

Hamid Reza Sobhi^{1,2}
Hadi Farahani³
Amir Kashtiaray¹
Mohammad Reza Farahani³

¹Department of Chemistry,
Tehran Payamenoor University,
Tehran, Iran

²Quantitative Mass Spectrometry
Facility, Centre Hospitalier
Universitaire Vaudois,
Lausanne, Switzerland

³Environment and Biotechnology
Research Division, Research
Institute of Petroleum Industry
(RIPI), Tehran, Iran

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Research Article

Tandem use of solid-phase extraction and dispersive liquid–liquid microextraction for the determination of mononitrotoluenes in aquatic environment

Solid-phase extraction (SPE) in tandem with dispersive liquid–liquid microextraction (DLLME) has been developed for the determination of mononitrotoluenes (MNTs) in several aquatic samples using gas chromatography–flame ionization (GC–FID) detection system. In the hyphenated SPE–DLLME, initially MNTs were extracted from a large volume of aqueous samples (100 mL) into a 500-mg octadecyl silane (C₁₈) sorbent. After the elution of analytes from the sorbent with acetonitrile, the obtained solution was put under the DLLME procedure, so that the extra preconcentration factors could be achieved. The parameters influencing the extraction efficiency such as breakthrough volume, type and volume of the elution solvent (disperser solvent) and extracting solvent, as well as the salt addition, were studied and optimized. The calibration curves were linear in the range of 0.5–500 µg/L and the limit of detection for all analytes was found to be 0.2 µg/L. The relative standard deviations (for 0.75 µg/L of MNTs) without internal standard varied from 2.0 to 6.4% ($n = 5$). The relative recoveries of the well, river and sea water samples, spiked at the concentration level of 0.75 µg/L of the analytes, were in the range of 85–118%.

Keywords: Aquatic samples / Dispersive liquid–liquid microextraction / Gas chromatography–flame ionization detection / Mononitrotoluenes / Solid-phase extraction

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1 Introduction

Mononitrotoluenes (MNTs) are a group of explosive compounds, being widely present in aquatic environments due to their widespread applications [1]. MNTs are used in the synthesis of intermediates for the production of dyes, rubber chemicals, pesticides and several industrial applications [2]. These compounds are highly toxic substances even in low concentrations and are suspected to be carcinogens [3]. Therefore, MNTs determination in various matrices at trace level is of great importance [4–7].

Sample preparation is an important analytical step especially for the determination of trace analytes in complex sample matrices, commonly encountered in environmental and biological analyses [8]. The objective of this challenging and critical step is to transfer the analyte into a form that is

prepurified, concentrated and compatible with the analytical system [9, 10]. Several procedures have been developed for the separation and preconcentration of contaminants from water sample matrices, such as liquid–liquid extraction [11, 12], homogeneous liquid–liquid extraction [7], solid-phase extraction (SPE) [13, 14], solid-phase microextraction [15–17] and liquid-phase microextraction [18–21].

SPE is a widely used sample-preparation technique for the isolation of selected analytes, usually from a mobile phase (gas, fluid or liquid). The principal goals of SPE are trace enrichment (concentration), matrix simplification (sample clean-up) and medium exchange. It is now the most common sampling technique in many areas of chemistry, including environmental, pharmaceutical, clinical, food and industrial chemistry [22].

Rezaee et al. have developed a simple and rapid preconcentration and microextraction method, named dispersive liquid–liquid microextraction (DLLME), which was initially applied for the determination of polycyclic aromatic hydrocarbons in water samples [23]. Recently, much more interest has been focused on the combination of SPE with DLLME as a hyphenated technique for the determination of a variety of contaminants in complex matrices [24–27].

This work evaluates the suitability of DLLME, in combination with reversed-phase SPE, for the extraction of MNTs in some water samples. The factors affecting the

Correspondence: Dr. Hadi Farahani, Environment and Biotechnology Research Division, Research Institute of Petroleum Industry (RIPI), P.O. Box 14665-1137, Tehran, Iran

E-mail: farahaniha@ripi.ir

Fax: +98-21-44739736

Abbreviations: DLLME, dispersive liquid–liquid microextraction; MNT, mononitrotoluene; PF, preconcentration factor

microextraction efficiency were investigated in detail and the optimal conditions were then established. The hyphe-nated technique was validated for quantitative purposes and then successfully applied for the analysis of real aquatic samples.

2 Materials and methods

2.1 Reagents and standard solutions

The studied MNTs including *o*-MNT, *m*-MNT and *p*-MNT were purchased from Merck (Darmstadt, Germany). The stock standard solutions (500 mg/L) were prepared in methanol. All of the standard solutions were kept in the refrigerator at 4°C. HPLC grade acetonitrile, acetone and methanol were supplied by Merck. Reagent grade carbon disulfide, carbon tetrachloride, tetrachloroethylene as well as sodium chloride were also purchased from Merck. Double distilled water was used for the preparation of the working solutions.

2.2 Real samples

River water sample was taken from one of the seasonal and local rivers in the southern part of Tehran, Iran. The depth at which the river water sample taken was almost 0.3 m. The well water sample was obtained from the northern part of Tehran, Iran, as well. It was taken from a 30-m depth. The source of the sea water sample was from Caspian Sea, close to coastline of Babolsar, North of Iran (almost 50 m off the seaboard). Amber glass bottles (250 mL) with PTFE screw caps were used for the collection of the samples. The bottles were completely filled to avoid the volatile organic compounds evaporation. All the above-mentioned samples were filtered right before the analysis, by using a 0.45- μ m nylon membrane filter (Whatman, Maidstone, UK).

2.3 Instrumentation

The analysis was performed on a Hewlett-Packard (Agilent Technologies, Palo Alto, CA, USA) HP 7890A series GC, equipped with FID and split/splitless injector. The chromatographic data were recorded using HP Chemstation software, which was controlled by Windows NT (Microsoft). Separation and identification of the analytes were performed using DB-5 fused-silica capillary column with a 30 m \times 0.32 mm id and 0.25 μ m film thickness. Separation conditions were as follows: initial 80°C (held 1 min) raised to 120°C at 5°C/min, then increased at 40°C/min to 250°C and finally held for 15 min. The injector and detector temperatures were 250 and 280°C, respectively. The injector split ratio was set at 1:1. Following gas flow rates were used: carrier (helium, 99.999%) 2.0 mL/min; make-up (nitrogen,

99.999%) 30 mL/min; hydrogen and air (for FID) 30 and 300 mL/min.

2.4 Extraction procedure

SPE of MNTs from the water samples was carried out using 500 mg of C₁₈ (octadecylsilane) sorbent (6 mL syringe barrel, Varian, Harbor City, CA, USA). Firstly, air was allowed in to make the solid phase dry by the use of a vacuum pump operating at 45 mL/min for 5 s. Secondly, the elution solvent (2.0 mL of acetonitrile) was used three times to wash the solid phase to remove possible interferences. For optimization process, 100 mL of the aqueous sample, in which the concentration level of each analyte was kept at 10 μ g/L, was used. The above sample was loaded at a flow rate of about 5 mL/min using a vacuum pump (Rotovac, Heidolph, Germany). After ventilating the solid phase, the analytes were eluted with 1.0 mL acetonitrile and then collected into a 10-mL screw cap glass test tube with conical bottom. Then, the mixture of 11 μ L of tetrachloroethylene (as extracting solvent) and the eluent was added to the conical test tube (5 mL double distilled water) by a syringe, leading to the dispersion process without shaking. The time needed for reaching the equilibrium is fast. The mixture was then centrifuged for 4 min at 5000 rpm. Afterwards, the dispersed fine tetrachloroethylene droplets were sedimented at the bottom of the conical test tube (6.0 ± 0.2 μ L). Of which, 2.0 μ L was then withdrawn into a 10.0- μ L microsyringe (SGE, Australia) and immediately injected into the GC system. All experiments were performed three times and for each experiment preconcentration factor (PF) and also extraction recovery (R%), as indices of extraction efficiency, were calculated and the mean of R% was used as the analytical signal.

3 Results and discussion

There are various parameters affecting the SPE-DLLME efficiency, which are discussed in detail. These parameters were investigated, and then the optimal conditions were established. One at a time strategy was employed to optimize the influential factors in this method. To screen the mentioned parameters, PF was calculated using the following expression:

$$PF = C_{\text{sed}}/C_0 \quad (1)$$

where C_{sed} and C_0 are the concentrations of the analyte in the sedimented phase and the initial concentration of the analyte in the aqueous sample solution, respectively. C_{sed} calculation was conducted by the direct injection of the MNT standard solutions in tetrachloroethylene with concentrations in the range of 1.0–50.0 mg/L. Based on Eq. (1), R% is then calculated

$$R\% = 100 \times PF \times (V_{\text{sed}}/V_{\text{aq}}) \quad (2)$$

where V_{sed} and V_{aq} are the volume of the sedimented phase and that of aqueous sample, respectively.

3.1 Optimization of the influencing parameters on extraction efficiency

3.1.1 Effect of the breakthrough volume

To study the breakthrough volume for all MNTs, different sample volumes (25, 50, 100, 150 and 200 mL) of the standard solution of the analyte mixtures were preconcentrated in the sorbent followed by DLLME procedure, as mentioned in Section 2. Based on Fig. 1, it can be seen that almost constant extraction recoveries were obtained for all MNTs with different sample volumes. But, in order to reduce the time of analysis, a sample volume of 100 mL was selected for the following tests.

3.1.2 Salt effect

To study the effect of salt addition on extraction efficiency, the experiments were conducted at different salt (NaCl) concentrations of the sample solution, ranging from 0 to 6% (w/v). The results exhibited that the salt addition demonstrated almost no significant effect on the extraction recoveries (Fig. 2). This is possibly because of two opposite effects of addition of salt in DLLME. One is to increase the volume of sedimented phase, which reduces PF; another is the salting-out effect, which increases PF. As a result, the extraction efficiency remains relatively constant when the amount of sodium chloride is increased. Therefore, all experiments were performed without the addition of the salt.

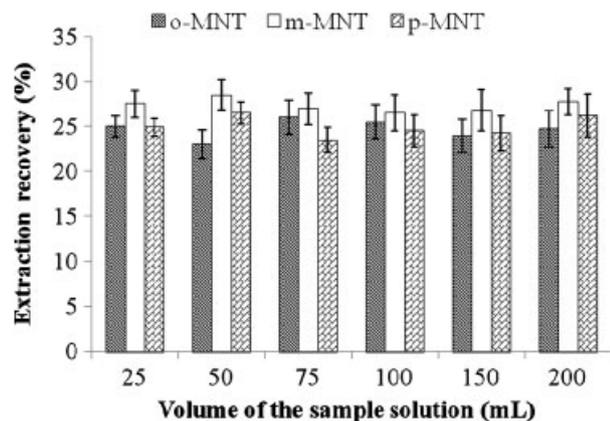


Figure 1. The influence of the volume of sample solution (breakthrough volume) on the extraction efficiency of MNTs obtained from SPE-DLLME. Extraction conditions: sample solution flow rate, 5 mL/min; elution solvent (acetonitrile) volume, 1.0 mL; extracting solvent (tetrachloroethylene) volume, 11.0 μL ; the target analytes concentration, 10.0 $\mu\text{g/L}$; volume of the aqueous solution for DLLME, 5.0 mL; sedimented phase volume, $6.0 \pm 0.2 \mu\text{L}$. The data are based on the average extraction recovery of each analyte for three replicate analyses with standard deviation for error bars in the range of 1.0–2.4.

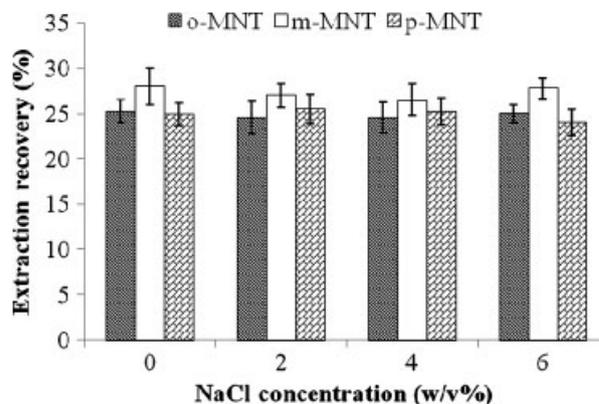


Figure 2. The effect of salt (NaCl) concentration on the extraction efficiency. Extraction conditions: as with Fig. 1, breakthrough volume: 100 mL and standard deviation for error bars in the range of 1.0–1.8.

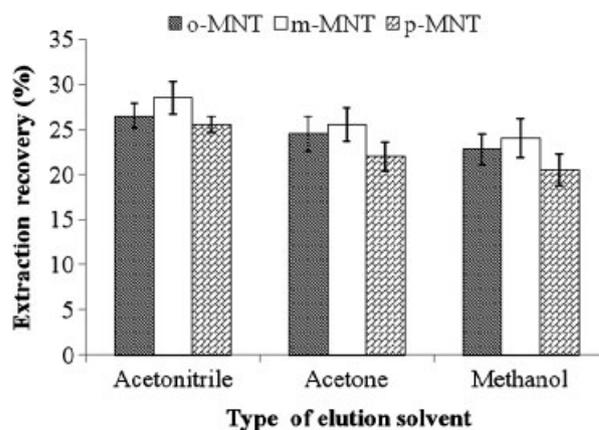


Figure 3. The effect of type of elution solvent on the extraction efficiency. Extraction conditions: as with Fig. 1, breakthrough volume: 100 mL and standard deviation for error bars in the range of 0.9–2.1.

3.1.3 Influence of the elution solvent type and volume

In SPE-DLLME method, the elution solvent of SPE must also play the role of the disperser solvent at the DLLME stage. Thereby, acetone, acetonitrile and methanol, demonstrating this ability, were chosen for this purpose [24]. The sorbent was eluted using 1.0 mL of each elution solvent. According to Fig. 3, acetonitrile was selected for the elution of the sorbent. For the evaluation of the required acetonitrile volume, the elution was investigated in the range of 0.5–2.0 mL of the selected elution solvent. As such, the best extraction results were achieved at the volume of 1.0 mL, as indicated by Fig. 4.

3.1.4 Selection of extracting solvent

The selection of an appropriate extracting solvent is very important to achieve good recovery, high PF for the target compounds. The extracting solvent has to meet several requirements. It should demonstrate (i) higher density than

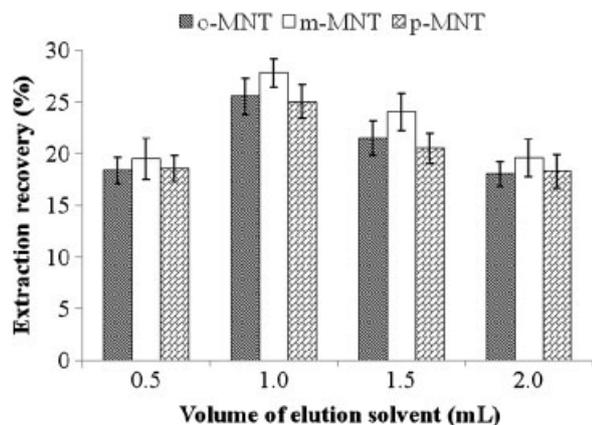


Figure 4. The effect of elution solvent (acetonitrile) volume on the extraction efficiency. Extraction conditions: as with Fig. 1, breakthrough volume: 100 mL and standard deviation for error bars in the range of 1.2–2.0.

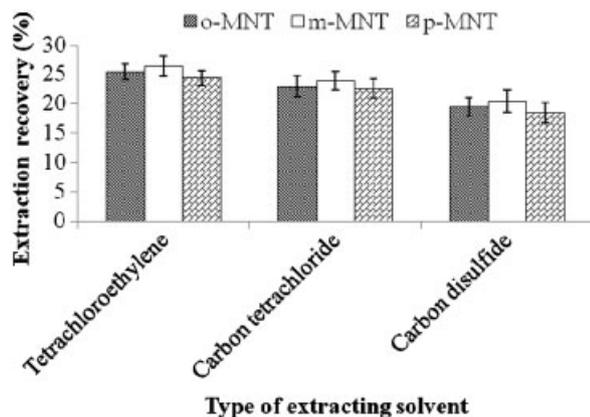


Figure 5. The effect of type of extracting solvent on the extraction efficiency. Extraction conditions: as with Fig. 1, breakthrough volume: 100 mL and standard deviation for error bars in the range of 1.3–1.9.

water, so that it will be possible to be withdrawn after going down at the bottom of conical glass tube, (ii) good chromatographic behavior, (iii) extraction capability of the interested compounds, (iv) low solubility in water and (v) being miscible with eluting solvent and on the contrary, immiscible with water which makes it to be extremely dispersed in the water system, so that the surface interface is sharply increased leading to gaining high PFs [23]. Carbon disulfide, carbon tetrachloride and tetrachloroethylene were initially tested in order to find the most suitable extracting solvent. For this purpose, a series of sample solutions were studied, using 1.0 mL acetonitrile containing different volumes of the extracting solvent to achieve almost 6.0 μL of the sedimented phase. Based on Fig. 5, tetrachloroethylene presented the highest extraction recoveries in comparison with the other two. For this reason, it was selected as the optimal extracting solvent.

Table 1. Figures of merit of the proposed method for the determination of the MNTs

Analyte	DLR ($\mu\text{g/L}$)	Regression equation	R^2	LOD ($\mu\text{g/L}$)	PF
<i>o</i> -MNT	0.5–500	$Y_a = 61.58X_b - 54.12$	0.9999	0.2	4247
<i>m</i> -MNT	0.5–500	$Y = 74.82X - 59.18$	0.9998	0.2	4570
<i>p</i> -MNT	0.5–500	$Y = 63.42X - 52.45$	0.9998	0.2	4141

a) Peak area.
b) Concentration in $\mu\text{g/L}$.

Table 2. Results obtained for the analysis of the MNTs in three different spiked aquatic real samples

Sample ^{a)}		<i>o</i> -MNT	<i>m</i> -MNT	<i>p</i> -MNT
River water	Recovery (%)	85	96	93
	RSD (%) ($n = 5$)	2.0	4.3	6.2
Well water	Recovery (%)	113	116	110
	RSD (%) ($n = 5$)	4.4	3.7	5.6
Sea water	Recovery (%)	115	112	118
	RSD (%) ($n = 5$)	6.4	6.3	5.9

a) The concentration level of the MNTs added to each sample was 0.75 $\mu\text{g/L}$.

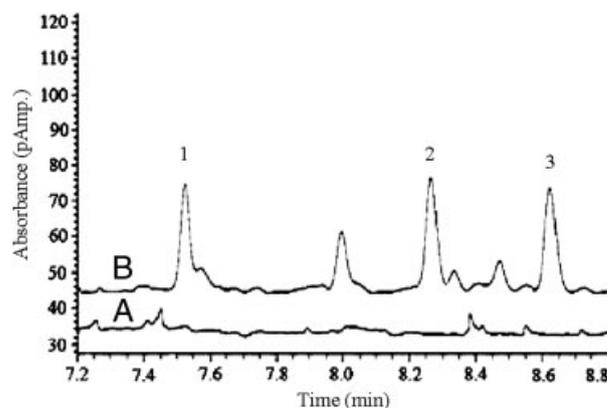


Figure 6. The SPE-DLLME-GC/FID chromatograms of MNTs at the concentration level of 0.75 $\mu\text{g/L}$ in the well water sample before (A) and after spiking (B). Extraction conditions: as with Fig. 1, breakthrough volume: 100 mL. The peak number corresponds to (1) *o*-MNT, (2) *m*-MNT and (3) *p*-MNT.

3.2 Figures of merit

To assess the practical applicability of the SPE-DLLME method, the optimized conditions were adopted for the evaluation of dynamic linear range, correlation of coefficients (r^2), limit of detection (LOD) and PF. For each level, three similar extractions were performed. LOD defined as the analytical signal, which is larger than the blank by multiple 3 of the variation in the blank, was found to be 0.2 $\mu\text{g/L}$ for all analytes. Furthermore, the mean value of PF, at the concentration level of 10 $\mu\text{g/L}$, was found to be in the range of 4141–4570. The mentioned range corresponds to a

Table 3. The comparison of the method (SPE-DLLME/GC-FID) with the other microextraction methods

Ref.	Ex/Det ^{a)}	LOD ($\mu\text{g/L}$)	DLR ($\mu\text{g/L}$)	RSD (%)	Extraction time (min)	Date
This work	SPE-DLLME/GC-FID	0.2	0.5–500	<6.4	~30	2011
[4]	SPME/GC-MS	0.03	20–1000	<3.6	15	2001
[5]	SDME/GC-MS ^{b)}	0.08–0.11	20–1000	<11.3	15	2001
[6]	HSSME/GC-FID ^{c)}	0.02–0.06	1–600	<12.9	15	2007
[7]	HLLME/GC-FID ^{d)}	0.09–0.10	0.5–500	<13.2	≤ 5	2007
[28]	DLLME(CCD ^{e)} /GC/FID	0.09–0.5	0.5–300	<10.1	≤ 5	2009
[29]	DLLME/GC/FID	0.5	1–1000	<9.4	≤ 5	2010

a) Extraction/detection.

b) Single drop microextraction.

c) Head space solvent microextraction.

d) Homogenous liquid–liquid microextraction.

e) Central composite design.

group of measured means ($n = 3$) for all analytes, falling in the above particular range. The results are summarized in Table 1.

3.3 Validation of the method

The developed method was used to determine MNTs concentration in three different spiked aquatic samples. The initial results proved that analyte concentrations in the samples were below the method detection limits. All the real water samples were spiked into with the same concentration level to assess the matrix effects. The results of relative standard deviations (RSDs %) based on five replicate determinations were within the range of 2.0–6.4%, presented in Table 2. Having drawn the calibration curve for the real sample, the sample was then spiked with the analytes to reach the concentration of 0.75 $\mu\text{g/L}$. The resulted SPE/DLLME/GC-FID response was compared with the corresponding concentration response derived by the calibration curve (in terms of percentage recovery). The data demonstrated a good recovery in the range of 85–118% (Table 2).

Figure 6 depicts the SPE-DLLME-GC/FID chromatograms of the MNTs at the concentration level of 0.75 $\mu\text{g/L}$ in the well water sample before and after spiking. As shown in Table 3, comparing the proposed method with other analytical techniques employed for the determination of MNTs in water samples, the present work has got low superiority over the other techniques in terms of RSDs and dynamic linear ranges. But, when it comes to the comparison of the other parameters such as extraction time and LODs, it seems not to be interesting.

4 Concluding remarks

The results from this work show that the SPE-DLLME technique in combination with GC-FID is a valid means of enrichment and quantification of MNTs at trace levels in environmental water samples. The established

procedure demonstrates good sample clean up with high sensitivity and PF. Excellent extraction efficiency is achieved almost independent of the matrix in the actual application. Subsequently, it can be extended to other applications.

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The authors have declared no conflict of interest.

5 References

- [1] Sachdev, A., Todd, J., *J. Loss Prevent Proc.* 2005, 18, 531–536.
- [2] Groggins, P. H., *Unit Processes in Organic Synthesis*, 5th Edn, McGraw Hill Book Company, New York 1958.
- [3] Walsh, M. E., Raney, T. A., Special Report 98-2, US Army Cold Regions Research and Engineering Laboratory, Hanover, NH 1998.
- [4] Psillakis, E., Kalogerakis, N., *J. Chromatogr. A* 2001, 938, 113–120.
- [5] Psillakis, E., Kalogerakis, N., *J. Chromatogr. A* 2001, 907, 211–219.
- [6] Ebrahimzadeh, H., Yamini, Y., Kamarei, F., Khalili-Zanjani, M., *Talanta* 2007, 72, 193–198.
- [7] Ebrahimzadeh, H., Yamini, Y., Kamarei, F., Shariati, S., *Anal. Chim. Acta* 2007, 59, 93–100.
- [8] Saito, Y., Ueta, I., Ogawa, M., Hayashida, M., Jinno, K., *J. Pharm. Biomed. Anal.* 2007, 44, 1–7.
- [9] Ulrich, S., *J. Chromatogr. A* 2000, 902, 167–194.
- [10] Melwanki, M. B., Fuh, M. R., *J. Chromatogr. A* 2008, 1198–1199, 1–6.
- [11] Liu, H., Dasgupta, P. K., *Anal. Chem.* 1996, 68, 1817–1821.
- [12] Makuch, B., Gazda, K., Kamiski, M., *Anal. Chim. Acta* 1993, 284, 53–58.
- [13] Rodriguez, I., Turnes, M. I., Mejuto, M. C., Cela, R., *J. Chromatogr. A* 1996, 721, 297–304.

- [14] Jauregui, O., Moyano, E., Galceran, M. T., *J. Chromatogr. A* 1997, 787, 79–89.
- [15] Llompарт, M., Lourid, M., Landin, P., Garcia-Jares, C., Cela, R., *J. Chromatogr. A* 2002, 963, 137–148.
- [16] Buchholz, K. D., Pawliszyn, J., *Anal. Chem.* 1994, 66, 160–167.
- [17] Arthur, C. L., Pawliszyn, J., *Anal. Chem.* 1990, 62, 2145–2148.
- [18] Jeannot, M. A., Cantwell, F. F., *Anal. Chem.* 1996, 68, 2236–2240.
- [19] Psillakis, E., Kalogerakis, N., *Trends Anal. Chem.* 2002, 21, 54–64.
- [20] Psillakis, E., Kalogerakis, N., *Trends Anal. Chem.* 2003, 22, 565–574.
- [21] He, Y., Lee, H. K., *Anal. Chem.* 1997, 69, 4634–4640.
- [22] Poole, C. F., *Trends Anal. Chem.* 2003, 22, 362–373.
- [23] Rezaee, M., Assadi, Y., Milani Hosseini, M. R., Aghaee, E., Ahmadi, F., Berijani, S., *J. Chromatogr. A* 2006, 1116, 1–9.
- [24] Fattahi, N., Samadi, S., Assadi, Y., Milani Hosseini, M. R., *J. Chromatogr. A* 2007, 1169, 63–69.
- [25] Wu, Q., Wang, C., Liu, Z., Wu, C., Zeng, X., Wen, J., Wang, Z., *J. Chromatogr. A* 2009, 1216, 5504–5510.
- [26] Liu, X., Li, J., Zhao, Z., Zhang, W., Lin, K., Huang, C., Wang, X., *J. Chromatogr. A* 2009, 1216, 2220–2226.
- [27] Montes, R., Rodriguez, I., Ramil, M., Rub, E., Cela, R., *J. Chromatogr. A* 2009, 1216, 5459–5466.
- [28] Ebrahimzadeh, H., Yamini, Y., Kamarei, F., *Talanta* 2009, 79, 1472–1477.
- [29] Sobhi, H. R., Kashtiaray, A., Farahani, H., Javaheri, M., Ganjali, M. R., *J. Hazard. Mater.* 2010, 175, 279–283.