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Khalil Farhadi[1]
Ramin Maleki[1,2]
Raheleh Tahmasebi[1]

[1]Department of Chemistry, Faculty of Science, Urmia University, Urmia, Iran
[2]Research Department of Chromatography, Iranian Academic Center for Education, Culture & Research (ACECR), Urmia Branch, Urmia, Iran

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Research Article

A sol-gel based solid phase microextraction fiber for the analysis of aliphatic alcohols in apple juices

A new fiber based on titania-chitin sol-gel coated on a silver wire for the headspace solid phase microextraction of aliphatic alcohols from apple juice samples was developed. The influences of fiber coating composition and microextraction conditions (extraction temperature, extraction time, and ionic strength of the sample matrix) on the fiber performance were investigated. Also, the influence of temperature and time on desorption of analytes from fiber were studied. Under the optimized conditions, a porous fiber with a high extraction capacity and good thermal stability (up to 250°C) was obtained. The proposed headspace solid-phase microextraction-GC method was successfully used for the analysis of aliphatic alcohols in apple juice and concentrate samples. The recovery values were from 92.8 to 98.6%. The RSD ($n = 5$) for all analytes were below 7.8%.

Keywords: Aliphatic alcohols / Apple juice / GC / Headspace solid phase microextraction / Sol-gel fiber coating
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1 Introduction

Apple juice is one of the most favored juices all over the world because of its unique flavor characteristics. Aliphatic alcohols and esters are the most important components of the aroma profiles of apple juices. In spite of the basic knowledge, provided previously, on variations of the genuine volatiles during apple juice and aroma production [1, 2], information about quantitative analysis of aliphatic alcohols in apple juices is rather scarce.

Headspace solid phase microextraction (HS-SPME) coupled with capillary GC is an attractive alternative to conventional techniques for the determination and identification of aliphatic alcohols from aqueous samples because of its fast, simple, low-cost, solvent-free properties [3]. In SPME, the coating of fiber has an important role in extraction and back extraction efficiency, selectivity, sensitivity, and reproducibility, so that the future developments of SPME will greatly depend on the coating technology. SPME fibers were developed and evaluated for the analysis of aliphatic alcohols based on polyaniline [4], polydimethylsiloxane [5], polyacrylate [6], carbowax-divinylbenzene [7] anodized aluminum wire [8], alumina doped in polyvinyl chloride [9], and polydimethylsiloxane divinylbenzene [10].

Sol-gel technology provides surface coating and also an efficient incorporation of organic components into inorganic polymeric structures in solution under extraordinarily mild thermal conditions. Sol-gel coatings have been used for the preparation of SPME fibers with high thermal stability and solvent stability [11–13] in the development of methodology for volatile and semi-volatile organic compounds analysis [14–18]. Recently, zirconia, alumina and titania based hybrid organic–inorganic sol-gel coatings have been developed for SPME [19–21]. The coatings demonstrated good stability and extraction performance.

Titania has also gained great interest in analytical chemistry because of its high chemical stability, durability, corrosion resistance, non-toxicity, and cost effectiveness. Coatings based on titania sol-gel were developed for capillary microextraction of polyaromatic hydrocarbons [21], phenol derivatives, and aromatic amines [22].

We have recently introduced SPME fibers for the analysis of aromatic hydrocarbons in soil and water samples [23, 24] and alcohols in human body fluids and beer samples [25, 26]. In this work, the preparation of a new SPME fiber with high extraction capacity based on titania sol-gel cross-linked with chitin was described. The efficiency of the proposed fiber was investigated and the developed method was successfully used for the determination of aliphatic alcohols in concentrate and apple juice samples by HS-SPME-GC.

Correspondence: Dr. Khalil Farhadi, Department of Chemistry, Faculty of Science, University of Urmia, Urmia, Iran
E-mail: khalil.farhadi@yahoo.com; kh.farhadi@mail.urmia.ac.ir
Fax: +98-441-3443442

Abbreviations: HS, headspace; **SPME,** solid phase microextraction; **TBOT,** tetrabutylorthotitanat; **TEA,** triethanolamine

2 Materials and methods

2.1 Chemicals and reagents

Methanol, ethanol, 2-propanol, 1-butanol, 2-butanol, 2-pentanol, nitric acid, and tetrabutylorthotitanat (TBOT) were purchased from Merck (Darmstadt, Germany). Triethanolamine (TEA) was prepared from Romil (Cambridge, UK). Chitin was obtained from Sigma (St. Louis, MO, USA); it was ground and sieved to 80–100 mesh followed by purifying with solvent extraction and sonication in *n*-hexane. A stock standard solution of alcohols studied was prepared in water at a concentration level of 100 mg/L. Working standard series (1, 2.5, 5, 10, 50, 100, 250, and 500 μg/L) were prepared daily by diluting the stock solution of each alcohol with double distilled water. The standard solutions were stored in refrigerator at 4°C. Nitrogen and hydrogen (99.999% purity) were from Roham (Middle East Dubai, United Arab Emirates). The apple juice and concentrate samples were provided by Pakdis Fruit Juice Company (Urmia, Iran).

2.2 Apparatus

Separation, detection, and quantitation of volatile organic compounds after HS-SPME were performed with a 6890 N GC–FID (Agilent Technologies, Wilmington, DE, USA) fitted with a J&W DB-Wax capillary column (30 m × 0.25 mm id, 0.25 μm film thickness), a split–splitless injector with Agilent tapered liner (4 mm id) and flame ionization detector. The initial column temperature was maintained at 40°C for 3 min and then raised at 15°C/min to 140°C and held for 2 min. Nitrogen was used as carrier and makeup gas, at flow rates of 1.0 and 45 mL/min, respectively. The injector and detector temperature were held at 200 and 250°C, respectively. ChemStation software was used for online data collection and processing. A Leo 440i scanning electron microscope (Leo Electron Microscopy, Cambridge, England, UK) was used for the investigation of the fiber surface. A laboratory-made SPME device was used in all experiments. Injections were done in splitless mode.

2.3 Preparation of SPME fiber

TBOT as initial alkoxide, TEA as stabilizer, nitric acid as acid catalyst, 2-propanol as solvent, and chitin as binder were utilized for the preparation of titania sol-gel. It was prepared according to the following procedure:

Aliquots of 250 μL of TBOT were dissolved in 0.5 mL of 2-propanol. This solution was slowly added to a solution containing a mixture of 0.5 mL of 2-propanol and 0.5 mL deionized water under vigorous stirring in a Teflon beaker for 10 min. To this mixture, 250 μL of TEA as stabilizer and 50 mg of chitin as binder were added and the pH of the mixture were adjusted at 2 using sufficient amount of 1.0 mol/L nitric acid. The mixture was stirred at 3000 rpm for about 2 h to obtain a viscose suspension at 25 ± 1°C.

A 1 cm length of silver wire (total length 2 cm) was placed in the laboratory-made SPME device and after washing with acetone, it was dipped vertically into the sol-gel solution for 1 min and dried under nitrogen gas. For each fiber, this coating process was repeated several times until the desired thickness (about 20 μm) of the coating was obtained. The proposed SPME fiber was then conditioned at 250°C for 8 h to remove any fiber contaminations.

2.4 HS-SPME of aliphatic alcohols

The Brix degree of apple juice and concentrate samples were adjusted to 11.2 before analysis. Aliquots of 20 mL of apple juice sample were placed in a 25 mL vial containing 1.5 g NaCl, then the vial sealed with a silicone septum and the vial contents were magnetically stirred at 600 rpm with 10 mm × 4 mm magnetic stir bars. SPME from HS of the sample was carried out at 50 ± 1°C. After 5 min, the SPME fiber was removed from the vial and for thermal de-sorption it was immediately inserted into the hot injection port of GC in splitless mode and stayed for 10 s. The standard addition method was used for quantification of aliphatic alcohols in apple juices.

3 Results and discussion

In our previous works, we introduced a titania sol-gel modified with PEG (6000)-TEA coated on anodized aluminum support as a new SPME fiber for the extraction of BTEX compounds [24]. In the next work, we used polymethylmethacrylate as a new binder in the construction of a titania sol-gel coated SPME fiber for HS-SPME of polar compounds such as alcohols in non-alcoholic beer samples [26]. In the present work, we focused our interest on the effect of other modifiers such as chitin on the behavior of titania sol-gel for the extraction of polar molecules. Therefore, the ability of the proposed sol-gel fiber coating was studied in extraction of aliphatic alcohols from apple juices. During the HS-SPME procedure, distribution and adsorption equilibrium of the analytes must be established between the aqueous and gaseous phase, and between the gaseous and solid phase. The equilibrium is affected by various factors, such as the chemical nature of analytes and fiber, the extraction time, sample volume, agitation speed, and the extraction time. The optimal experimental conditions should be investigated for each compound and for the fiber selected.

3.1 Optimization of coating composition

The effect of coating composition on extraction efficiency of aliphatic alcohols was investigated. For this purpose, fibers with various compositions of coating material (weight

percent) were prepared, and extraction of alcohols from HS of apple juice samples was performed (Fig. 1). The obtained results show that, among four prepared compositions, fiber no. 1 (89.75% TBOT, 10.25% chitin) has the maximum extraction efficiency in comparison to the other compositions. It should be mentioned that the fiber constructed with TBOT and without chitin (fiber no. 4) has a minimum extraction so that the use of chitin in preparation of titania sol-gel fiber coating increases the fiber efficiency in HS-SPME of aliphatic alcohols. The micrograph of the sol-gel fiber no. 1 (Fig. 2) clearly shows the porous structure of the sol-gel coating. A high surface area will be able to provide large stationary phase loading and therefore, high extraction capacity. So, fiber no. 1 was selected as optimum fiber coating composition.

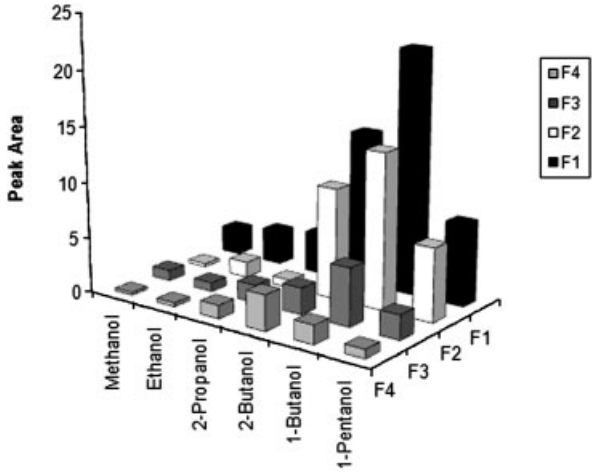

Figure 1. Effect of fiber coating composition (w/w%) on alcohols microextraction (fiber 1, 89.75% TBOT, 10.25% chitin; fiber 2, 83.60% TBOT, 16.40% chitin; fiber 3, 93.85% TBOT, 6.15% chitin; fiber 4, 100% TBOT).

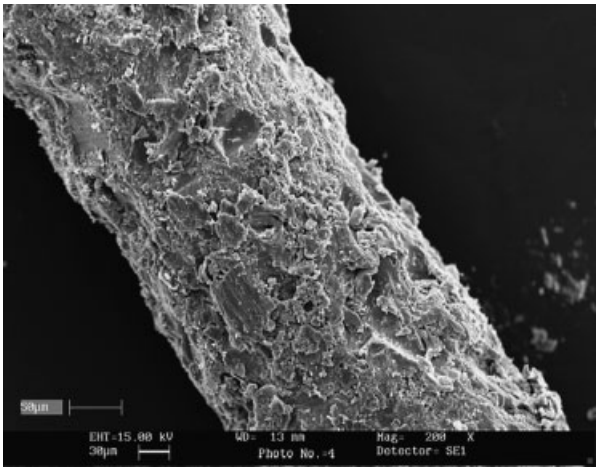

Figure 2. Scanning electron micrograph of the sol-gel based fiber (fiber 1, 89.75% TBOT, 10.25% chitin).

3.2 Optimization of the microextraction temperature

In HS-SPME for quantitative extraction of analytes, optimization of the extraction temperature is necessary. For this purpose, the extraction of alcohols was performed from 20 mL of apple juice sample at various temperatures (25–70°C). The obtained results are presented in Fig. 3. As can be seen, temperature has dual effect, the use of high temperature is suitable for increasing volatility of analytes and establishing the analytes distribution equilibrium between the sample solution and gaseous phase but adsorption of the analytes on the fiber is less favored at high temperatures. Therefore, 50°C was selected as a suitable experimental temperature in further studies.

3.3 Optimization of microextraction time

Exposure time of the fiber in gaseous samples is an important parameter in achieving distribution equilibrium of analytes between fiber and sample; it is a decisive factor for improving the extraction efficiency. Therefore, the proposed extraction procedure was carried out at different times, ranging from 1 to 10 min. As shown in Fig. 4, at 50°C, the amount of adsorbed analytes reaches a maximum level within 5 min and then it stays constant. Thus, in further extractions the fiber was exposed to HS of sample for 5 min.

3.4 Optimization of desorption temperature and time

In order to find the optimum desorption temperature, injections were carried out at various temperatures ranging from 170 to 220°C. As can be seen from Fig. 5, all of the analytes are completely desorbed from the fiber at 200°C, so it was selected as optimum desorption temperature. The desorption time profile is illustrated in Fig. 6.

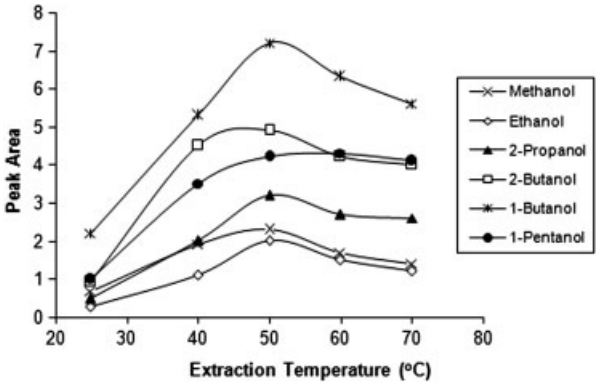

Figure 3. Effect of temperature on microextraction of alcohols (microextraction time, 5 min).

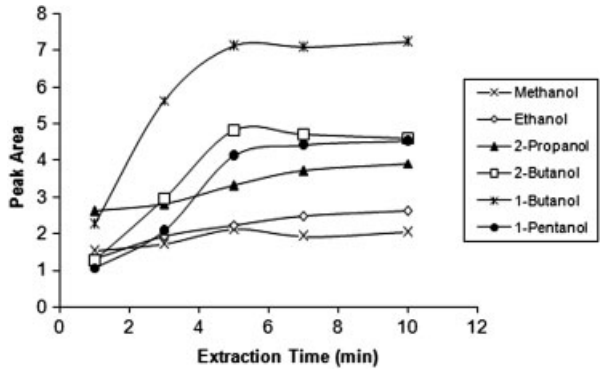

Figure 4. Effect of time on microextraction of aliphatic alcohols (microextraction temperature, 50°C).

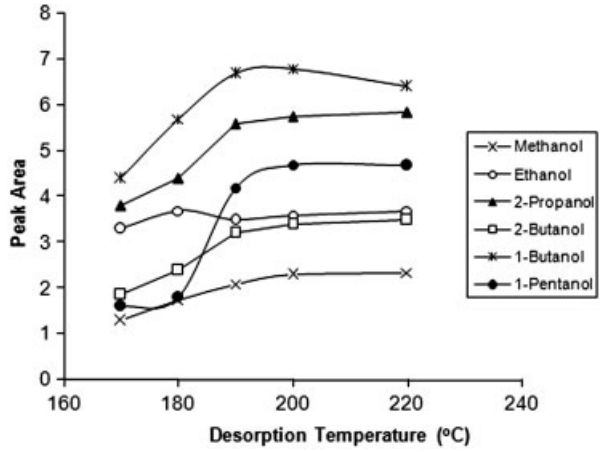

Figure 5. Effect of desorption temperature on desorption of alcohols from fiber.

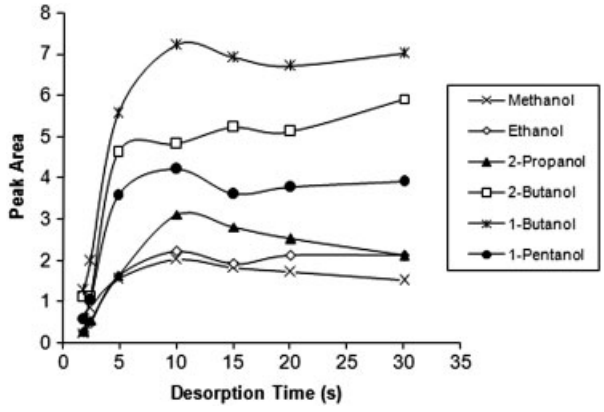

Figure 6. Effect of time on desorption of alcohols from the fiber at 200°C.

All analytes that were adsorbed by the porous layer diffuse rapidly from fiber into carrier gas. As Fig. 6 shows, the time required to complete desorption process is less than 10 s for all analytes. Such short desorption equilibration times arise from the porous structure of the sol-gel TBOT-chitin fiber and result in a short analysis time as well as sharp chromatographic peaks.

3.5 Effect of salt

In order to study the salt effect on HS-SPME efficiency, the extraction was performed in the presence of different concentrations of NaCl (0–10% w/v). The results demonstrated that the highest extraction efficiency was reached by adjusting the NaCl concentration at 6% w/v. The salt addition represents a crucial parameter because the water solubility of aliphatic alcohols is reduced by the presence of NaCl in aqueous sample solution. Ions of the dissolved salt attract and hold water molecules, and thus make them less free to interact with the alcohols.

3.6 Study of reproducibility

To assess the repeatability of the method, five replicate determinations have been carried out using a single fiber and the RSD% were calculated. The RSD values were less than 7.8% for all alcohols, which indicates that the proposed method is repeatable. Inter-day precision was determined by analysis of similar standards on three different days over a period of 1 wk and the RSD values for the studied alcohols were in the range of 5.7–8.2%. Also, reproducibility studies were performed on three different fibers, and the fiber to fiber RSD values were lower than 9.5% for all compounds.

3.7 Quantitative characteristics of the proposed method

The quantitative characteristics of the proposed method such as calibration curve equations, correlation coefficients, method linearity, and LODs were studied. The linearity is ideal in the range: 1.5–450 μg/L for methanol, 0.3–300 μg/L for ethanol, 0.3–450 μg/L for 2-propanol, 0.3–400 μg/L for 2-butanol, 0.5–450 μg/L for 1-butanol and 1.5–450 μg/L for 1-pentanol. For these compounds, LOD values calculated based on $S/N = 3$ are between 0.3 and 1.5 μg/L. To demonstrate the suitability of proposed method for the quantitative analysis of alcohols, we studied the recovery of alcohols from apple juice and concentrate samples matrix (Table 1). The recovery values were in the range of 92.8–98.6% for all of the studied alcohols, which indicates that the matrix effect is negligible.

The accuracy of the method has also been checked; the mean value of the three replicate analyses can be seen in Table 1. No significant difference was obtained between the added standard concentration values (10.0 μg/L) and the SPME results.

Analysis of aliphatic alcohols in apple juice and concentrate samples was carried out using the standard

Table 1. Results obtained from the analysis of aliphatic alcohols in apple juice samples and recovery studies

Analytes	Apple juice X ± SD[a)] (μg/L)	RSD (%)	Added in apple juice (μg/L)	Found (μg/L)[a)]	Recovery (%)
Methanol	2.5 ± 0.2	7.8	10.0	11.9	92.8
Ethanol	3.4 ± 0.2	5.9	10.0	12.8	94.1
2-Propanol	3.7 ± 0.2	5.4	10.0	13.0	93.5
2-Butanol	6.9 ± 0.5	7.3	10.0	15.9	93.0
1-Butanol	19.5 ± 1.1	5.6	10.0	29.8	98.6
1-Pentanol	11.4 ± 0.7	6.1	10.0	21.1	95.7

a) Three-replicate determination.

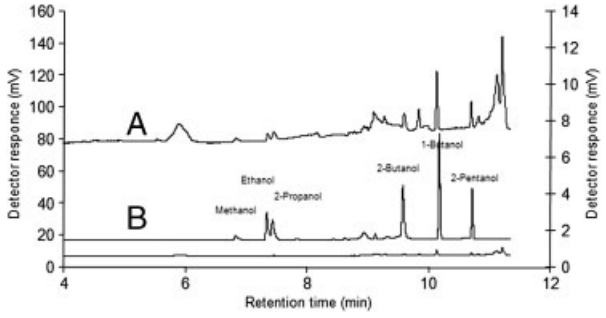

Figure 7. Chromatograms obtained from apple juice sample (A) and standard sample solution (B) through HS-SPME-FID analysis.

addition method followed by the proposed HS-SPME-GC procedure. A typical chromatogram is shown in Fig. 7.

4 Concluding remarks

This study presents a new sol-gel coated SPME fiber based on inorganic polymeric matrix (titania sol and chitin), which is coated on silver wire and reports its application to the HS-SPME of aliphatic alcohols from apple juice samples prior to capillary GC analysis. The porous structure of sol-gel coating increases the surface area on the fiber, speed of extraction and desorption steps, and sample capacity. Wide linear ranges, low detection limits (0.3–1.5 μg/L, $S/N = 3$), fast sampling, and good mechanical stability (used for more than 100 samplings) are the unique characteristics of the proposed SPME fiber.

The authors have declared no conflict of interest.

5 References

[1] Kato, T., Shimoda, M., Suzuki, J., Kawaraya, A., Igura, N., Hayakawa, I., *Food Res. Int.* 2003, *36*, 777–785.

[2] Elss, S., Preston, C., Appel, M., Heckel, F., Schreier, P., *Food Chem.* 2006, *98*, 269–276.

[3] Pawliszyn, J., *Application of Solid Phase Microextraction*, R.S.C. Chromatography Monograph, Cambridge 1999.

[4] Djozan, Dj., Bahar, S., *Chromatographia* 2004, *59*, 95–99.

[5] Tankeviciute, A., Panavaite, D., Kazlauskas, R., Vickackaite, V., *Chemija* 2004, *15*, 39–44.

[6] Fitzgerald, G., James, K. J., Macnamara, K., Stack, M. A., *J. Chromatogr. A* 2000, *896*, 351–359.

[7] Tankeviciute, A., Panavaite, D., Kazlauskas, R., Vickackaite, V., *Chemija* 2003, *14*, 207–211.

[8] Djozan, Dj., Assadi, Y., Hosseinzadeh-Haddadi, S., *Anal. Chem.* 2001, *73*, 4054–4058.

[9] Farajzadeh, M. A., Rahmani, N. A., *Anal. Sci.* 2004, *20*, 1359–1362.

[10] Yang, C., Wang, Y., Liang, Z., Fan, P., Wu, B., Yang, L., Wang, Y., Li, S., *Food Chem.* 2009, *114*, 1106–1114.

[11] Li, X. J., Zeng, Z. R., Gao, S. Z., Li, H. B., *J. Chromatogr. A* 2004, *1023*, 15–25.

[12] Li, X. J., Zeng, Z. R., Zhou, J. J., Gong, S. L., Wang, W., Chen, Y. Y., *J. Chromatogr. A* 2004, *1041*, 1–9.

[13] Li, X. J., Gong, S. L., Zeng, Z. R., *Chromatographia* 2005, *62*, 519–525.

[14] Liu, M., Zeng, Z., Lei, Y., Li, H., *J. Sep. Sci.* 2005, *28*, 2306–2318.

[15] Bagheri, H., Aghakhani, A., Es-haghi, A., *Chromatographia* 2007, *66*, 779–783.

[16] Azenha, M., Malheiro, C., Silva, A. F., *J. Chromatogr. A* 2005, *1069*, 163–172.

[17] Zuin, V. G., Lopes, A. L., Yariwake, J. H., Agusto, F., *J. Chromatogr. A* 2004, *1056*, 21–26.

[18] Wang, D., Xing, J., Peng, J., Wu, C., *J. Chromatogr. A* 2003, *1005*, 1–12.

[19] Alhooshani, K., Kim, T. Y., Kabir, A., Malik, A., *J. Chromatogr. A* 2004, *1062*, 1–14.

[20] Liu, M. M., Liu, Y., Zeng, Z. R., Peng, T. Y., *J. Chromatogr. A* 2006, *1108*, 149–157.

[21] Li, X., Gao, J., Zeng, Z., *Anal. Chim. Acta* 2007, *590*, 26–32.

[22] Kim, T. Y., Alhooshani, K., Kabir, A., Fries, D. P., Malik, A., *J. Chromatogr. A* 2004, *1047*, 165–174.

[23] Farhadi, K., Mamaghanian, M., Maleki, R., *J. Hazard. Mat.* 2008, *152*, 677–682.

[24] Farhadi, K., Tahmasebi, R., Maleki, R., *Talanta* 2009, *77*, 1285–1289.

[25] Maleki, R., Farhadi, K., Matin, A. A., *Anal. Sci.* 2006, *22*, 1253–1255.

[26] Maleki, R., Farhadi, K., Tahmasebi, R., *Chromatographia* 2009, *69*, 775–778.

