A multiple hollow fibre-liquid phase microextraction method for the determination of halogenated solvent residues in olive oil

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Present paper describes a method based on the extraction of analytes by multiple hollow-fibre liquid phase microextraction and detection by ion-trap mass spectrometry and electron capture detectors after gas chromatographic separation. The limits of detection are in the range of 0.13-0.67 µg Kg^{-1}, five orders of magnitude lower than those reached with the European Commission Official method of analysis, with three orders of magnitude of linear range (from the quantification limits to 400 µg Kg^{-1} for all the analytes) and recoveries in fortified olive oils in the range of 78-104 %. The main advantages of the analytical method are the absence of sample carryover (due to the disposable nature of the membranes), high enrichment factors in the range of 79-488, high throughput and low cost. The repeatability of the analytical method ranged from 8 to 15 % for all the analytes showing a good performance.

Keywords: Membrane extraction, hollow-fibre, multiple extraction, gas chromatography, electron capture detection, mass spectrometry, olive oil.

Introduction

Halogenated solvents are employed to extract crude olive-pomace oils from the solid residue obtained during the pressing of olive oils since this procedure is cheaper than forcing oil, but its quality is lower and it can be harmful to human health. The European Union (EU) [1] established the parameters for olive oil quality which limit the presence at levels less or equal to 0.1 mg Kg^{-1} (individual compounds) and 0.2 mg Kg^{-1} (total content).

Because of the high volatility of these solvents, the analytical methods are mainly based in the isolation of compounds from the oil by headspace (HS) with gas chromatography coupled to electron capture detector (ECD) [2] or headspace solid-phase microextraction (HS-SPME) with gas chromatography and mass spectrometry (MS) [2]. However, membrane based extraction techniques have become a promising alternative against miniaturised techniques such as solid phase microextraction and liquid phase microextraction (LPME) [3]. In addition, multiple hollow-fibre (MHF) can increase the capabilities of HF since it implies a step-wise extraction procedure from a single sample. In this way, the concentration of the analyte decays exponentially and the total peak area, corresponding to an exhaustive extraction of the analyte, can be calculated as the sum of areas of each individual extraction. The main advantage is that the matrix effect can be overcome and calibration can be performed in aqueous solutions even if solid matrixes are analysed. Multiple headspace (MHS) using SPME [4] and two-step liquid-liquid-liquid microextraction [5] has been proposed in the literature, the later using two different membrane extraction approaches for the analysis of nonsteroidal antiinflammatory drugs in wastewaters.

In the present paper an optimized method for the determination of halogenated solvents (bromoform, chloroform, trichloroethene, tetrachloroethene, dibromochloromethane and bromodichloromethane) in olive oils using MHF-LPME followed by gas chromatography with ECD and MS detection, will be presented.

Experimental

Standard solutions, reagents and samples.

All solvents used for sample preparation were of the highest available purity (HPLC grade). Isooctane, cyclohexane, n-decane and acetone were obtained from Teknokroma (Barcelona, Spain). 1-octanol and toluene were supplied by Merck KGaA (Darmstadt, Germany).

Methanol, n-hexane, bromoform (99 %), chloroform (99.9 %), trichloroethene (99.9 %), tetrachloroethene (99.9 %), dibromochloromethane (98.7 %) and bromodichloromethane (99 %) were obtained from Sigma-Aldrich GmbH (Steinheim, Germany). Individual standard solutions (1,000 mg L^{-1}) were prepared by exact weighting of pure compounds and dissolution in n-hexane. All standard solutions were stored in vials without headspace at -22 ºC until analysis, and they were stable during overall experiment. Ultrapure water (18 MΩ cm) obtained from a Milli-Q® Gradient system (Millipore UK Ltd., Watford) was used throughout. Olive oil samples were purchased from a local supermarket.

Instrumentation

Analyses were carried out using a 6890N GC-μECD with a Chrompack CP-Sil™ 24 CB chromatographic column (30m × 0.25mm × 0.25 μm) (Hewlett Packard, Wilmington, USA). Carrier gas was helium at a flow-rate of 1.5 mL min^{-1}. The oven temperature was optimized and programmed at 40 ºC for 1 min, subsequently increased to 200 ºC at 85 ºC min^{-1} held for 1.12 min. The make up gas was nitrogen at 50 mL min^{-1} and detector temperature was set at 280 ºC. The injector temperature was set at 280 ºC. ChemStation software package (version A0903) was used for data acquisition and evaluation.

Samples were simultaneously analyzed in a gas chromatograph model Trace GC Ultra equipped with an ion trap (model ITQ 900, Thermo Fisher Scientific SpA, Rodano, Italy). Xcalibur 2.1 software package was used for data acquisition and evaluation. The chromatographic conditions were the same above described and transfer line temperature was set at 200 ºC. Data acquisition was carried out in the electron impact (EI) mode at 70 eV. For selected ion monitoring (SIM), the following ions were chosen (relative intensity in parenthesis): (i) Chloroform: m/z 47:50 (29); 82:86 (100); 118:120 (6); (ii) Trichloroethene: m/z 60:62 (50); 95:97 (96); 130:134 (100); (iii) Bromodichloromethane: m/z 47:49 (19); 127:131 (14); 80:84 (100); (iv) Tetrachloroethene: m/z 94:98 (35); 129:135 (74); 164:170 (100); (v) Dichloromethane: m/z 79:86 (9); 127:131 (100), (vi) Bromoform: m/z 79:81 (11); 171:175 (100); 250:253 (18). Helium and nitrogen, used as carrier and make-up gas, respectively, were of high-purity grade (>99.999%). A centrifuge model 5804R (Eppendorf AG, Hamburg, Germany) and an orbital shaker (Heidolph Rotamax 120) were used for sample preparation.

Procedures

Multiple Hollow Fibre-Liquid Phase Microextraction

A mixture of methanol (total volume of 10 mL) and 10.00 g of olive oil was placed in a centrifuge tube. After that, the mixture was shaken until the cloud point was observed, and then centrifuged at 6,245 *g* and 25 ºC for 3 minutes allowing a complete separation of the phases. The polar phase (methanol extract, 5 mL) was placed into a 20 mL vial with 15 mL of ultrapure water and analytes were extracted by MHF-LPME.

The porous hollow fibre used to support the organic phase and for containing the acceptor solution was Q3/2 polypropylene (Accurel Q3/2, Membrana, Wuppertal, Germany) with an internal diameter of 600 µm, 200 µm of wall thickness and 0.2 µm pores. The extraction procedure used has been published elsewhere [3] with several modifications. Briefly, the assembly consists of an Eppendorf GELoader pipette tip for filling microinjection capillaries with a volume range of 0.5-20 µL, 3 cm of HF membrane and a 20 mL vial covered with a septum. First of all, the HF is cut and one end closed by means of a hot soldering tool. The pipette tip end is cut allowing a perfect connection with the open end of the membrane and then, the HF is introduced in the extraction solvent (n-hexane) during 1 minute to open the pores. The membrane is filled with the solvent and the HF-tip pipette assembly introduced into a vial containing 5 mL of sample extract (methanol) plus 15 mL of ultrapure water. The extraction was carried out during 3 minutes at 25 ºC and 5.5 *g*, using a magnetic stirrer. After that, the sealed end of the HF is cut and 1 µL is injected into the GC analysis. The procedure is illustrated in Fig. S1 (see Electronic Supplementary Material Fig. S1).

For MHF-LPME, exponential decays of the analytes with the successive extractions were calculated by plot the total area versus the analytes mass using solutions of the analytes in olive oil. Three repeated extractions were performed per solution and the total area was calculated using the linear regression of the logarithms of the individual peak areas (Equation I and II, where “A1” is the peak area of the first extraction and “β” is calculated from linear regression of the logarithms of the individual peak areas) [4].

Results and discussion

Optimization of the HF-LPME method

Initially, olive oil samples were directly extracted by MHF-LPME but, due to the complexity of the samples, the procedure was considerably enhanced with a previous extraction with

methanol. Seven different solvents were tested for the extraction of analytes: acetone, toluene, n-decane, n-hexane, isooctane, cyclohexane and 1-octanol. The preconcentration of analytes is only possible with the four last solvents. Figure 1 shows the effect of solvents used for HF-LPME on the peak area of analytes. It can be observed that preconcentration of tetrachloroethene is very efficient using any of these solvents, but especially with isooctane. However, a compromise is adopted for all the analytes using n-hexane as extraction solvent that was selected for further experiments. The extraction time was optimized from 1 to 10 minutes and although, some improvement in the peak areas can be observed after 3 minutes, especially in the case of tetrachloroethene, the repeatability is very poor and for this reason the extraction time was set at 3 minutes for further experiments. For the same reason, the stirring speed was set at 5.5 *g*.

Performance of the method and application to real samples

Table 1 collects the parameters of quality of HF- and MHF-LPME followed by GC-ECD analysis. Using HF-LPME, all the analytes (except tetrachloroethene) showed exponential decay of the peaks areas, and the corresponding correlation coefficients (R) of the linear plot ln A_i versus (i-1) obtained were in all the cases higher than 0.99. As above mentioned, tetrachloroethene response was considered as non-linear since it showed an increase of the ln A_i value while increasing (i-1). Therefore, it cannot be quantified by MHS-SPME. Figure 2 shows overlapped chromatograms of four subsequent extractions of the analytes at 10 µg Kg^{-1} using HF-LPME. At the top of Fig. 2 are shown two insides with the exponential decay obtained for bromodichloromethane; it can be observed very good correlation coefficient of the linear plot ln A_i versus (i-1).

Linear ranges for calibration curves were obtained from the quantification limits to 400 µg Kg^{-1} for all the analytes with correlation coefficients higher than 0.999. But because correlation coefficient cannot be used as a suitable tool for testing linearity, the lack-of-fit test has been applied [6]. If this F value is lesser than the tabulated one for p-2 and N-p degrees of freedom at a given confidence level, namely 95%, one can conclude that the plot is significantly linear. In our work, and from triplicate measurement in each calibration point, the lack-of-fit test indicates a good linearity in all cases.

The detection and quantification limits (LODs and LOQs, respectively) were calculated as the analyte concentration that corresponds to a signal equal to “a + 3 Sy/x” and “a + 10 Sy/x” respectively, where “a” is the origin ordinate, and “Sy/x” indicates the random errors for the slops. As can be seen in Table 1, the LODs using HF-LPME ranged from 0.75 to 1.5 µg Kg^{-1}

and using MHF-LPME, from 0.13 to 0.67 μg Kg^{-1}, which demonstrate the higher sensitivity of the MHF method, especially in relation with the official methods such as the EEC method which is 10 μg Kg^{-1} [1].

The enrichment factors were calculated as described elsewhere [3] at 10 μg Kg^{-1} for all the analytes (Table 1). The matrix effect was evaluated by the comparison of the slopes of the calibration curves obtained by both external and standard addition calibration methods. Since a good correlation was observed between the slopes for all the analytes and samples, external calibration was selected for further experiments. The relative recoveries were determined as the ratio of the concentrations found in ultrapure water and fortified samples at the same concentration. Recovery experiments were carried out in samples fortified at three different concentrations 5, 10 and 25 μg Kg^{-1} and the results are in the range of 78-104 %. The recovery of tetrachloroethene was calculated using HF-LPME and is 66 % (Table 1). As can be seen, the recoveries for all the analytes are very similar at the different concentrations showing a good performance of the HF-LPME assembly when it is applied to real matrices, such as olive oil. Precision was evaluated at three different concentrations (5, 10 and 25 μg Kg^{-1}) by performing repeatability (instrument and method precision), intermediate precision and reproducibility. The injection precision of the method was evaluated by performing ten replicate injections of the same sample extract. The relative standard deviation (% RSD) of the peak area was always below 0.5 % for all the analytes and concentrations, which was considered acceptable. The % RSD of the sample response factor was calculated for five separate extracts. The results ranged from 8 to 15 % for all the analytes that were considered acceptable at these low levels (Table 1). Intermediate precision was performed by two analysts, each testing five sample extracts of five different fortified samples on five separate days. Fresh sample and standard solutions were independently prepared on each day of analysis. The intermediate precision results ranged from 10-15 % for all the analytes and concentrations that was considered acceptable. Finally, the reproducibility of the method was calculated as the intermediate precision, but in this case using two different chromatographic columns. The % RSD varies from 12-17 % that was considered acceptable.

Finally, twenty five commercial olive oil samples were analysed with this method including five samples of virgin olive oil, ten refined olive oils and ten olive-pomace oils. According to the olive oil production process, the halogenated solvents could be present in the olive-pomace oil samples, but the analytes were under the detection limits in all the samples.

Conclusions

The new analytical approach described in this paper constitutes a powerful tool to determine halogenated solvent residues in olive oils, with sensitivities under the limits established by the EU for this kind of samples.

In addition, unequivocal identification of analytes is obtained by MS detection even if they are coeluting with other compounds present in the oils. HF-LPME is cheap and overcome the use of organic solvents needed in canonical extraction techniques like Soxhlet extraction. On the other hand, sample throughput is considerably high. The absence of sample carryover (due to the disposable nature of the membranes) and high reproducibility are the main advantages of the present method in comparison with SPME. The stepwise extraction proposed in this work eliminates a matrix effect and decreases the limits of detection that is a critical point in the analysis of olive oil. Finally, the approach is very simple and can be used in routine analysis of halogenated solvents in olive oils to assure their quality to consumers.

Acknowledgements

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213 Table 1. Features of the HF-LPME and MHF-LPME/GC-ECD methods for the extraction of halogenated solvents. Recovery values (%) for the analytes extracted from fortified olive oils (%RSD,
214 repeatability n=5, m=3).
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Name	LOD/LOQ (µg Kg^{-1})		Log k_{ow}	Ef	RSD (%) n = 5	MHF-LPME					
	HF-LPME	MHF-LPME				5 µg Kg^{-1}		10 µg Kg^{-1}		25 µg Kg^{-1}	
						Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Chloroform	1.28/4.27	0.67/2.23	1.94	79	13	78	12	80	13	85	10
Trichloroethylene	1.44/4.80	0.44/1.47	2.57	257	11	80	12	80	11	90	8
Bromodichloromethane	1.38/4.60	0.13/0.43	2.04	97	11	98	11	100	11	104	10
Tetrachloroethene	0.75/2.50	-	3.07	488	15	[a]67	15	[a]66	15	[a]72	11
Dibromochloromethane	1.35/4.50	0.19/0.63	2.21	136	13	95	13	92	13	99	10
Bromoform	1.50/5.00	0.24/0.80	2.44	207	11	85	10	86	11	88	9

[a]Data obtained with HF-LPME.

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Fig. 1. Effect of the extraction solvents used for HF-LPME in the peak area of organohalogen solvent. The error bars indicate the standard deviation (n=3). Relative peak area = peak area/first peak area corresponding to the lowest experimental value of the abscissa.

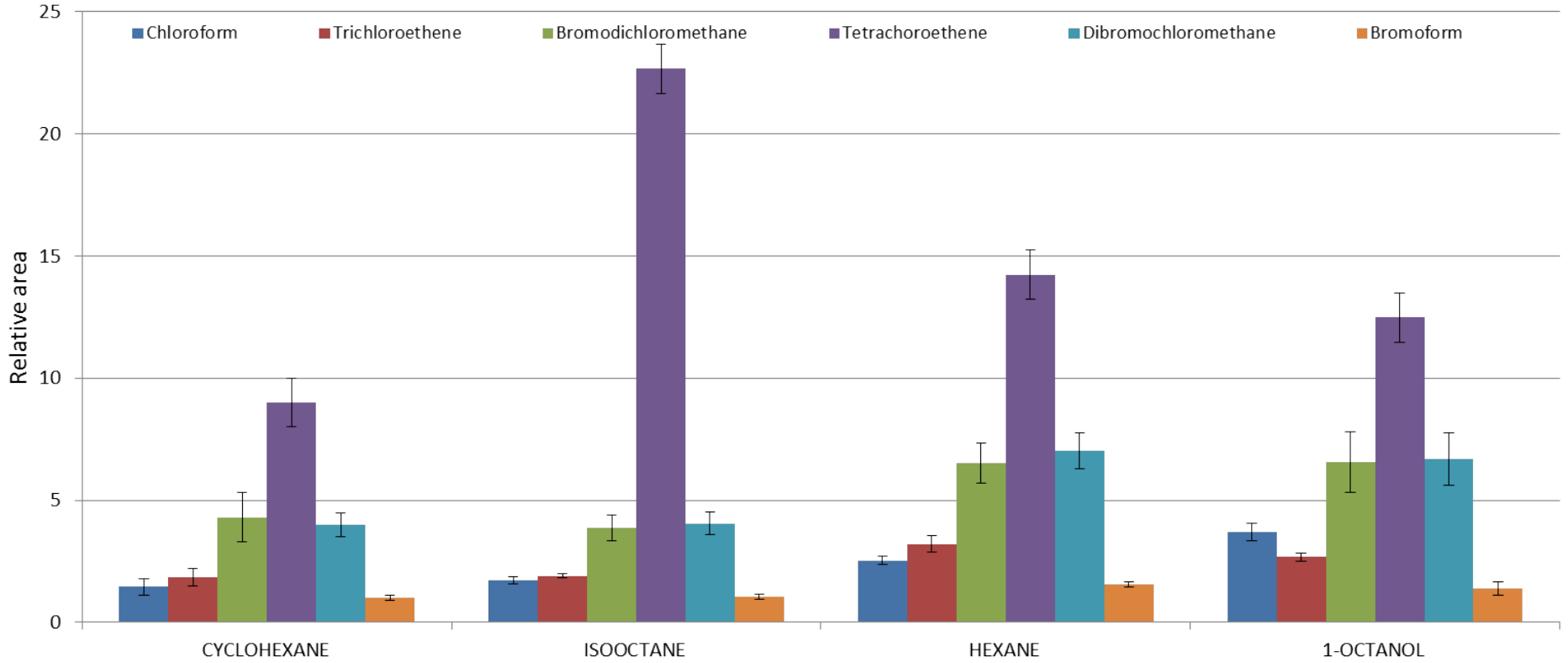


Fig. 2. Chromatograms obtained from a mixture of halogenated solvents in blank olive oil at 10 μg Kg^{-1} using MHF-LPME and analysis by GC-ECD. At the top the exponential decay of bromodichloromethane is showed as an example. Peak assignment: (1) chloroform, (2) trichloroethene, (3) bromodichloromethane, (4) tetrachloroethene, (5) dibromochloromethane, (6) bromoform.

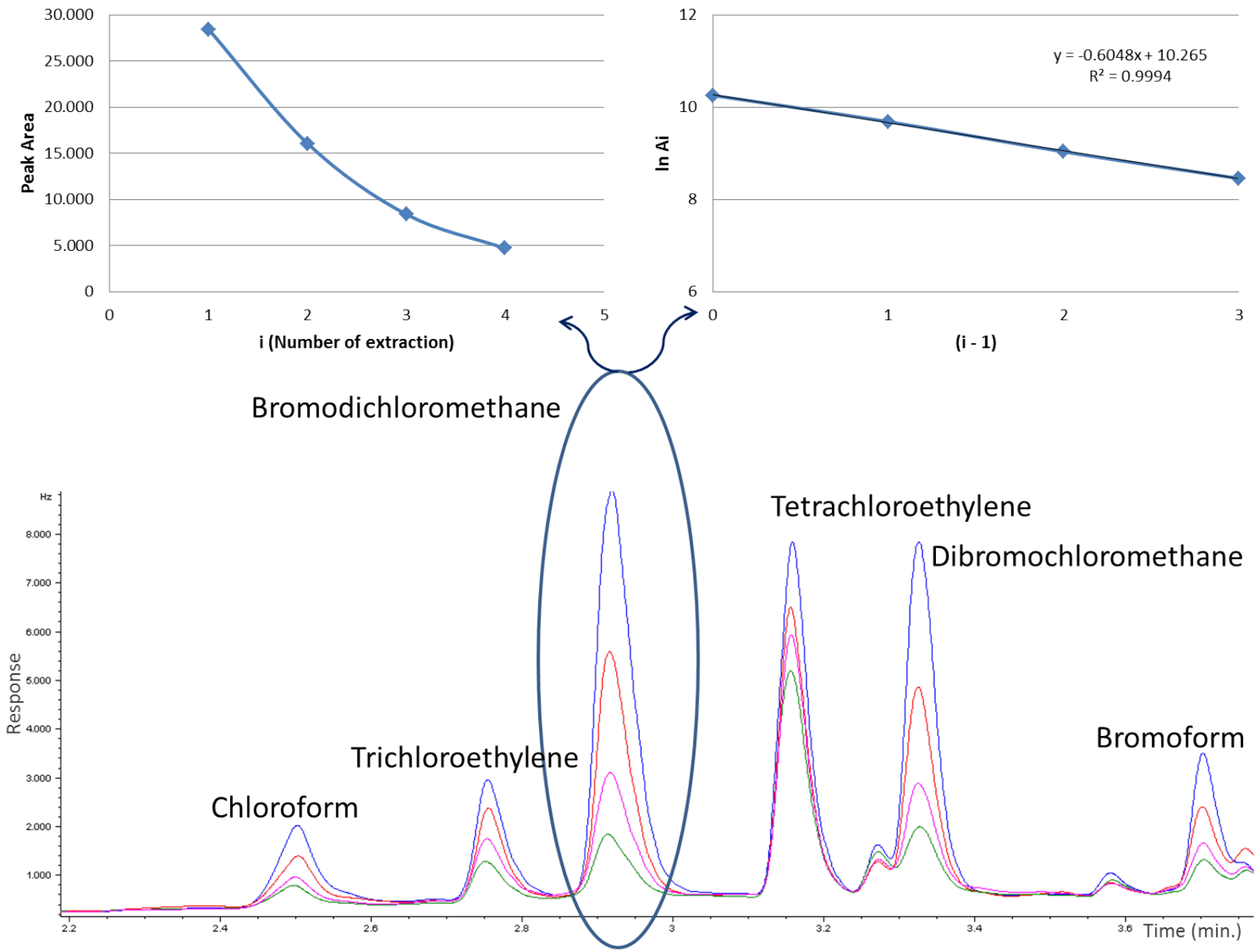
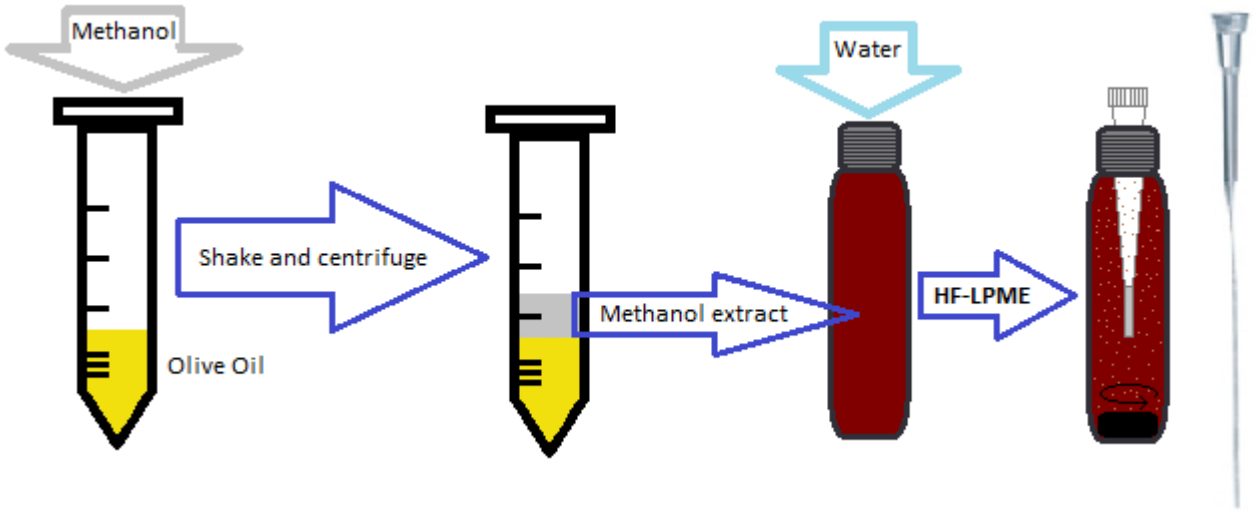

30.000
25.000
20.000
15.000
10.000
5.000
0
Peak Area
0
1
2
3
4
5
i (Number of extraction)
12
10
8
6
ln Ai
y = -0.6048x + 10.265
R² = 0.9994
0
1
2
3
(i - 1)
Bromodichloromethane
Tetrachloroethylene
Dibromochloromethane
Bromoform
Trichloroethylene
Chloroform
Hz
8.000
7.000
6.000
5.000
4.000
3.000
2.000
1.000
Response
2.2
2.4
2.6
2.8
3
3.2
3.4
3.6
Time (min.)

Figure S1. Ouline of the experimental procedure



EQUATIONS

Equation I

$$A_T = \frac{A_1}{1-\beta}$$

Equation II

$$\ln A_i = (i-1)\ln\beta + \ln A_i$$