

Short communication

Dynamic ultrasound-assisted extraction of environmental pollutants from marine sediments for comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection

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Abstract

A dynamic ultrasound-assisted extraction (UAE) of marine sediments has been optimized using experimental design methodology. Comprehensive two-dimensional gas chromatography (GC × GC) using a cryogenic modulator, and time-of-flight-mass spectrometry (TOF-MS) were used to separate and identify environmental pollutants. Six compounds from three different chemical classes were used to optimize the extraction parameters.

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1. Introduction

Sample preparation (involving extraction, clean-up, pre-concentration, derivatization, etc.) is generally required before subjecting a sample to separation-plus-detection under the appropriate conditions. In general, solid samples require more laborious treatment than liquids. Conventional sample preparation methods are well-established; however, they often require the use of large volumes of organic solvents and time-consuming multi-step procedures without the possibility of automation. Ultrasound-assisted extraction (UAE) is a fast and efficient alternative to conventional extraction techniques [1] for the isolation of a number of analytes from different types of sample [2–4]. There are two devices for UAE, bath and probe units. Ultrasonic baths are more widely today [5–7], but ultrasonic probes have the advantage of focusing their energy on the sample zone, thus providing more efficiency and better experimental repeatability [1]. Moreover, ultrasonic probes are more versatile as they can be incorporated in continuous extraction systems, with miniaturization of the extraction step, reducing the consumption of sample

and reagents and facilitating the coupling of extraction with other steps of the analytical process.

Marine sediments from a bay where an urban wastewater treatment plant discharges its effluents was selected to carry out the present study. These marine sediment samples are commonly very complex because, next to the compounds discharged by the plant additional contaminants from ship fuel will be present. That is, a large number of pollutants differing in polarity and chemical nature can be expected to be present [8–10]. One-dimensional capillary gas chromatography (1D-GC) generally does not provide enough separation for complete qualitative and quantitative analyses, because the high concentration of matrix constituents in the extracts can easily obscure the trace-level analytes of interest. Admittedly, gas chromatography–mass spectrometry (GC–MS) in the selected ion monitoring (SIM) mode and GC–MS–MS are valuable analytical tools to solve many interference problems, but comprehensive two-dimensional gas chromatography (GC × GC), a new technique, is an excellent means to enhance the separation of the analytes of interest from each other and/or the matrix background. In the past few years, GC × GC has shown its capability to considerably improve the analysis of complex samples such as, e.g., cigarette smoke [11,12], contaminated air [13], and petrochemical products

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[14]. Impressive results have been obtained in terms of separation efficiency and, also, compound identification mainly based on (i) the increased peak capacity due to the use of two columns, (ii) the improved analyte detectability due to peak compression during modulation, and (iii) the presence of structured chromatograms, which facilitates the recognition of unknown compounds.

The aim of this study was to develop a UAE procedure for the extraction of mainly hydrophobic pollutants from marine sediments, with final analysis by GC × GC with time-of-flight-mass spectrometric (TOF-MS) detection to ensure sufficient resolution and, consequently, clean mass spectra. That is, the main goal was not the full characterisation of the sediment, but the efficient monitoring of the optimization of the extraction procedure.

2. Experimental

2.1. Extraction

Ultrasonic irradiation was applied by means of a Branson (Danbury, CT, USA) 450 digital sonifier equipped with a cylindrical titanium alloy probe, which was immersed in a water bath in which the extraction cell was placed. An extraction chamber consisting of a stainless-steel cylinder (12 cm × 10 mm I.D.) closed with screws at both ends was used, allowing circulation of the leaching solvent through it. The screw caps were covered with a cellulose filter to ensure that the sample remained in the extraction chamber.

A Gilson (Worthington, OH, USA) Minipuls-3 low-pressure programmable peristaltic pump, programmed for changing the rotation direction at preset intervals, a low-pressure injection valve (Rheodyne, Cotati, CA, USA) and PTFE tubing of 0.8 mm I.D. were used to build the flow extraction manifold (Fig. 1).

2.2. GC separation and detection

An Agilent gas chromatograph Model 6890 (Agilent Technologies, Palo Alto, CA, USA) coupled to a Leco Pegasus II

TOF-MS (St. Joseph, MI, USA) was used. One microliter of extract was injected through an Optic 2 PTV injector (ATAS, Zoetermeer, The Netherlands) operated in the splitless mode at 300 °C (1 min splitless time). Helium (99.999% purity; Hoekloos, Schiedam, The Netherlands) was used as carrier gas at a pressure of 200 kPa. The temperature of the GC oven was programmed from 70 °C (2 min hold) to 300 °C (8 min hold) at 5 °C/min.

A 20 m × 0.25 mm I.D., 0.25 μm DB-5 column (5% phenyl–95% dimethylpolysiloxane) (J&W, Folsom, CA, USA) was used as first-dimension column and a 1 m × 0.1 mm I.D., 0.1 μm BGB-1701 column (14% cyanopropylphenyl–86% dimethylpolysiloxane) (BGB-Analytik, Zürich, Switzerland) as second-dimension column. The columns, housed in the same oven, were connected with a press-fit connector (Varian universal quick seal, Varian-Chrompack, Palo Alto, CA, USA). Thermal modulation was performed with a dual-stage CO₂ jet modulator [15] using a modulation time of 5 s.

TOF-MS was operated in the electron ionisation (EI) mode at a spectrum storage rate of 50 Hz, using a mass range of *m/z* 50–600 and a multi-channel plate voltage of –1800 V. The transfer line and ion source were kept at 300 and 250 °C, respectively.

Raw data were exported as csv-formatted files. For data transformation and visualization two additional programmes were used: a programme to convert the raw data into a two-dimensional array (software provided by Ph. Marriott, RMIT, Melbourne, Australia [16]) and a programme to generate contour plots from this array (“Transform”, part of Noesys software package; Research Systems International, Crowthorne, UK).

2.3. Reagents and samples

HPLC-grade hexane and acetone (Panreac, Barcelona, Spain) were used as solvents in the extraction step. Marine sediments collected at the outflow of an urban wastewater treatment plant into the Mediterranean (Almería, Spain) were used to carry out the study. Sediment containers were closed

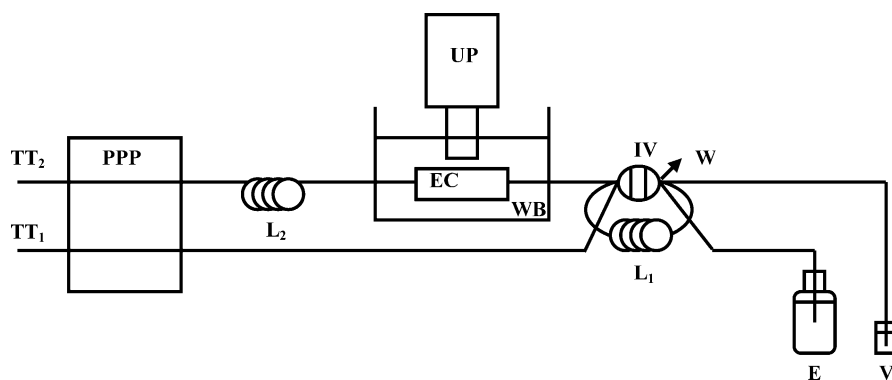


Fig. 1. Experimental set-up used for dynamic UAE. TT, transport tubing; PPP, programmable peristaltic pump; L, loop; UP, ultrasonic probe; EC, extraction chamber; WB, water bath; IV, injection valve; W, waste; E, extractant; V, vial.

and stored at 4 °C. Once in the laboratory, the samples were homogenised (by sieving and mixing), frozen and lyophilised with the help of a freeze-drier equipped with a three-plate tower (Edwards, Sussex, UK) and, finally, stored in closed flasks at room temperature until extraction.

2.4. Conventional Soxhlet extraction

Three grams of lyophilised sample were placed in a cellulose thimble (25 mm × 88 mm; Albet, Barcelona, Spain), which was capped with cotton wool and placed in the Soxhlet chamber. The overall Soxhlet glassware was fitted to a distillation flask containing 80 ml of extractant and two or three glass-boiling regulators. Extraction with hexane was carried out for 24 h.

2.5. Ultrasound-assisted extraction

The dynamic system of Fig. 1 was used. A preset volume of extractant is pumped in the forward and backward direction through the solid sample by programming a peristaltic pump. In this way, undesirable compression of the sample in the extraction chamber and an increase of pressure are avoided.

One gram of lyophilised sediment was mixed with 3 g of sand and placed in the extraction chamber, which was assembled and filled with the extractant (hexane) aspirated by the programmable peristaltic pump (via transport tubing TT₁), in order to avoid passage of the organic solvent through the pump tubes. After filling the loop L₁ and the extraction chamber with a total volume of 6 ml of hexane, the chamber was immersed in the water bath at room temperature. Then, the hexane was circulated through the solid sample with the help of TT₂ (at 0.7 ml/min flow rate) under ultrasonic irradiation (60% duty cycle and 50% amplitude, with the probe placed 1 mm from the top surface of the extraction chamber). After extraction, the extract was removed by draining it to the vial.

Preconcentration was required before injection of the extracts, which consisted of evaporation of the extracts until dryness under a nitrogen stream (Hoekloos) and subsequent reconstitution of the residues with 200 μl of ethyl acetate (J.T. Baker, Deventer, The Netherlands). Finally, 1 μl was injected into the GC.

3. Results and discussion

3.1. GC × GC–TOF–MS

Fig. 2 shows a typical example of the analysis of the sediment used in this study. The total ion current contour plot shows the presence of a large number of compounds. The real complexity is even greater: many more compounds are present at much lower concentrations than those seen here and can be visualised only by using other colour intensity settings. Actually, with the present sample, up to 1500

spots could be observed. The GC × GC plot clearly demonstrates that, in these environmental samples, many compounds are present that would show co-elution in a 1D separation. In other words, the improved separation efficiency of GC × GC is required to create the possibility of identification on the basis of clean mass spectra. For reasons of clarity, for the examples described below, relevant parts from the total separation space are shown as reconstructed-ion contour plots.

The strategy for non-target analysis of Dallüge et al. [17] was used, which comprises of automated data processing (including peak finding, deconvolution and library searching) and generation of a peak table. An extract obtained with the center point settings of the experimental design (see below) was subjected to the GC × GC–TOF–MS analysis and the mentioned strategy.

A beneficial aspect is the presence of ordered structures or clusters in the contour plots. For example, the region in the contour plot between 1250 and 1800 s (indicated by a box around ²t_R of 1.2–1.5 s) showed remarkable structures. Here, one compound was identified to be 1-butylheptylbenzene, which is a compound present in, for instance, diesel fuel. Its characteristic fragment ions are *m/z* 91, 105, 119 and the molecular ion, *m/z* 232. After construction of the contour plots for these ions, spots near the identified compound appeared to have high similarity and could be identified as isomers with a different degree of branching (insert of Fig. 2; label, C₁₁-isomers). Other compounds with similar elution patterns were also observed in the contour plots of this region. By using the same strategy of selecting a specific spot (e.g. at ¹t_R 1469.9 s, ²t_R 1.10 s) and using the mass spectral information, three C₁₂- and four C₁₃-substituted benzenes could be identified. They are all shown in the insert labelled *m/z* = 91. In a similar way, but now using the reconstructed ion contour plot of *m/z* 133, the presence of another class of similar analytes could be detected, i.e. alkyl-substituted benzenes with a double bond in one of their chains.

Since nonylphenol was expected to be present, a target search using *m/z* 135 was made, which led to the identification of this compound at ¹t_R 1456.6 s and ²t_R 2.19 s. Again, structurally related compounds eluted in its vicinity. At least five other C₉-substituted phenols (see insert of Fig. 2, label *m/z* = 135) could be identified. This is, in addition, a good example of a, seemingly, ‘empty box’ which is, next, found to contain a series of analytes of interest. Using *m/z* 202, the characteristic ion for pyrene, immediately led to the positive identification of this compound as well as its isomer, fluoranthene.

From amongst the various compounds that were identified, six target analytes were selected to optimize the extraction: two polycyclic aromatic hydrocarbons (fluoranthene and pyrene), a surfactant (nonylphenol) and three dialkylated benzenes (butylheptylbenzene, pentylheptylbenzene and pentylloctylbenzene). All these analytes were identified with similarity factors higher than 800. Due to the stability of the retention times in both dimensions, they

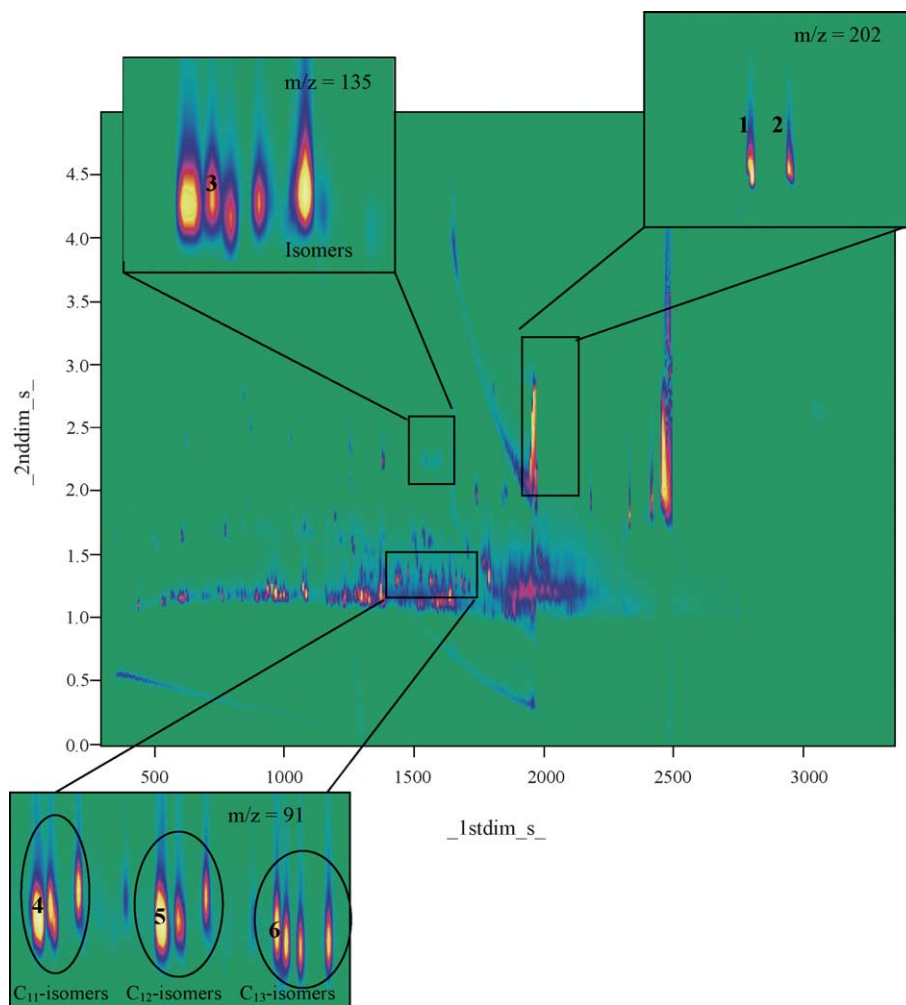


Fig. 2. Full-scan (m/z 50–600) GC \times GC–TOF-MS contour plot of an extract obtained under optimum conditions and partial contour plots of the m/z traces 202, 135 and 91. Peak assignment: 1, fluoranthene; 2, pyrene; 3, nonylphenol; 4, butylheptylbenzene; 5, pentylheptylbenzene; 6, pentyloctylbenzene.

were easily located in the contour plots. As indicated below, tetradecanoic acid was added to the test set as a (polar) compound representative for long-chain carboxylic acids which – because of their severe tailing – often disturb sediment analysis.

3.2. Optimization of the extraction

The variables affecting the dynamic UAE were optimized in order to obtain the highest peak areas of the six selected analytes. In order to see the influence on the selectivity of the system, the peak area of tetradecanoic acid was monitored as well. Selectivity was best determined by using the ratios “peak area of the target compounds over the peak area of tetradecanoic acid” for all extracts used in the experimental designs.

The experimental variables optimized were the irradiation time, the percentage of duty cycle of ultrasonic exposure, the ultrasound radiation amplitude, the probe position, the extractant composition, the extractant flow rate and the extractant

volume. The probe position was established as the distance between the tip horn of the ultrasonic probe and the top surface of the extraction chamber. A multifactorial design methodology [18] was used for the optimization of these factors, as they were presumably interrelated.

A Plackett-Burman design ²⁷ involving 12 experiments plus 3 center points [19] was built for a screening study of the behaviour of the variables (Table 1a). The key factor appeared to be the composition of the extractant. This factor had a significant and negative effect on the extraction of the target analytes, which means that the peak areas of the target analytes massively changed with solvent polarity (as indicated by the ρ -value of the ANOVA). The highest peak areas were obtained with *n*-hexane as extraction solvent. Thus, hexane was selected as extractant for further experiments. The extractant volume, the duty cycle and the amplitude were statistically non-influential factors; that is, no significant changes were observed in the peak areas. However, better results were obtained with the upper values tested, which were selected for subsequent experiments (6 ml of extractant,

Table 1
Optimization of the ultrasound-assisted extraction

Parameter	Tested range	Result	Effect on analyte extraction	Optimum value
(a) Screening study				
Probe position (mm)	1–20	Non-significant	Negative	1
Radiation amplitude (%)	10–50	Non-significant	Positive	50
Duty cycle (%)	20–60	Non-significant	Positive	60
Extractant flow rate (ml/min)	1–3	Significant	Negative	–
Extraction time (min)	3–7	Significant	Positive	–
Extractant volume (ml)	4–6	Non-significant	Positive	6
Extractant composition (hexane:acetone)	1:0–0.5:0.5	Significant	Negative	Hexane
(b) Full factorial design				
Extraction time (min)	7–15	Non-significant	Positive	15
Extractant flow rate (ml/min)	0.5–1	Non-significant	Positive	1

60% duty cycle and 50% amplitude). The probe position was also a statistically non-influential factor; however, its effect on the extraction was negative. Therefore, the lowest value tested, 1 mm, was selected for further experiments.

The flow rate of the extraction solvent and the irradiation time were influential factors, with a negative and positive effect on the extraction, respectively. Therefore, lower values for the flow rate and higher values for the irradiation time were tested using a 2² full factorial design involving four runs plus three center points [19] (Table 1b). The result was that neither factor was statistically significant in the new ranges studied: the values were near their optima and the peak areas hardly changed. However, higher signals were obtained with the upper values tested, which were selected for subsequent work.

Finally, the experimental designs of the optimization study were analyzed for tetradecanoid acid in order to check if the optimum extraction conditions obtained are selective for the target analytes. The results from the first design (2⁷ involving 12 experiments plus 3 center points) showed that the irradiation time and volume of extractant had a positive effect on the extraction of this compound, as was the case for the target analytes. However, for the other variables, the effect was opposite to that for the analytes of interest. When analyzing the second design for the matrix compounds, the peak areas for the acid were smaller, while those of the analytes of interest increased, i.e. the ratios increased. This demonstrates that the selected extraction conditions are more favourable for the target analytes and result in a more selective extraction.

3.3. Analytical performance data

To demonstrate the performance of the GC × GC system, one extract obtained under the optimum conditions was analysed three times. Data on the retention times and peak areas of the selected target compounds are shown in Table 2. The values of the first-dimension retention times are averages of the three to four modulations in which the analytes eluted. It should be added that all analytes always eluted in the same modulations, so that it can be concluded that the first-dimension retention times are very reproducible from run to run. The repeatabilities of the peak areas expressed as relative standard deviations (RSDs) were below 10%. This is a fully satisfactory result. The precision was evaluated by three measurements of the analytes performed on different days. In each case, 1 g of sediment was subjected to extraction and, then, the extract was analyzed by GC × GC. Peak-area precisions expressed as RSD were 4% for fluoranthene, 4% for pyrene, 2% for nonylphenol, and 3 to 5% for the dialkylated benzenes.

3.4. Comparison of UAE and conventional Soxhlet extraction

The optimized UAE was compared with conventional Soxhlet extraction in terms of peak area and precision. The results reported in Table 3 show that the peak areas calculated for UAE are higher than those obtained with the Soxhlet procedure and that the results are more precise. The probable explanation is that the efficiency of the extraction process is

Table 2
Data on retention times and peak areas for the target analytes obtained under the optimum analytical conditions

Analyte	<i>m/z</i>	Retention time (s)			Peak area (a.u.)		RSD of peak areas (%; <i>n</i> = 3)	
		¹ _{tr} (s)	² _{tr} (s)	SD ² _{tr} (s)	UAE	Soxhlet	UAE	Soxhlet
Butylheptylbenzene	91	1338.5	1.15	0.08	1160	500	5.1	6.8
Nonylphenol	135	1456.6	2.19	0.10	3450	2200	2.2	11
Pentylheptylbenzene	91	1469.9	1.10	0.08	1880	510	3.0	7.0
Pentylloctylbenzene	91	1593.1	1.06	0.08	1850	1380	2.0	8.9
Fluoranthene	202	1875.9	2.20	0.14	78	31	4.1	6.5
Pyrene	202	1935.8	2.21	0.11	46	38	3.9	12

Table 3
Comparison of the proposed method with conventional Soxhlet extraction

	Peak area		RSD ^a (%)	
	UAE	Soxhlet	UAE	Soxhlet
Benzene, (1-butylheptyl)-	1155308	496661	5.1	6.8
Phenol, nonyl-	3454705	2201891	2.2	11
Benzene, (1-pentylheptyl)-	1881741.5	508483	3	7
Benzene, (1-pentylloctyl)-	1846755	1383894	2	8.9
Fluoranthene	78280	31251	4.1	6.5
Pyrene	46229	38084	3.9	12

^a $n = 3$.

better when auxiliary energy is applied to break the strong retention of part of the analytes by the heterogeneous matrix [21]. The energy of the condensed extractant is less powerful. Therefore, the reproducibility of Soxhlet extraction is worse because the extraction is not complete.

4. Conclusions

The qualitative analysis of pollutants in complex samples requires the combined use of an efficient and selective extraction and a subsequent powerful separation/detection technique. In the present study, dynamic UAE using an ultrasonic probe was used for the first time in combination with GC × GC–TOF–MS for the analysis of different types of pollutant from marine sediments. This study convincingly shows that UAE combined off-line with GC × GC is a fast and efficient multi-residue screening tool. The results from GC × GC–TOF–MS – which were obtained without injecting standards – confirm the usefulness of dynamic UAE: fully satisfactory separation and identification of the analytes of environmental interest present in the sample was achieved. In this context, two aspects of special interest are the use of sophisticated peak finding and deconvolution algorithms and improved separation of the analytes, not only from each other but, also, from the interfering matrix compounds.

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