SCIENTIFIC PAPERS
OF THE UNIVERSITY OF PARDUBICE
Series A
Faculty of Chemical Technology
8 (2002)

APPLICATION OF PRESSURISED FLUID EXTRACTION TO SAMPLE PREPARATION

Martin ADAM, Karel VENTURA[1] and Aleš EISNER
Department of Analytical Chemistry, The University of Pardubice,
CZ–532 10 Pardubice

Received September 30, 2002

The article is focused on the pressurised fluid extraction - PFE technique using of a onePSE device and its application to isolation of various organic compounds from solid samples. In this paper optimisation procedure for individual analytes is described and discussed. Applicability of PFE is tested on isolation procedures of phthalates, PAHs and TPHs from environmental materials (soils, river sediments), and the additives in smokeless gunpowders.

Introduction

The preparation of a sample represents the most time-consuming and the most complicated step in analysis of solid samples. In general, sample preparation is being considered as an operation causing the most serious errors in the whole analytical procedure. An ideal method for extracting solid samples should be rapid, simple, inexpensive and providing the quantitative yield of the analytes

[1] To whom correspondence should be addressed.

without their loss or unwanted disintegration or degradation [1].

Classical procedures for extraction of solid matrices by liquid solvents do not meet the above-mentioned criteria. For instance, extraction time is typically counted in hours or even days and the resultant extracts are diluted requiring thus re-concentrating. Moreover, there is a necessity to use a relatively large amount of solvents of high purity that are expensive and usually considerably harmful [2].

In the recent years, some attempts have been made to replace traditional extraction techniques by new ones that would allow lowering of the consumption of extraction agents and reduction of extraction time. Some of them were merely adapted classical procedures others, however, brought a novelty to the field when being based on new technologies [3].

Pressurised fluid extraction (PFE) appears to be a technique perspective for isolating organic compounds from solid matrices. Its principles [4] are based on the solid/liquid extraction system when the whole process takes place under conditions of increased temperature (up to 200 °C) and pressure (up to 20 MPa) and within an acceptably short time interval (max. 20 min). Due to this, the extraction rate is considerably accelerated and, because of the increased pressure, the whole operation always proceeds in liquid solvent when temperatures are set above the atmospheric boiling point of the extracting solvent used. In contrast to classical methods employing Soxhlet extractor, one can choose freely any mixture of mutually miscible solvents thanks to operational conditions in liquid phase. Shortly speaking, PFE method permits to extract a wider spectrum of compounds compared to that extractable in Soxhlet extractor [5].

Since, originally, the ASE (Accelerated Solvent Extraction) is a trademark registered for Dionex Corporation, it is possible to find out that literature sources quote this technique under various names: Fast Extraction, Pressurised Liquid Extraction, Pressurised Fluid Extraction, Pressurised Solvent Extraction, Enhanced Solvent Extraction or High-Pressure Solvent Extraction [3].

There are two main reasons for highly effective extractions with liquids at increased temperatures and pressures. It is a solubility effect connected with mass transport processes and displacements (alterations) in the surface equilibria.

PFE methods can be used to extract solid samples and are especially effective for dry materials with very small particles. Using PFE, semivolatile basic, neutral and acidic compounds (BNA) can be extracted as well as some organophosphate and chlorinated pesticides and herbicides, PAHs and PCBs from soils, clays, sediments and sludge [3]. Furthermore, PFE is suitable also for additives in explosives [6] and in polymers, fats in foodstuff, active formula in pharmaceuticals or as a useful tool for investigating soil contaminated with crude oil [7].

Another promising technique for isolating organic compounds from solid matrices is supercritical fluid extraction (SFE), which is an extraction technique based on specific properties of a solvents above the critical point. The physical

properties of a supercritical fluids are favourable factors for the separation of substances from solid samples. The most frequently used fluid for SFE is carbon dioxide. Details about comparison of PFE and SFE can be found in the literature [5,8].

Experimental

Instrumentation

The PFE extractions were carried out using of a *one*PSE apparatus (Applied Separations, USA). This extractor allows operating at temperatures from 50 to 150 °C, at a pressure up to 15 MPa being limited by construction of switching multi-channel valve.

Quantitative determination of crude-oil contamination (total petroleum hydrocarbons, TPH) was performed by infrared spectroscopic method using an Equinox 55 (Bruker Analytische Messtechnik, Ltd., Germany). This apparatus operates in Fourier transformation mode and is capable of measuring the spectra in near (NIR) and medium (MIR) infrared region.

The extracts obtained from smokeless gunpowders were analysed using HPLC. For separation, a column with Biospher SI C18 filling (7 μm, 250 × 4 mm) and a Separon SGX C18 protective pre-column (7 μm, 30 × 3.3 mm; all products of Tessek, Prague, Czech Republic) were used. Into column, the sampling was performed through a valve with a 20 μl outer loop. Mobile phase adjusted at a flowrate of 0.8 ml min^{-1} was delivered by a LCP 4000 high-pressure pump. The mobile phase gradient was formed by a GP 5 programming unit. A LCD 2084 UV detector (all purchased from Ecom s.r.o., Prague, Czech Republic) completed the device employed.

Based on UV spectra measured with a GBC 916 (GBC Scientific Equipment, Australia) apparatus, the wavelength for UV detection in HPLC analysis was optimised. The GBC 916 measures the spectra in the range from 190 nm to 1000 nm.

Soil and river sediment extracts (for the determination of phthalate esters and polycyclic aromatic hydrocarbons - PAHs) were analysed by a GC 17A gas chromatograph with a QP 5050A mass detector (both Shimadzu, Japan) and a Combi Pal autosampler (CTC Analytics, Switzerland). For GC-MS measurements, a standard configuration consisted of an Ultra 2 fused silica capillary column (phenylmethylsilicone (5 %), length: 25 m, i.d.: 0.2 mm, film thickness: 0.11 μm; Hewlett-Packard), GC/MS interface, and electron ionisation source.

Samples and Reagents

The samples of soils and river sediments for determination of phthalates were collected in various localities in the vicinity of factories producing PVC near Napajedla and Lomnice nad Popelkou (Czech Republic). Samples for determining the contamination background were taken near the hydrometeorologic station in Košetice (region Pelhřimov, Czech Republic).

The soils were sampled in a manner characterising the sampling locality and care was taken to collect a material free of grass and of possible manipulation in the recent times. Only the surface layer was sampled and always in a dry period when, at least, three previous days were without rain. The samples quantity was from 0.5 to 1.0 kg

River sediments were collected approx. 1.5 – 2 m off the banks in a mild flowing stream; the sample volume of each sample being ca 700 –1000 ml.

Three samples of soils for the determination of TPH were collected in the areas of crude-oil centre in a region of South Moravia

- Sample A — its matrix was formed by a sandy soil. The major component of this material can be characterised by grain diameter of the order 10^{-4} – 10^{-3} m.
- Sample B — taken from a depth of 3 m beneath the land and being similar to the sample A, however, formed by smaller grains.
- Sample C — a mixed sample material macroscopically reminding clay. This material was sampled from a heap of contaminated soil obtained from different places and diverse depths.

Five samples of river sediment for determination of PAHs were collected from the bed of Elbe river in unspecified localities. In these samples, both fluoranthene and pyrene were extracted and analysed as their content was known from a certificate of Bioanalytika CZ, s.r.o., Czech Republic.

The samples of smokeless gunpowders were obtained from Synthesia (Pardubice, Czech Republic) under denotation as R-5027 and LOVEX S-062. Both are single-base powders produced in a form of small cylindrical particles whose surface is modified by grafitisation. As additives, the R-5027 contains dinitrotoluenes (DNT) and diphenylamine (DPA) with a declared content of 4.46 % DNT and 1.17 % DPA, respectively. LOVEX S-062 was declared to contain 0.98 % diphenylamine (DPA) and 3.03 % Centralite I (CI I).

PFE Conditions

After pre-treatment, the weighted amount of sample was placed into extraction cell with a portion of glass wool on the bottom. Next portion of wool was laid on the surface of the sample and the remaining volume of the cell was filled up with glass

balls. After inserting so prepared cell into extractor heating oven, the desired value of both temperature and pressure were adjusted by means of extraction solvent.

Two extraction steps were performed when the extract was being entrapped into the same collecting vessel. The conditions for extraction of the individual samples were as follows

- Samples for the determination of phthalates: Analysed material was dried at 60 °C and passed through a sieve with a mesh of 2 mm; a weighted amount of 5 g was extracted at 120 °C and 15 MPa into *n*-hexane for 10 + 5 min.
- Samples for the determination of TPH: Weighted amount of 0.5 g underwent extraction at 100 °C and 10 MPa in tetrachloromethane for 10 + 5 min.
- Samples for the determination of PAH: Weighted amount of 1 g was extracted at 120 °C and 15 MPa in dichloromethane for 5 + 5 min.
- Smokeless gunpowders: Sample of R-5027 ground to ca 1 mm particles, LOVEX S-062 taken without any pretreatment; weighted 1 g of each then extracted at 120 °C and 10 MPa in methanol for 5 + 5 min.

Analysis of Standards and Extracts

FTIR analysis: All solutions to be analysed were measured in a quartz cuvette (optical length: 1 cm) versus tetrachloromethane as a background. Quantification was then performed by the calibration-curve method when evaluating the spectrum within a region of 3100 – 2700 cm^{-1}. NM4 diesel fuel was selected as a standard being available.

UV analysis of standards: In order to find out suitable wave-lengths for UV-detected HPLC analysis of gunpowders, the standards of 2,6- a 2,4-dinitrotoluene, nitrosodiphenylamine, diphenylamine and centralite I were selected. Methanolic solutions of the individual standards were measured in a range of 200 – 400 nm in a quartz cell (1 cm) versus pure solvent.

HPLC analysis: By employing a HPLC method with UV detection and gradient elution, extracts of gunpowders were analysed. To perform analysis under gradient condition, pure methanol represented solution A and 30 % CH_3OH in water was then solution B. The gradient programme was as follows. Initial composition of the mobile phase: 100 % of A kept constant for 3 min, a linear gradient was used for 8 min until the concentration had attained 29 % A and 71 % B. Then, this composition was kept constant for 10 min, follow again by linear gradient for 12 min and finished at the moment when the mobile phase composition was 5 % A and 95 % B. Flow-rate of the mobile phase was set at 0.8 ml min^{-1}.

To analyse LOVEX S-062 extract, UV detection was initiated at 280 nm for 14 min; then, the wave-length was changed by a programme to 247 nm and prior to the end of the measurement, the wave-length was set back to 280 nm. For R-

5027 extracts, the wave-length was kept constant at 280 nm. By evaluating the corresponding peak areas, the determination of target compounds was carried out again by means of calibration curves. Sample R-5027 was analysed for the content of dinitrotoluenes (DNT) and of diphenylamine (DPA), sample LOVEX S-062 was analysed for the content of Centralite I (CI I) and of diphenylamine (DPA).

GC/MS analysis: Extracts of samples analysed for phthalates and PAHs were subjected to gas chromatography with mass detection. The extracts were concentrated to a volume of 0.5 ml in helium stream, transferred to vessel for automated sampler and diluted to 1 ml. For the proper GC/MS analysis, a DB 5 capillary column (30 m × 0.25 mm; 0.25 μm film, J & W Scientific, Folsom, USA) was chosen. Helium of high purity was used as a carrier gas.

The temperature programme for the determination of phthalates by GC/MS was: initial temperature at 200 °C kept for 2 min and changed with a gradient of 40 °C min^{-1} up to 330 °C; temperature of sampler: 185 °C, temperature of detector: 185 °C; mobile phase flow-rate: 0.6 ml min^{-1}. For analysis, 2 μm of extracts were sampled being reduced in a splitter at a ratio of 1:50. Among phthalates, diethylphthalate (DEP), dibutylphthalate (DBP), benzylbutylphthalate (BBP) and bis(2-ethyl-hexyl)phthalate (2-EHP) were sought. However, only DBP a 2-EHP were found and determined in all samples being analysed.

In the case of analysis of PAHs, the column was pre-heated at a constant temperature of 230 °C, temperature of sampler: 220 °C, temperature of detector: 230 °C; flow-rate of mobile phase: 0.6 ml min^{-1}. For analysis, 1 μl of extract was sampled and reduced in the splitter to 1:100.

GC/MS analysis of substances of interest was carried out in a SIM mode when a mass ratio with m/z = 149 was used for detecting phthalates, m/z = 202 for pyrene plus fluoroanthene, and m/z = 153 for acenaphthene. The quantity of the individual components was evaluated again from the peak areas using the inert standard method.

Results and Discussion

Prior to use PFE for routine analysis of a series of samples, it is necessary to optimise the individual extraction parameters such as the amount of a sample, its pretreatment before the extraction proper, the solvent or a mixture of solvents, temperature, pressure, number of extraction steps, and the way of analysing the extracts, inclusive of their treatment.

Suitable analytical methods have been selected in an effort to meet desired criteria for the determination of compounds of interest and, at the same, to have them as simple as possible. Therefore, it had to be taken into account whether the results reflect only the total content of substances in a wide group (e.g. TPHs) or the sample contains the individual predicted compouds (e.g. phthalates). Based

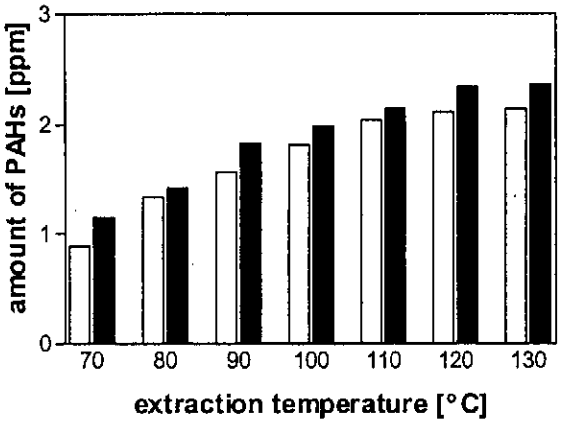

Fig. 1 Optimisation of extraction temperature for determination of PAHs: ■ – fluoranthene: □ – pyrene

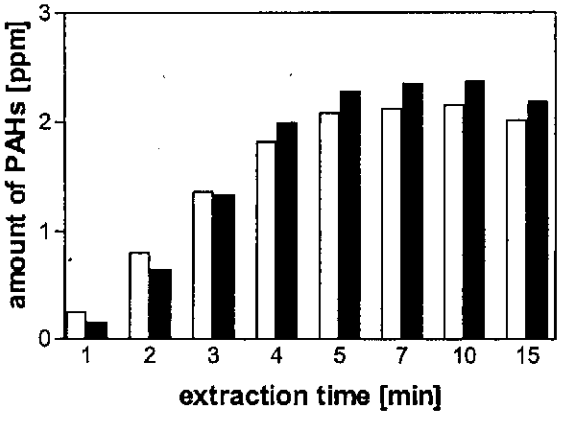

Fig. 2 Optimisation of extraction time for determination of PAHs: ■ – fluoranthene: □ – pyrene

on these assumptions, the individual analytical methods were selected as those listed in Experimental together with their experimental characteristics.

The amount of a sample was pre-determined by the performance of methods selected for analysis of the extracts, i.e., mainly by their sensitivity and selectivity. Important factors were, of course, the representativeness and a sufficient reproducibility of the results. Respecting this, the individual samples were weighed in a quantity to meet the above-mentioned criteria and all of them are declared in Experimental.

For extracting the individual materials, the choice of a solvent was in accordance with that used in Soxhlet apparatus [9]. Furthermore, some mixed solvents were tested as well to improve the performance of the process. Beside a maximal recovery of extraction, a factor co-determining the choice of a solvent was also its selectivity to the analyte of interest, i.e., minimisation of co-extracting other species along with.

Temperature was optimised always in intervals from 70 °C upwards by choosing the consecutive steps of 10 °C. The minimum temperature beyond which no evident improvement of extraction had been ascertained was taken as the value of choice. As an illustration, the optimisation of extraction temperature for determination of PAHs is shown in Fig. 1.

With respect to the fact that, in principle, there is no direct effect of the pressure upon the extraction process (a certain pressure is however necessary to keep the solvent in liquid state), its value was not optimised, but always chosen in accordance with the previous experience.

The time necessary for reaching the equilibrium (one step period) as well as the minimum number of steps for completing the quantitativeness of the extraction process were investigated.

Regarding the first one, the establishment of equilibria was tested on a series of five extractions lasting from 1 to 20 min. The time period for one step was then evaluated graphically by means of a dependence between the recovery of extraction and its time. A time period whose prolonging did not lead to any improvement in the recovery was chosen as the optimum. As an illustration, the optimisation of extraction time for determination of PAHs is shown in Fig. 2.

The optimum number of extraction steps was evaluated next. By measuring a series of five types of extractions, with the same sample amount, three consecutive extractions (i.e. steps) were performed. Each extract was evaluated separately. In all the cases, it was found out that the results of two-step and three-step extractions differs only very slightly. Hence, based on this, two-step extractions were considered as the optimum from economical point of view. An addition of further steps would not improve the performance very significantly whereas the consumption of time, energy and chemicals would rise dramatically.

After having found out the optimal conditions for extraction of all the samples, PFE technique was applied to their extraction and previously described analytical methods for their analysis. Tables I to IV below summarize the individual results of analyses performed after the corresponding extractions. Typical GC chromatogram of extract from soil contamined with phthalates is shown in Fig. 3.

From the results presented in Tables I to IV is evident that all results obtained by pressurised fluid extraction are in good accordance with those accomplished by using classical extraction techniques (Table II) or with values to be declared (Tables III and IV).

Table I Environmental specimens for the determination of phthalates[a]

Content of phthalates[b], ppm			Content of phthalates[b], ppm		
Sample	DBP	2-EHP	Sample	DBP	2-EHP
N1-soil	0.63 ± 0.02	1.08 ± 0.08	LP1-soil	0.37 ± 0.01	0.74 ± 0.05
N2-soil	0.29 ± 0.01	0.65 ± 0.09	LP2-soil	0.31 ± 0.01	0.72 ± 0.06
N1-sediment	0.43 ± 0.01	1.34 ± 0.10	LP1-sediment	0.84 ± 0.02	7.92 ± 0.59
N2-sediment	0.30 ± 0.01	0.63 ± 0.04	LP3-sedimnt	0.48 ± 0.01	8.48 ± 0.64
blanc0	0.06 ± 0.00	0.17 ± 0.01	-	-	-

[a] 5 g of sample, 120 °C, 10 MPa, 10 + 5 min, *n*-hexane, GC/MS analysis, $n = 10$
[b] calculated for the weighted amount of dry sample

Table II Soil samples for the determination of TPHs

Sample of soil	THP content, g kg^{-1}		
	PSE[a]	SFE[b]	Soxhlet extraction[c]
Sample A	36.09 ± 1.48	33.40 ± 1.27	32.80 ± 0.59
Sample B	34.37 ± 1.41	31.93 ± 1.21	32.45 ± 0.58
Sample C	33.85 ± 1.39	31.02 ± 1.18	31.83 ± 0.57

[a] 0.5 g of sample, 100 °C, 10 MPa, 5 + 5 min, tetrachloromethane, FTIR analysis, $n = 10$
[b] 0.2 g of sample, 100 °C, 40 MPa, static mode: 5 min, dynamic mode: 25 min, pure CO_2, FTIR analysis, $n = 10$
[c] 1 g of sample, 12 hours, tetrachloromethane, FTIR analysis, $n = 5$

Table III River sediments for the determination of PAHs

Sample of sediment	Content of PAHs, ppm			
	PSE[a]		Certified	
	fluoranthene	pyrene	fluoranthene	pyrene
3A	2.37 ± 0.12	2.15 ± 0.08	2.12	1.76
9A	1.91 ± 0.09	1.96 ± 0.12	2.46	2
14A	2.47 ± 0.11	2.15 ± 0.11	2	1.8
17A	2.11 ± 0.10	1.91 ± 0.08	1.88	1.74
33A	2.42 ± 0.13	2.48 ± 0.14	2.05	1.91

[a] 1 g of sample, 120 °C, 15 MPa, 5 + 5 min, dichloromethane, GC/MS analysis, $n = 10$

Table IV Smokeless gunpowders

Sample	Compound	Content of additive, %		
		PSE[a]	SFE[b]	Declared
R-5027	DNT	4.05 ± 0.17	4.32 ± 0.12	4.46
	DPA	1.05 ± 0.09	1.14 ± 0.02	1.17
LOVEX S-062	CII	3.08 ± 0.06	2.99 ± 0.05	3.03
	DPA	0.98 ± 0.06	1.06 ± 0.02	0.98

[a] 0.5 g of sample, 100 °C, 10 MPa, 5 + 5 min, tetrachloromethane, HPLC analysis, n = 10
[b] 0.2 g of sample, 100 °C, 40 MPa, static mode: 10 min, dynamic mode: 50 min, CO_2 modified with acetonitrile, addition of 1,6-hexandiamine into cell, HPLC analysis, n = 10

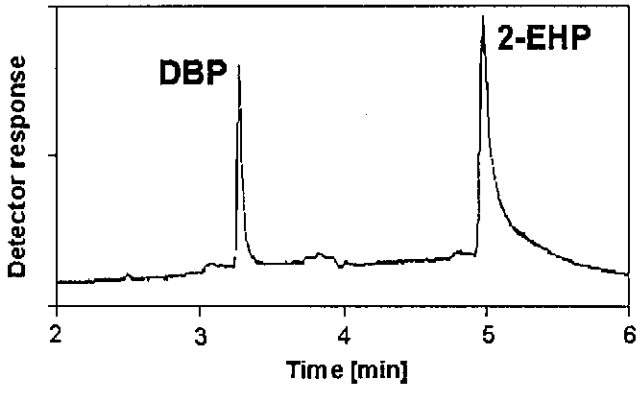

Fig. 3 Typical GC chromatogram of PFE extract from soil contaminated with phthalates

Conclusion

The work has been focused on the development of methods of pressurised fluid extraction using a *one*PSE device and its applicability to the above-mentioned extraction technique for isolation of organic compounds from various types of solid samples.

Applicability of PFE has been tested on procedures of isolating phthalates, PAHs and TPHs from environmental materials (soils, river sediments), and the additives in smokeless gunpowders.

For all these types of samples, the corresponding optimisation of experimental conditions has first been made. The results and observations obtained have revealed some general trends and rules that can be summarised in the

following way:

- the amount of a sample (material) to be extracted should be chosen adequately to the analytical performance of a method (technique) selected for the determination of the analyte(s) of interest — prior to extraction, the sample has to be dried and, if possible, pre-treated to obtain the most favourable ratio between the amount of sample and its active surface (e.g., utilising manual operations to reduce too large pieces or particles),
- some materials that are formed by matrices not very resistant to heat and may thus be melted should be mixed with a suitable additive such as sea sand,
- the solvent should be chosen accordingly to that used for related materials in classical Soxhlet arrangement [9]. Some undesirable properties of organic solvents are partly compensated by adding small amounts of a modifier or, eventually, one can use a mixture of miscible solvents,
- when seeking a suitable temperature, it is advisable to go from lower to higher values in regular intervals until the temperature increase gives further improvement in the recovery of extraction; the end of this trend then indicates the optimum temperature,
- pressure limits for extracting solvents can be found in the literature; however, according to the previous experience, a value of 10 MPa seems to be suitable to be chosen as the minimum capable of effective pressing the molecules of solvent to the pores of solid sample.
- time period of extraction step can be ascertained via the recovery; the moment when further prolonging of extraction process leads to no significant improvement in the recovery is again considered as that indicating the optimum extraction time,
- in practice, it is convenient to apply at least two extraction steps; a majority of analytes being isolated already during the first extraction whereas the repetitive step serves for extracting the remaining traces and for rinsing the system.

Experiments performed in this work have shown that methods employing PFE represent a promising and perspective way of extracting solid samples for organic analysis. Being confronted to traditional extraction techniques, PFE is more economical, less time-consuming, working with more friendly chemicals, and applicable to a wider spectrum of substances and materials. Owing to this, PFE appears to be especially convenient for routine use in laboratories where a large series of solid samples are to be analysed.

The application of PFE technique is more convenient in comparison with SFE, as it is not limited only to the extraction of substances of low polarity, but the selectivity of this technique is lower than that utilising supercritical carbon dioxide [10].

At present, more dynamic development and applications of PFE are being somewhat hampered by relatively high investments to the equipment in

comparison with simple Soxhlet devices. Also, PFE is still quite a new technique and, therefore, there is a lack of practical procedures and methods. Nevertheless, when one considers the above-mentioned advantages, it is believed that PFE will soon become the prevailing technique in routine processing of solid samples in organic analysis.

Acknowledgments

All experiments were performed thanks to financial supports from the Ministry of Education, Youth and Sports of the Czech Republic (Project MŠM 253100002) and from the Grant Agency of the Czech Republic (Project 203/02/0023).

References

[1] Majors R.E.: LC-GC Int. **9**, 638 (1996).
[2] Lambert P., Fingas M. Goldthorp M.: J. Hazard. Mater. **83**, 65 (2001).
[3] Kurečková K., Ventura K., Eisner A., Adam M.: Chem. Listy **95**, 415 (2001).
[4] Richter B.E., Pohl C.A., Porter N.L., Jones B.A., Ezzell J.L., Avdalovic N.: *Accelerated Solvent Extraction Method* US Patent 5,843,311, December 1, 1998.
[5] Dean J.R.: *Accelerated Solvent Extraction. In Extraction Methods for Environmental Analysis*, (Dean J.R., Ed.), John Wiley & Sons Ltd., Chichester, 1998.
[6] Dionex Application Note 328: *Extraction of Explosives from Soils by Accelerated Solvent Extraction (ASE)*, LPN 0888 5M 1/97, Dionex Corporation, 1997.
[7] Dionex Application Note 324: *Accelerated Solvent Extraction (ASE) of Hydrocarbon Contaminants (BTEX, Diesel, and TPH) in Soils*, LPN 0731 5M 7/96, Dionex Corporation, 1996.
[8] Dean J.R.: *Supercritical Fluid Extraction. In Extraction Methods for Environmental Analysis* (Dean J.R., Ed.), John Wiley & Sons Ltd., Chichester, 1998.
[9] Lo T.C., Baird M.H.I., Hanson C.: *Handbook of Solvent Extraction*, (Hanson C., Ed.), Krieger Bublishing Company, 1991.
[10] Ventura K., Adam M., Dostálek J.: J. Liq. Chrom. & Rel. Technol. **26**, 243 (2003).