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Short communication

Supported liquid membrane extraction for sample work-up and preconcentration of methoxy-*s*-triazine herbicides in a flow system

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Abstract

A method for sample preparation of methoxy-*s*-triazine herbicides using supported liquid membrane extraction has been developed. The analytes were selectively extracted from the donor solution of pH 7.0 into a porous polytetrafluoroethylene (PTFE) membrane impregnated with di-*n*-hexyl ether. After diffusion through the hydrophobic membrane the analytes were irreversibly trapped in the acidic acceptor phase of pH 1.0. The donor waste was monitored for estimating the amount of sample trapped at certain time intervals. Comparison of the selectivity with solid-phase extraction has been performed. A low detection limit, ca. 15 ng/l, has been obtained with liquid membrane extraction.

Keywords: Supported liquid membrane; Pesticides; Methoxy-*s*-triazines

1. Introduction

The selective sample handling technique based on supported liquid membrane (SLM) extraction in a flow system, developed in our research group [1] has been successfully utilized for preparation of samples in various matrices. The liquid membrane is prepared by impregnation of a porous polytetrafluoroethylene (PTFE) membrane with a water-immiscible organic solvent, and when housed in a membrane separator forms a selective barrier between two aqueous phases. The analytes in uncharged form are first extracted from the aqueous donor phase into the organic membrane liquid. After diffusion through the membrane, the analytes are irreversibly trapped in a charged form in the second aqueous solution, the acceptor. Analyte enrichment can be brought about by pumping the sample solution through the donor channel keeping the acceptor stagnant [2,3].

SLM methodology has been shown to give high sample enrichment factor besides the high degree of clean-up [4]. Using SLM various analytes in complex matrices as urine [5], blood plasma [6,7] and animal manure [8] have been efficiently enriched and quantified. The application of SLM for sample extraction of pesticides, viz., chlorophenols [9], sulphonylurea herbicides [10], phenoxy acids [11,12] and *s*-triazines [13,14] from environmental waters was successful.

The objectives of the present study are two-fold. The first one is to develop a methodology which

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enables the selective extraction of the particular class of triazine, methoxy-*s*-triazines, from environmental water samples. The second is to compare the extraction potential and selectivity of the SLM with solid-phase extraction (SPE) for the same model compounds.

2. Experimental

2.1. Chemicals

Atratone, 99% (2-methoxy-4-ethylamino-6-isopropylamino-*s*-triazine), secbumetone, 95.7% (2-methoxy-4-ethylamino-6-isopropylamino-*s*-triazine), simetone, 99.6% [2-methoxy-4,6-bis(ethylamino)-*s*-triazine] and terbumetone, 99.8% (2-methoxy-4-ethylamino-6-tertbutylamino-*s*-triazine) were from Promochem (Wesel, Germany). Structural information and related physical constants are shown in Table 1 [15].

Di-*n*-hexyl ether and *n*-undecane (Sigma, St. Louis, MO, USA) were used as membrane solvents. All other chemicals including acetonitrile and phosphate salts used for adjusting the donor pH were from Merck, (Darmstadt, Germany), and all were of analytical-reagent grade or better. Reagent water was purified using a Milli-Q/RO 4 unit (Millipore, Bedford, MA, USA). River water samples for spiking were collected from the Höje river, located ca. 2 km south of Lund, filtered through a 0.22-μm filter (Millipore) and stored in a refrigerator at 4°C.

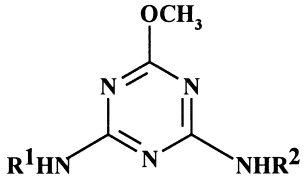
2.2. Liquid membrane extraction and separation system

Construction of the membrane separator, preparation of the liquid membrane, general procedures for membrane extraction, processes of solution transfer to the donor phase and liquid chromatographic separation system employed in this study are discussed in earlier works [8,14]. The chromatographic data, based on the peak height, were collected and handled with a personal computer using the JCL 6000 Chromatographic Data System (Jones Chromatography, Hengoed, Mid-Glamorgan, UK).

The mobile phase was composed of 56% acetonitrile and 44% 0.05 mol/l sodium acetate, adjusted to pH 7.0 using 0.5 mol/l sulphuric acid, and was degassed by bubbling helium gas. A 25-μl aliquot of the processed sample of the *s*-triazine mixture was introduced into the separation system, and the analytes were monitored at a wavelength of 220 nm. All analyses were carried out at a mobile phase flow-rate of 1.0 ml/min.

A series of sample solutions in the concentration range 0.2–2.0 mg/l was prepared by diluting the 100 mg/l standard solution prepared in acetonitrile. A 0.5 mg/l aqueous solution of the sample mixture was prepared in reagent water for extraction. The extent of sample enrichment is expressed by the extraction efficiency, *E*, which is defined as the ratio of the amount of analyte extracted in the stagnant acceptor channel to the initial concentration entering the donor phase [8,14]. At a given flow-rate and ionic strength it is constant, and is given by:

Table 1
Structures and physical constants [15] of the methoxy-*s*-triazine herbicides of study



Common name	R^1	R^2	Solubility (ppm)	pK_a value
Simetone	$-C_2H_5$	$-C_2H_5$	–	4.15
Atratone	$-C_2H_5$	$-CH(CH_3)_2$	1650	4.20
Secbumetone	$-C_2H_5$	$-CH(CH_3)C_2H_5$	620	4.40
Terbumetone	$-C_2H_5$	$-C(CH_3)_3$	130	4.60

$$E = n_a/n_d = C_a \cdot V_a/(C_d \cdot V_d) \quad (1)$$

Here, n_a and C_a are number of moles and the concentration of the extracted sample collected from the acceptor channel, respectively. V_a is the final volume after pH adjustment and V_d is the total volume of the sample of concentration C_d and number of moles n_d which has passed the donor channel.

2.3. Carry over effects from the membrane extraction

To investigate the total quantity left-over due to adsorption to various parts of the extraction system, the following procedures were followed: the sample mixture was first extracted, which was followed by extraction of the blank reagent water in a similar manner. The carry over effect (COE) was evaluated from the peak heights, using the following equation:

$$\text{COE} = P_b/(P_b + P_s) \quad (2)$$

where P_b and P_s are peak heights of the blank extraction and sample mixture, respectively.

2.4. Determination of the enrichment factor from the donor waste

To determine the enrichment factor from the donor waste, 0.10 mg/l of the aqueous mixture of the herbicides was first mixed with equal volume of the phosphate buffer (ionic strength of 0.01 *M*), and was pumped with the donor flow-rate of 7.5 ml/min for 200 min. The donor waste was collected for 1 min, at certain time intervals, to estimate the permeation through the liquid membrane.

The extraction efficiency, *E*, was evaluated from the donor waste using the following equation:

$$E = 1 - n_w/n_d \quad (3)$$

where n_w is the total number of moles in the donor waste collected from the beginning of the experiment.

The enrichment factor, E_e, of the extraction process was determined from the following equation:

$$E_e = C_a/C_d = (1 - n_w/n_d)V_d/V_a \quad (4)$$

2.5. Solid-phase extraction

The SPE column used was a C_{18} EC ISOLUTE (International Sorbent Technology, Hengoed, Mid-Glamorgan, UK) packed with 500 mg of the sorbent in a 6-ml polypropylene syringe barrel. Percolation of all solutions and solvents through the column was carried out using a vacuum manifold. For preconditioning 3 ml acetonitrile was used, followed by rinsing with 3 ml of reagent water for equilibration. Sample solutions were percolated at about 15 ml/min. The column was then flushed with 3 ml of water. For elution of the sample, 3 ml (2×1.5 ml) acetonitrile was used. The extracts were introduced into the separation system without any further treatment.

3. Results and discussion

3.1. Optimization of the liquid membrane extraction

Selection of the membrane solvent for immobilization in the support material is one of the most critical step in SLM extraction. In the present study, di-*n*-hexyl ether, *n*-undecane and a mixture of both solvents (50:50, v/v) were tried as membrane solvents, and the extraction efficiencies, calculated using Eq. (1), were compared, Table 2. The membrane solvent composed of 100% di-*n*-hexyl ether exhibited by far better efficiency, particularly for more polar compounds, simetone and atratone, than the other membrane solvents tried. The same solvent proved to be useful for extraction of chloro-*s*-triazines [13], but less effective for extraction of relatively less polar alkylthio-*s*-triazines [14].

The effect of the acceptor pH on the efficiency of the membrane extraction was examined by varying the concentration of sulphuric acid, Fig. 1. All the methoxy-*s*-triazine herbicides studied exhibited similar behaviour towards the change in the acceptor acid concentration. The efficiency was higher at pH 1.0, which also agrees with the theoretical predictions [16], and this pH was used through out this study.

Extraction efficiencies were determined for various donor solutions pH values (pH 3–8) and the results are shown in Fig. 2.

Table 2
Extraction efficiency, *E*, of 0.5 mg/l methoxy-*s*-triazine herbicides of study in the various membrane solvents chosen

Compound	Extraction efficiency[a], in		
	100% di-*n*-hexyl ether; $n=9$	50% di-*n*-hexyl ether in *n*-undecane; $n=10$	100% *n*-undecane; $n=10$
Simetone	0.45±0.05	0.32±0.01	0.20±0.02
Atratone	0.59±0.04	0.48±0.01	0.40±0.04
Secbumetone	0.65±0.04	0.57±0.02	0.52±0.05
Terbumetone	0.68±0.04	0.61±0.02	0.57±0.02

Donor pH: 7.0; acceptor pH: 1.0; donor flow-rate: 1.0 ml/min.
[a] Mean±95% confidence interval.

In the liquid membrane extraction of the basic compounds, the pH of the sample solution has to be kept 2.0 pH units more than the highest pK_a, to keep the analytes in uncharged form [16]. The extractability of these compounds was satisfactory at any pH above the pK_a values. The highest efficiency was obtained at pH 7.0 and this pH was used for all the extraction works. The decrease in efficiency above pH 7.0 was an unexpected observation, even though hydrolysis of the compounds is known in highly basic solutions [17]. This may be attributed to the greater basic character of this class of *s*-triazines when compared with chloro- and alkylthio-*s*-triazines [17].

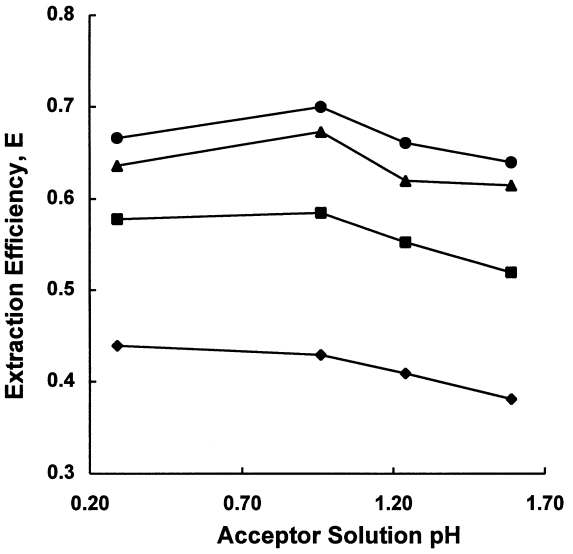


Fig. 1. Extraction efficiency, *E*, of the 0.5 mg/l of the methoxy-*s*-triazine herbicides in various concentrations of the acidic acceptor solution: 20 min extraction; donor pH 7.0; donor flow-rate of 1.0 ml/min; membrane solvent: 100% di-*n*-hexyl ether. A 25-μl aliquot of the enriched sample was injected into the separation system. Symbols: ♦ Simetone; ■ atratone; ▲ secbumetone; ● terbumetone.

3.2. Analyte adsorption in the extraction system

To determine the amount of molecules left in the flow system, a reagent water blank was pumped in the same way after collecting the enriched sample plug. The COE was calculated using Eq. (2), and the results fall between 2 and 3%. It can be concluded that by rinsing the flow system it is possible to transfer maximum fraction of the analyte molecules, and the amount determined in this study were all below the uncertainty of the measurements, Table 2.

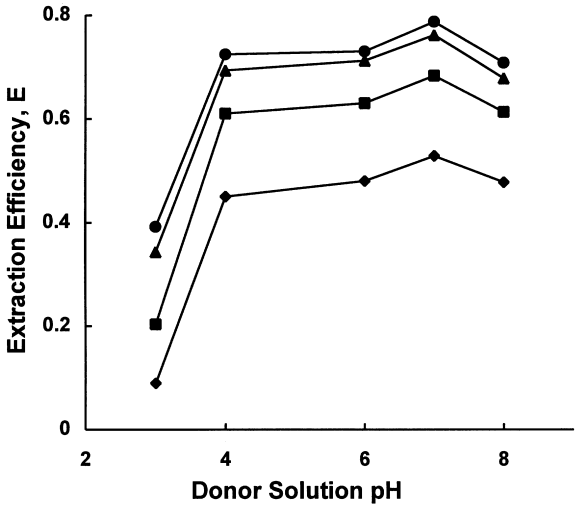


Fig. 2. Extraction efficiency, *E*, versus the donor pH. Acceptor pH 1.0; Donor pH was varied with 85% H_3PO_4–NaH_2PO_4, NaH_2PO_4 and NaH_2PO_4–Na_2HPO_4. Other conditions and symbols as in Fig. 1.

Table 3
Effect of washing time of the donor stream on the extraction efficiency of 0.5 mg/l of the methoxy-*s*-triazine herbicides

Compound	Extraction efficiency[a] after washing time for				
	0 min	5 min	10 min	20 min	40 min
Simetone	0.43	0.46	0.46	0.47	0.48
Atratone	0.56	0.61	0.62	0.62	0.63
Secbumetone	0.63	0.66	0.66	0.67	0.70
Terbumetone	0.65	0.67	0.68	0.69	0.71

Membrane solvent: 100% di-*n*-hexyl ether. Other conditions as in Table 2.
[a] Mean of four extractions.

The results obtained by washing the flow system for different times, Table 3, are also in good agreement with those of the COE when the system is left to stand for 10 min. Therefore, in all the subsequent extractions the flow system was flushed for 20 min, unless otherwise mentioned.

To investigate the membrane memory effect (MME), the enriched extract was collected after allowing to stand for varied times, 0 to 20 min. Based on the results obtained, Table 4, it was concluded that 10 min was sufficient to get the most fraction of the molecules transferred to the acidic acceptor solution. However, when complex samples of unknown concentrations, e.g., river water, are extracted a waiting time of 20 min or more may be required to ensure that the MME from the earlier extractions are insignificant.

3.3. Dependence of E on donor flow-rate

The extraction efficiency was also determined as a function of the donor flow-rate. The decrease in efficiency with increasing donor flow-rate for 20 min extraction is shown in Fig. 3. With a higher flow-rate there is an increase of the number of moles coming in contact with the liquid membrane per unit time which offsets the decrease in *E*. This results in an increased accumulation of analytes [2,14,16]. Therefore, when large sample volume is available for analysis, pumping at higher flow-rate will reduce the analysis time and decrease the detection limit.

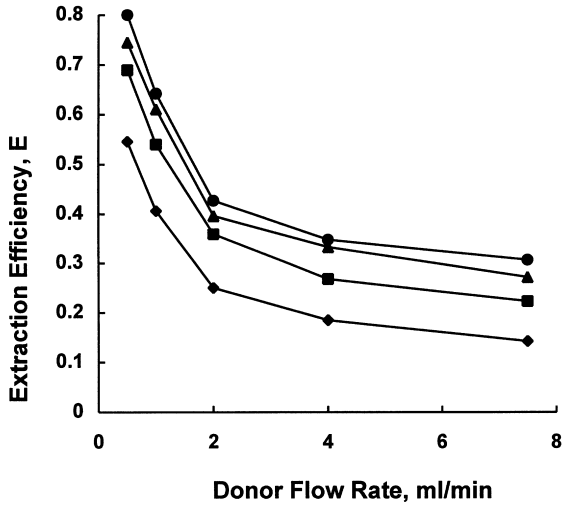


Fig. 3. Change of the extraction efficiency, *E*, with the donor flow-rate, in ml/min, for 20 min extraction of the methoxy-*s*-triazine herbicides. Acceptor pH 1.0. Other conditions and symbols as in Fig. 1.

The problem associated with increasing the donor flow-rate is shortening of the life time of the membrane. The reason for this may be the larger volume of the sample to be processed, allowing the dissolution of the membrane solvent [18]. Thus, unless specifically noted, all studies were performed at a flow-rate of 1.0 ml/min.

Table 4
Study of the effect of waiting time, after 20 min sample extraction and 20 min flushing of the donor stream with the donor buffer, on the extraction efficiency of 0.5 mg/l of the mixture of the methoxy-*s*-triazine herbicides

Compound	Extraction efficiency[a] after waiting time of				
	0 min	2 min	5 min	10 min	20 min
Simetone	0.39	0.42	0.43	0.43	0.42
Atratone	0.55	0.56	0.53	0.59	0.61
Secbumetone	0.56	0.59	0.59	0.63	0.67
Terbumetone	0.59	0.60	0.61	0.65	0.67

All other conditions as in Table 2.
[a] Means of three extractions.

3.4. Determination of enrichment factor from the donor waste

In almost all the SLM extractions so far reported, extraction efficiencies have been calculated from the enriched sample collected from the acceptor, except for the recent work on *s*-triazines and aniline derivative compounds [19]. In the present work the donor wastes collected during 1 min were directly trans-

ferred to the separation system without any further treatment. The number of moles passed the donor waste since the beginning of the experiment was calculated using numerical integration and this was used to estimate the extraction efficiency at each interval, using Eq. (3).

One important observation here was that during the first few minutes, E was relatively high and then becomes nearly unaffected throughout. This time may most probably be the time required to establish a steady-state mass transfer through the membrane separation system [1]. It may also be useful to employ this procedure, during optimization of the SLM extraction, to determine the optimal time of extraction for routine works. Estimation of the efficiency from the donor waste may be amenable only when the compounds under study are quantitatively transferred and completely trapped in the acceptor solution.

The enrichment factor, E_e, during the whole extraction period was calculated using Eq. (4). The problem encountered with this work was that quantification of the more polar compound, e.g., simetone, was not reliable since it overlaps with the peaks from the ions of the donor buffer solution. Fig. 4 illustrates the increase in E_e throughout the experiment. It is also evident that if the extraction efficiencies calculated using Eq. (1), agree with the values estimated using Eq. (3) from the donor waste monitoring, then there will not be analyte accumulation effects in the membrane and MME will be negligible.

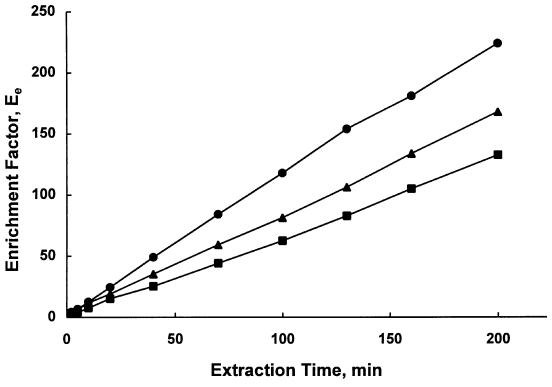


Fig. 4. Enrichment factor, E_e, determined from the donor waste at certain time intervals shown at the donor flow-rate of 7.5 ml/min. Donor pH of 7.0, acceptor pH 1.0, and 25 μl was introduced into the separation system. Symbols as in Fig. 1.

3.5. Solid-phase extraction of methoxy-s-triazines

The recoveries of the methoxy-*s*-triazines, concentrations of 1.0 μg/l each, from reagent water and spiked in river water sample are given in Table 5. The chromatogram for the sample spiked in natural water, and enriched using a C_{18} disposable column, is shown in Fig. 5a. The clean-up of the sample is limited by the interfering compounds, as it was observed in the quantification of simetone. To minimize the effect of ions of the buffer, as also noted from the donor waste monitoring, the ionic strength was kept minimum (0.01 M). This process could not remove the additional peaks appearing in the early stage of the chromatogram, Fig. 5a.

3.6. Selectivity and detection limit

Comparison of the chromatograms obtained by SPE, Fig. 5a, and liquid membrane extraction, Fig. 5b, of the *s*-triazines mixture spiked in river water samples indicated that the selectivity of the latter is larger than that of the former. This may be due to the rejection of the potentially interfering ionic solutes from entering the acceptor compartment of the liquid membrane.

Quantitative studies of the performance of the liquid membrane extraction of the model compounds have been done in the concentration range of 0.2–2.0 mg/l, at five points. All calibration graphs were linear with insignificant intercepts at the 95% confidence level. The precision was in the order of 5%

Table 5
Mean recovery (%) for solid-phase extraction of 1.0 μg/l sample mixture in a litre of solution both in reagent water and spiked in river water

Compound	Recovery in reagent water ($n=4$)	Recovery in river water ($n=3$)
Simetone	68.9	63.9
Atratone	86.0	74.7
Secbumetone	95.8	92.7
Terbumetone	106.0	99.7

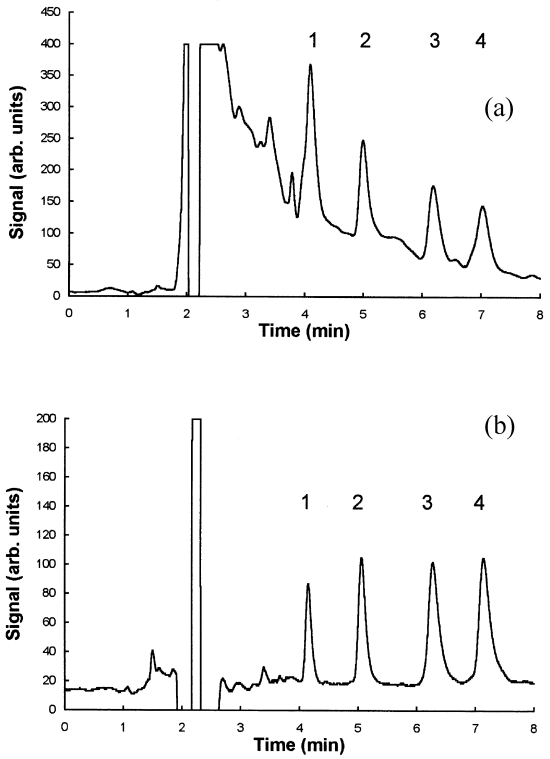


Fig. 5. Chromatograms (LC–UV) of the methoxy-*s*-triazine herbicides used as model compounds in this study. (a) SPE of spiked river water (1.0 μg/l of each analyte in 1 l), extracted at a flow-rate of about 15 ml/min; (b) SLM extraction of spiked river water (0.5 μg/l of each analyte), extracted for 270 min at a donor flow-rate of 7.5 ml/min; 50 μl of the extract was introduced into the separation system. Peaks: 1=Simetone; 2=atratone; 3=secbumetone; 4=terbumetone.

and all the graphs gave linear correlation coefficients of 0.9995 or better.

To determine the limit of detection (LOD) of the compounds of study, low concentrations of the sample mixture, 0.5 μg/l, were prepared both in reagent water and spiked in river water, and extracted at the donor flow-rate of 1.0 ml/min and 7.5 ml/min for about 270 min. As shown in Table 6, the LOD values in both water samples are not significantly different. By increasing the donor flow-rate from 1.0 ml/min to 7.5 ml/min, the LOD, calculated as twice the noise level, has been lowered twice or three times. Thus, with liquid membrane extraction, there is a possibility of much lowering the LOD, when large sample volume is available, at the expense of time, particularly when the pollutants in question are present in trace level.

4. Conclusions

In this work a method based on supported liquid membrane for selective extraction of the particular class of triazine compounds, methoxy-*s*-triazines, from complex matrices has been developed. An alternative way of determining the efficiency of the extraction process – by collecting the donor waste at certain time interval – has been investigated. The selectivity has also been compared with SPE for sample mixture prepared under similar conditions. A sub-ppb amount of the sample compounds has been determined after selective extraction with liquid

Table 6
Determination of the LOD for extraction of 0.5 μg/l of the sample mixture for 4.5 h at the flow-rates of 1.0 and 7.5 ml/min

Compound	LOD in ng/l at 1.0 ml/min, $n=5$		LOD in ng/l at 7.5 ml/min, $n=4$	
	Sample in reagent water	Sample in river water	Sample in reagent water	Sample in river water
Simetone	38	39	15	19
Atratone	42	45	19	19
Secbumetone	42	47	18	18
Terbumetone	56	62	17	17

The flow system was rinsed for 30 min, and then left to stand for 10 min. Injection volume was 50 μl.

membrane. The SLM sample preparation method is, therefore, a promising technique for enrichment of trace level of ionizable organic pollutants in environmental samples.

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