using our developed LC-qTOF-MS method. However, none of the compounds of interest was found in the studied samples.

Summary

We developed, optimized and validated an UHPLC-qTOF-MS analytical methodology for the quantification of cyclic PET oligomers in food matrices. Furthermore, we applied the method in virgin olive oil and water sample analysis after their storage in new and recycled PET containers.

References

[1] https://doi.org/10.1016/j.foodchem.2021.129040
[2] https://doi.org/10.1016/j.fpsl.2019.100441
[4] https://doi.org/10.1007/s00216-021-03741-6

Conclusions and Future perspectives

- The method presented in this work can be seen as a contribution for the analytical challenge of compliance checking of FCM products regarding PET oligomers.
- Using a LLE step and an efficient QuEChERS clean-up protocol, the quantification of 4 common polyester (PES) cyclic oligomers was achieved.
- LOQs were sufficiently low to allow quantitative statements, while method accuracy and precision showed acceptable ranges.
- This is the first reported method for the quantification of cyclic PET oligomers in virgin olive oil samples.
- None of the tested compounds were found in the samples after their storage in PET bottles under household conditions.
- The developed method will be applied in food simulants following the EU regulated migration testing conditions for compliance testing of plastic FCMs.

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