Screening Method for Mononitrotoluenes in Natural Waters Using Magnetic Solvent Bar Liquid-Phase Micro-extraction Followed by Gas Chromatography-Flame Ionization Detection

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Abstract

A simple andefficient hollow fiber-based method,viz magnetic solvent bar liquid-phase microextraction (MSB-LPME) combined with gas chromatography-flame ionization detection (GC-FID) has been successfully developed for the trace determination of mononitrotoluenes (MNTs) in natural water samples. The analytes were extracted from sample solution to the organic solvent immobilized in a fiber. Following the extraction, the analyte-adsorbed magnetic solvent bar can be easily isolated from the sample solution by a magnet which could significantlyfacilitate the operation as well asreducing the total pretreatment time. The bar was mainly eluted with acetonitrile, evaporated to dryness while the residue was dissolved in toluene and finally injected into GC-FID. At first, a series of parameters influencing microextraction performance were systematically investigated and optimized. The values of the detection limit were in the range of 0.05-0.08 µg L^{-1} and the RSD% for the analysis of 50.0µg L^{-1} of the analytes was below than 5.8% (n = 6). An acceptable linearity ($0.996 \geq r^2 \geq 0.995$) and a broad linear range (0.2-200 µg L^{-1}) were achieved. The method was eventually employed for the preconcentration and determination of the MNTs in environmental water samples and acceptable results were reached.

Keywords*:Gas chromatography-flame ionization detection;magnetic solvent barliquid-phase microextraction;mononitrotoluenes; natural water samples.*

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Introduction

Nitroaromatic explosives have commonly been applied with plenty of industrial applications as far back as the nineteenth century [1]. Discharge of contaminated manufacturing waste streams into the environment, burial of obsolete munitions, and military exercises intensify the availability of the chemicals for movement towards natural resources[2].Among the explosives, mononitrotoluenes (MNTs, including a group of three isomers) exist widely in aquatic environments considering their widespread usages[3]. They are employed in the synthesis of intermediates for the formulation of dyes, rubber chemicals, and pesticides along with the other industrial demands [4]. MNTs are highly toxic substances at trace concentration levels and suspected to be carcinogens[5]. Consequently, their sensitive determination has turned an emerging and vital topic of interest together with the increasing concern over the environmental issues worldwide [6-9].

High-performance liquid chromatography (HPLC) [10], gas chromatography (GC) [11], ion mobility spectrometry (IMS) [12], mass spectrometry (MS) [13], fluorescence spectroscopy [14] and electrochemical sensors [15] have been reported for the determination of nitroaromatic explosives in different matrices. Moreover, the extraction techniques which are applied before the instrumental analysis are liquid-liquid extraction (LLE) [16] and solid phase extraction (SPE) [17]. Nevertheless, these classical sample pretreatment methods need either a large amount of sample and toxic organic solvents while they are considered as time-consuming, labor-intensive and expensive which frequently results in strong blank values [18, 19].

Liquid-phase micro-extraction (LPME) is a sample pretreatment method that utilizes negligible volumes of organic solvents to extract a wide range of analytes from several matrices before instrumental analysis[20]. The development of these techniques centers on providing accessible, low-priced and green chemistry approaches for sample preparation [21, 22]. Through the various modes of LPME and to improve its capabilities, Pedersen-Bjergaard and Rasmussen reported hollow fiber liquid-phase micro-extraction (HF-LPME) [23, 24]. It used a HF to keep steady and protect the extraction solvent, and the small pore size of the fiber prevents large molecules and particles from entering into the acceptor phase, resulting in a clean-up of sample matrix in addition to the extraction [25]. Reasoning slight amounts of the solvent are used, consumption of toxic organic solvents is minimized while the technique combines merely extraction and pre-concentration as well as sample introduction into a single step [26, 27].

Recently, Wu et al. have reported a simple, efficient and novel HF-LPME based method, named magnetic solvent bar liquid-phase micro-extraction (MSB-LPME) that was first applied for the determination of organophosphorus pesticides in fruit juice samples[28]. In MSB-LPME, some

modifications of HF-LPME were presented for assisting the practical operations as well as enhancing the method efficiency.

Present work intends to evaluate the MSB-LPME technique suitability for the determination of three MNTs in the aquatic environment. The factors affecting micro-extraction efficiency were studied in detail, and the optimum conditions were set. The method was validated for quantitative purposes and employed to real samples analysis in combination with gas chromatography-flame ionization detection (GC-FID).

Experimental

Chemicals and materials

Three compounds analyzed in this study including *o*-MNT, *m*-MNT and *p*-MNTwere obtained from Merck (Darmstadt, Germany). Analytical reagent grade acetone, ethyl acetate, hexane, carbon tetrachloride, chlorobenzene, and 1-octanol were acquired from Merck Company (Darmstadt, Germany). Q3/2 Accurel PP hydrophobic polypropylene HF membrane (600 µm inner diameter, 200 µm wall thickness and 0.2 µm pore size) was bought from Membrana GmbH (Wuppertal, Germany). The extraction procedure was performed in the 22 mL screw top vials(Sigma-Aldrich, Steinheim, Germany) with the dimension of 23 mm (outer diameter) × 85 mm (height) × 18 mm (inner diameter). The stainless-steel wire (505 µm outer diameter) was just fit to HF membrane. HPLC-grade methanol and acetonitrile (Fisher Chemicals, Fair Lawn, NJ, USA) and ultrapure water (Millipore, Bedford, MA, USA) were used in all experiments. All solutions were stored at 4°C and protected from light.

Instruments

An Agilent 6890N gas chromatograph (Wilmington, DE, USA) equipped with a split/splitless inlet and flame ionization detector (FID) was used for the determination of the analytes. Helium (99.999%) was employed as carrier gas at the flow rate of 1.0 mL min^{-1}. The chromatographic data were recorded using an HP Chemstation, which was controlled by Windows NT (Microsoft). The analytes were separated by a 30 m × 0.25 mm i.d. × 0.25 m film thickness DB-5 gas chromatographic column (J&W Scientific, Folsom, CA, USA) with the following oven temperature program: initial 60°C, from 60°C (held 2 min) to 200°C at 10°C min^{-1}, then increased at 20°C min^{-1} to 280°C and held for 5 min.The analysis used a 1.0 µL sample injection in a 1:10 split ratio while the injection port and detector were operated 250 and 280°C, respectively.

Real samples collection

The performance of the proposed method was evaluated by analyzing MNTs in four natural water samples including Caspian Sea (Sari Coast, Iran), Persian Gulf (Bandar Lengeh Coast, Iran), Jajroud River (Tehran, Iran) and Latian Dam (Tehran, Iran). The samples were collected in amber glass bottles (1.0 L). The containers were rinsed several times with the pure water to be analyzed and filled till overflow to prevent loss of the volatile organic compounds in the presence of the headspace. The water samples were filtered before the analysis using a 0.45 µm nylon membrane filter (Whatman, Maid-stone, UK) to eliminate the particles. All the samples were stored in the refrigerator at 4 °C until their analysis time.
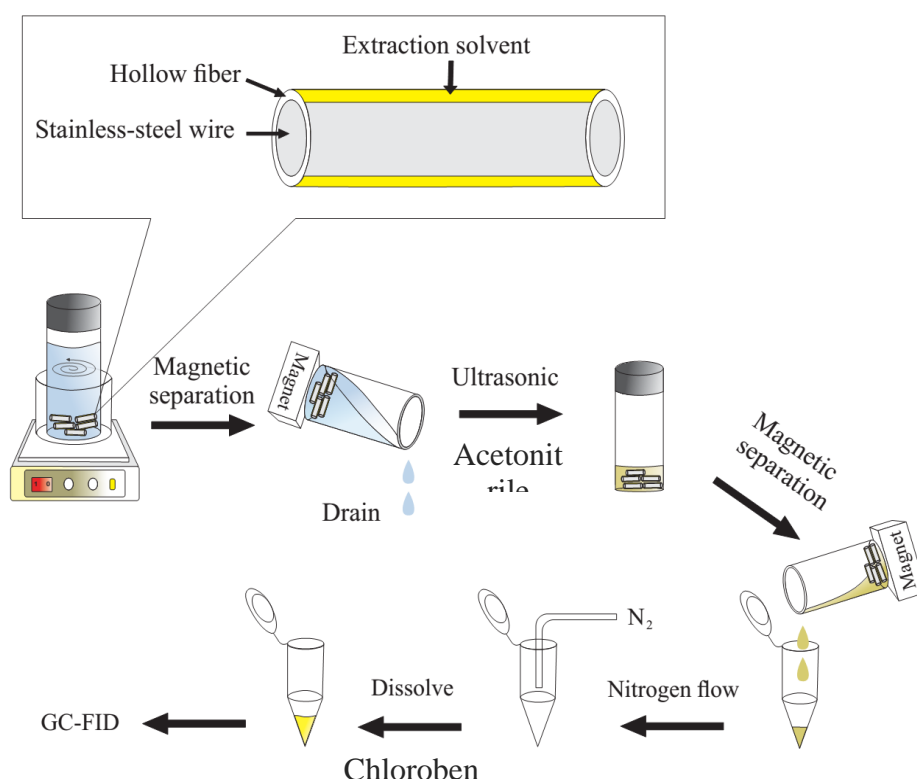

Figure 1.The schematic diagram of MSB-LPME-GC.

Extraction procedure

The MSB-LPME procedure has been introduced in detail [28]and presented in Figure 1. It contained a HF and stainless-steel wire with magnetic properties; they were manually cut into segments of 1.2 cm length. These sections were ultrasonically cleaned to remove impurities and dried in the air. Herein, the stainless steel wire was inserted into the hollow of the HF. The resulting fiber piece was immersed in an organic solvent for one min to impregnate pores of the fiber wall. With regard to remove the extra amount of the organic solvent from the surface of the fiber, it was carefully rinsed with water. After that, five MSBs were placed into the 22 mL screw top vial

containing 20 mL of aqueous sample. The vial was closed and put into a water bath with a temperature of 40 $^\circ$C on the magnetic stirrer for 20 min at 600 rpm. After the extraction, with the help of an external magnet, the MSBs were separated rapidly from the sample solution. Then, the bars were eluted with 400 µL of acetonitrile in an ultrasonic bath for 3 min. The eluate was separated from the MSBs also by a magnet. The eluate obtained was evaporated to dryness under a nitrogen stream, and the residue was dissolved in 100 µL of chlorobenzene. Finally, a 1.0 µL of the extracting phase was injected into GC-FID.

Results and discussion

A one at a time approach was employed to optimize the affecting parameters on the micro-extraction efficiency including the type of extraction solvent, extraction temperature, salt concentration, stirring speed, extraction time and desorption conditions. A fixed concentration of the analytes (50.0 µg L^{-1}) was used in the optimization process.All the quantifications were performed from the average of three replicate measurements. Blank samples were run to confirm the absence of interference.
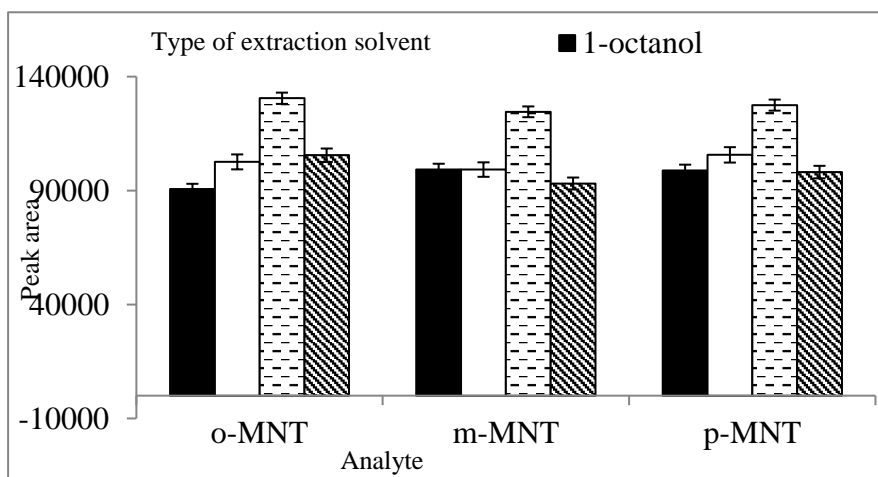
**Figure 2**. The effect of extraction solvent type on MSB-LPME efficiency.

The selection of extraction solvent

The selection of aproper organic solvent in HF-LPME is of great importance for efficient analyte pre-concentration [29]. There are some criteria for organic solvent selection as follows. Firstly, it should be effortlessly immobilized in the HF pores. Secondly, it needs to be nearly nonvolatile to avoid solvent loss during the extraction. In the third order, the organic solvent should be immiscible with water because it serves as a barrier via the two aqueous phases, the source and the receiving phases. Besides, the organic solvent is used to promote analyte diffusion from the source phase into

the receiving phase by the pores of HF[30]. By these regards, 1-octanol, carbon tetrachloride, chlorobenzene, and hexane were investigated in the initial experiments. As it can be seen in Figure2, the highest extraction efficiency for all the analytes was attained with chlorobenzene. Therefore, chlorobenzene was selected as the extraction solvent.

The effect of extraction temperature

The extraction temperature could obviously influence the extraction efficiency in two opposing ways; to begin with, it may enhance the mass transfer of the analytes, and secondly, it can decline the partition coefficients (K_{ow}) between the organic and aqueous phase. Hence, the extraction efficiency will be higher or lower according to the dominant factor [31, 32]. The effect of sample solution temperature was studied in the range of 20-50°C. It is demonstrated that the extraction efficiency for all the target analytes was raised with the increase of temperature (Figure 3). Nonetheless, increasing the extraction temperature upper the mentioned value would result in the dissolution and volatilization loss of the extracting solvent and formation of air bubbles adhering to HF, which would effect on the extraction operation and precision. Thereupon, to achieve better extraction efficiency and reproducibility, the temperature of 40°C was used in further experiments.
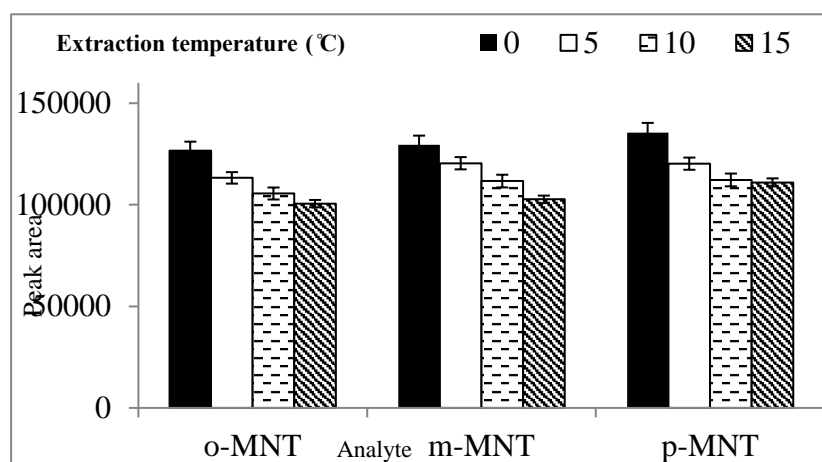

Figure 3.The effect of extraction temperature on MSB-LPME efficiency.

The effect of salt addition

The salt added to sample solution often amplifies the ionic strength and so, improves the extraction efficiency owing to the salting-out effect. This effect has been accounted to decline the solubility of target analytes in the aqueous phase and increasing partitioning into the organic phase [33, 34]. For this purpose, different concentrations (0%-20% w/v) of NaCl were added to the sample solution to

evaluate its influence on the extraction efficiency (Figure4). As it can be seen, the salt addition has no significant impact on the pre-concentration factors. Therefore, the pre-concentration factor is nearly steady by increasing the amount of NaCl, and the extraction experiments were carried out without adding salt.
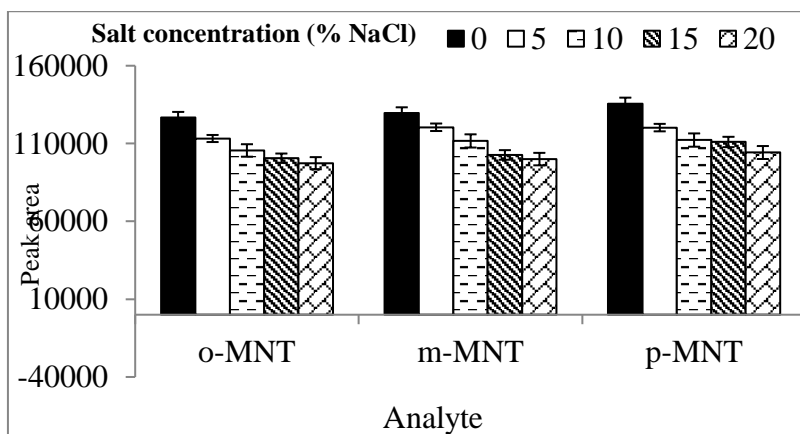
Figure 4. The effect of salt concentration on MSB-LPME efficiency.

The effect of stirring speed

The magnetic stirring improves extraction efficiency and diffusion of the analytes into the interfacial layer of HF while it reduces the time required to reach thermodynamic equilibrium [35, 36]. Herein, the solvent is protected by HF and as a result quicker stirring rates could be applied. The effect of this parameter on the extraction efficiency of the system was studied in the range of 350-750 rpm.
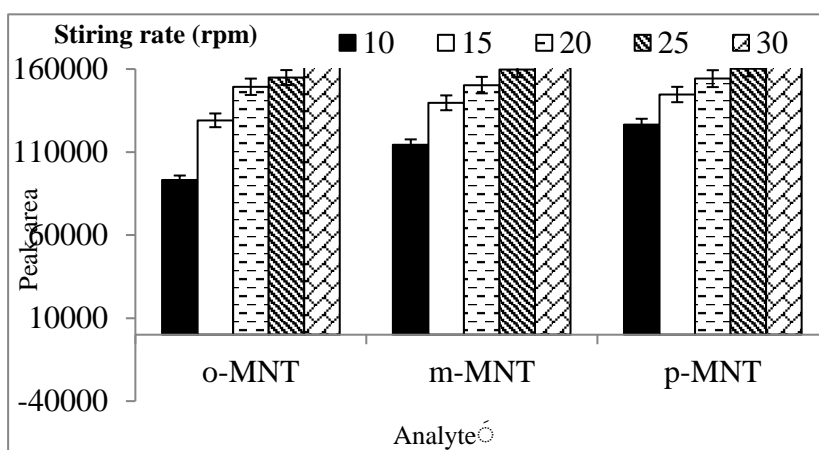

Figure 5. The effect of stirring rate on MSB-LPME efficiency.

As shown in Figure5, the results confirmed that the agitation of the sample substantially boosted the extraction efficiency. However, higher stirring rates were not evaluated as they mightcause the excessive formation of air bubbles on the surface of HF or would lead to solvent dissolution, which conducted to poorer precision and experimental failure. Hence, 750 rpm was selected as the optimum condition in the following experiments.

The effect of extraction time

The mass transfer in MSB-LPME is a process dependent on equilibrium rather than exhaustive extraction and beneficial to achieve good extraction repeatability, it is essential to choose a suitable extraction time [37]. Herein, the extraction time profiles were investigated by recording the variation of the analytical signals of the analytes as a function of extraction time, in the range of 10-40 min. As shown in Figure6, by growing the extraction time up to 30 min, the extraction efficiencies rose quickly and afterward stayed approximately steady. It is remarkable that longer extraction time can result in the loss of the extracting solvent and contraction of the extraction yields. Accordingly, the exposure time of 30 min was selected as the optimum value in the subsequent experiments.
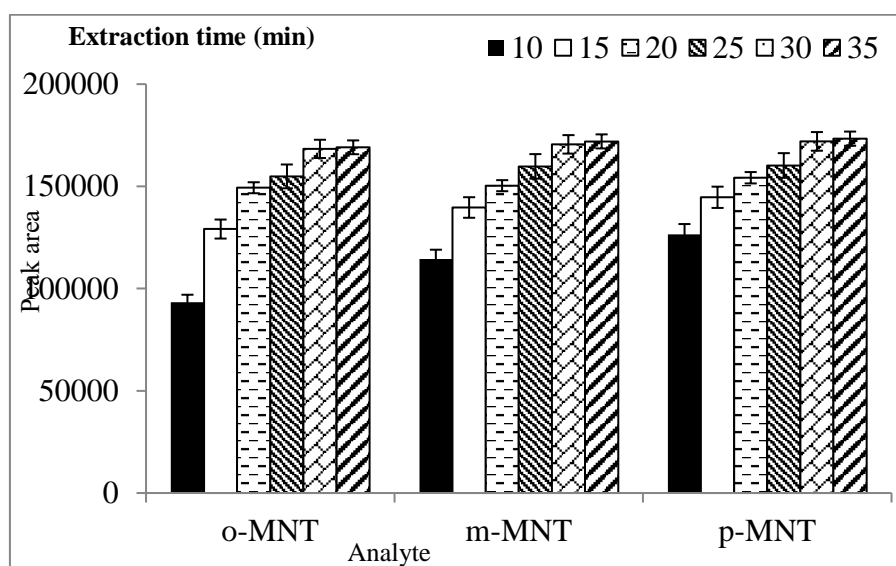
Figure 6.The effect of extraction time on MSB-LPME efficiency.

The effects of desorption solvent and time

Owing to the importance of desorption solvent and time on the extraction efficiency, the parameters were investigated and optimized. When the extraction process was completed, a 400 µL volume of desorption solvents including acetone, acetonitrile, methanol and ethyl acetate was applied to elute the analytes from the MSBs. The results indicated that the best desorption efficiencies were

obtained with acetonitrile. Therefore, it was chosen to be optimum for extraction of MNTs. To evaluate the other parameter, the analyte-enriched MSB was ultrasonicated in the range of 1-10 min. The result confirmed that 4 min was enough to get the best analytical signals. However, if the desorption time was too long, the analytes would be lost significantly. So, 4 min was chosen as the appropriate value.

The analytical performance

To assess the applicability of the method, calibration curves were plotted at the optimum conditions using different concentration levels of the analytes. The limit of detection (LOD) based on the signal-to-noise ratio (S/N) of 3, the determination correlation coefficient (r^2), the linear range (LR) and the relative standard deviation (RSD) were calculated. As shown in Table 1, LODs for the MNTs were in the range of 0.05-0.08 µg L^{-1} while LRs varied in the range of 0.2-200 µg L^{-1} with r^2 of 0.995 to 0.996. By applying a 50.0 µg L^{-1} of MNTs mixed standard solution, the RSDs were below than 5.8% ($n = 6$).

Table 1. Some quantitative data achieved using MSB-LPME and GC-FID for the determination of MNTs.

Analyte	*o*-MNT	*m*-MNT	*p*-MNT
LOD (µg L^{-1}) [a]	0.08	0.05	0.07
r^2	0.996	0.995	0.995
LR (µg L^{-1}) [b]	0.2-200	0.2-100	0.2-200
RSD% [c]($n = 6$)	5.6	5.8	5.5

[a]Limit of detection for S/N = 3
[b]Linear range
[c]Relative standard deviation at concentration level of 50.0 µg L^{-1} for each analyte

As shown in Table 2, comparing the proposed method with other analytical techniques employed for the determination of MNTs in water samples, the present work has low superiority over the other techniques in terms of RSDs and LODs. It should be noted that by applying the mass spectrometer as a detector, the LODs would significantly decrease while the analysis cost increase remarkably. On the other hand, when it comes to the comparison of the extraction time, the represented method nearly stands in last order.

Table 2. The comparison of the current method with the other microextraction methods for the determination of MNTs in water samples.

Method	LOD ($\mu g\ L^{-1}$)	LR ($\mu g\ L^{-1}$)	RSD%	Extraction time (minute)	Reference
MSB-LPME-GC-FID [a]	0.05-0.08	0.2-200	< 5.8	30	Represented method
SPE-DLLME-GC-FID [e]	0.2	0.5-500	<6.4	30	[3]
SDME-GC-MS [b]	0.08-0.11	20-1000	<11.3	15	[6]
SPME-GC-MS [c]	0.08-0.11	20-1000	<3.6	15	[7]
HSSME-GC-FID [d]	0.02-0.06	1-600	<12.9	15	[8]

(a) Magnetic solvent bar liquid-phase microextraction-gas chromatography-flame ionization detection
(c) Single drop microextraction-gas chromatography-mass spectrometry
(b) Solid-phase extraction-dispersive liquid-liquid microextraction-gas chromatography-flame ionization detection
(d) Solid phase microextraction-gas chromatography-mass spectrometry
(e) Headspace solvent microextraction-gas chromatography-mass spectrometry

Analysis of environmental watersamples

Set at the optimum conditions, the method performance was tested by analyzing the analytes in the four different environmental water samples. The results are presented in Table 3 and showed that they were free of MNTs contamination.

Table 3. The results acquired from analysis of natural water samples.

Sample	*o*-MNT	*m*-MNT	*p*-MNT
Caspian Sea (Sari Coast, Iran), (10.0 $\mu g\ L^{-1}$ added)			
MNTs concentration ($\mu g\ L^{-1}$)	ND [a]	ND	ND
Found after spike ($\mu g\ L^{-1}$)	10.5	9.5	9.3
Relative recovery%	105	95	93
RSD% ($n = 6$)	6.1	6.7	5.9
Persian Gulf (Bandar Lengeh Coast, Iran), (25.0 $\mu g\ L^{-1}$ added)			
MNTs concentration ($\mu g\ L^{-1}$)	ND	ND	ND
Found after spike ($\mu g\ L^{-1}$)	26.3	24.1	23.8
Relative recovery%	105	96	95
RSD% ($n = 6$)	5.8	6.3	6.6
Jajroud River (Tehran, Iran), (50.0 $\mu g\ L^{-1}$ added)			
MNTs concentration ($\mu g\ L^{-1}$)	ND	ND	ND
Found after spike ($\mu g\ L^{-1}$)	52.1	50.9	48.3
Relative recovery%	104	102	97
RSD% ($n = 6$)	6.4	5.5	6.0
Latian Dam (Tehran, Iran), (100.0 $\mu g\ L^{-1}$ added)			
MNTs concentration ($\mu g\ L^{-1}$)	ND	ND	ND
Found after spike ($\mu g\ L^{-1}$)	104.1	94.9	97.2
Relative recovery%	104	95	97
RSD% ($n = 6$)	6.4	6.1	6.2

[a] Not detected.

It is noteworthy that MSB-LPME is a non-exhaustive extraction procedure and therefore the relative recovery (determined by the ratio of the concentrations found in the real environmental sample and reagent water sample, spiked with a similar quantity of the analytes), rather than the absolute recovery (used in exhaustive extraction procedures), was utilized. So, in next step and to evaluate the matrix effects, all the real samples were spiked with MNTs standards at different concentration levels and the relative recovery experiments of the analytes are calculated (Table 3). The obtained recoveries were between 93-105%, indicating that the method is not influenced by the matrix in actual applications while the RSD% values were below than 6.7 ($n = 6$).An overlay of two chromatograms obtained by performing MSB-LPME-GC-FID for the Caspian Sea (Sari Coast, Iran) sample before and after MNTs spiking are shown in Figure 7 and demonstrated no significant interference through the analytical procedure.
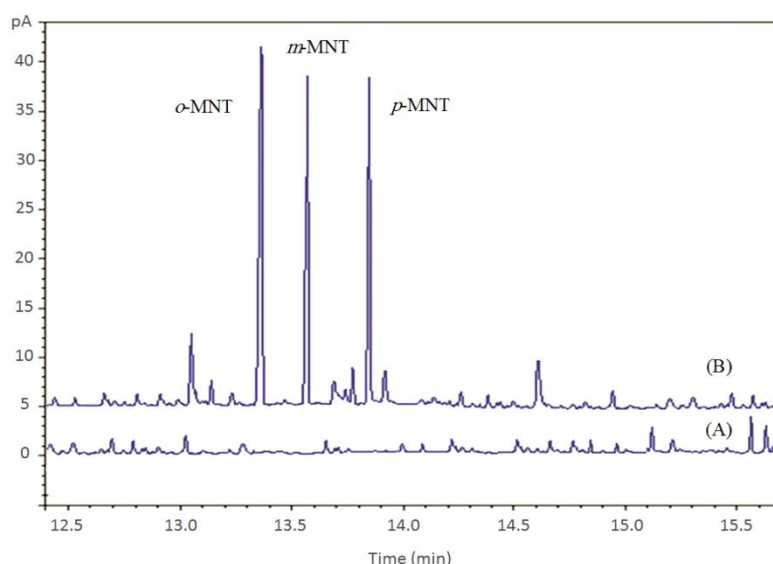
Figure 7. An overlay of two chromatograms obtained by performing MSB-LPME-GC-FID for the Caspian Sea sample (Sari Coast, Iran)before (A) and after MNTs spiking (B).

Conclusion

This work outlined the successful development and application of MSB-LPME method followed by GC-FID as a simplified mode of HF-LPME for the trace determination of the MNTs in environmental water samples. The satisfactory extraction efficiency, sufficient sensitivity and repeatability along with significant accuracy and linearity over a broad range were achieved, almost independent of the complex matrix in the real samples. Moreover, it needs just a little volume of organic extractants, being consequently an environmentally friendly approach of sample preparation. The entire analytical procedure presents a cost effective and quick way for the

screening purposes. Hence, putting all the advantages together, the method possesses great potential to be employed in the other applications.

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