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Combination of supported liquid membrane and solid-phase extraction for sample pretreatment of triazine herbicides in juice prior to capillary electrophoresis determination

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Abstract

A possibility of a combination of supported liquid membrane (SLM) and solid-phase extraction (SPE) for the determination of atrazine at microgram level in different type of fruit juices is presented. In comparison to SPE extraction from juice samples, the application of SLM–SPE enrichment provides much cleaner extracts and the possibility of lowering the limit of detection as low as 30 μg/l. However, it was also shown that by appropriate manipulation of SLM extraction conditions mainly flow-rate of donor phase and volume ratio between donor and acceptor phase, the level of detection can be further decreased to 10 μg/l. The results suggest that the application of SLM extraction prior to SPE is an alternative method for atrazine enrichment from complicated liquid matrices and could be used as routine method for the clean-up of such samples.

Keywords: Solid-phase extraction; Supporter liquid membrane extraction; Fruit juice; Atrazine; Triazines; Pesticides

1. Introduction

Since their introduction more than 40 years ago triazine herbicides has been widely used in crop protection. Among them atrazine, which is structurally based on the chlorinated 1,3,5-triazine ring, is one of the most extensively applied. This widespread application produces problems with the contamination of the environment with atrazine and its metabolites. Those substances can be found mainly in water matrices (e.g. field, soil water), which is a result of its physicochemical properties that determine high water solubility, high persistence and weak adsorptivity. Those properties cause migration of atrazine from the environment to the matrices that can directly influence our everydaylife (e.g. drinking water, feeds and foods). Therefore, it is necessary to monitor the level of triazine herbicides and their fate in the environment.

According to an European Union directive (EEC Drinking Water Directive, 1998) [1] the maximum limit concentration for atrazine is set at 0.1 μg/l (0.1 ppb) for a single substance and 0.5 μg/l (ppb) for the sum of all pesticides, if considering only water samples. For other matrices, including food samples, the triazine tolerance level is much higher and ranges between 100 and 250 μg/kg (100–250 ppb) in the EPA Tolerance Index [2].

In order to determine atrazine at the microgram level in water the most popular techniques applied so far are high-performance liquid chromatography

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(HPLC) and gas chromatography (GC) [3,4]. Other assays based on the application of capillary zone electrophoresis (CZE) [5] or micellar electrokinetic chromatography (MECC) are less commonly used [6]. The promising application of enzyme-linked immunosorbent assay (ELISA) has also been reported [7]. Despite the availability of the different techniques for determination of atrazines, there are still problems with the analysis. They may be attributed to the purity of the sample and limit of detection that is possible to reach by the particular analytical method. In order to avoid those difficulties, most commonly the reduction of the sample volume by various means has been introduced, but this is usually the main source of errors. The additional challenge is miniaturisation since it results in the reduction of time and solvent consumption. Therefore, special methods of sample pre-treatment have been applied. They are based on extraction steps and clean-up procedures that make the sample compatible with chromatographic or electrophoretic techniques of analysis.

The application of particular sample pretreatment method mainly depends on the matrix from which sample is acquired. In case of water samples the method of choice seems to be solid-phase extraction (SPE) [8]. The application of supercritical fluid extraction (SFE) for the determination of atrazine in eggs was also reported [9]. However, when more complex samples, originated for example from field waters, juices or wines, etc. were cleaned-up some difficulties can be observed. Often the matrix components are difficult to remove and interfere with the signals originated from the target analyte. For juice samples for example it may be necessary to dilute the sample up to 100-fold [8]. For more complex samples, for example food, it is necessary to use an additional sample clean-up prior to analysis. The relatively most extensively used for this purpose is liquid–liquid extraction (LLE) combined with column chromatography [10]. However, this method is labour and time consuming and requires the use of organic solvents.

The use of supported liquid membrane (SLM) extractions, for clean-up of sample prior to further analysis, offers a possibility to avoiding those problems. SLM extraction is a combination of three simultaneously processes: extraction of the analyte into an organic phase, membrane transport and re-extraction. The main advantages of SLMs are the simplicity of preparation and adjustment of extraction conditions [11]. Additionally, they can be characterised by an insignificant use of organic solvents or other, sometimes very expensive, substances facilitating extraction (e.g. chiral extractants) and the possibility of operating with a high volume ratio. Moreover SLM can be easily combined on-line with various analytical instruments [12]. In comparison to SPE, SLM extraction can provide a considerably cleaner extract that can lead to more reliable identification with a lower detection limits. Moreover, the SLM can be easily tuned in term of selectivity by application of specific carriers introduced into the liquid membrane.

SLM extraction has been successfully applied to determination and quantification of triazine herbicides in aqueous matrices. Chimuka et al. [13] described the application of supported liquid membrane extraction combined on-line with HPLC for analysis of triazine contents in natural waters. They were able to determine the triazines, including atrazine at 0.03–0.16 μg/l. SLM was also applied as a clean-up method in recently proposed Immuno-SLM technique, a combination of supported liquid membrane extraction and flow injection immunoassay [14]. In this technique the SLM is applied to separate the target analyte and transfer it to the acceptor phase containing the solution of an analyte-specific antibody. This method was successfully used for the separation and determination of phenols in water samples [14].

The mostly currently used sample pretreatment technique for atrazine enrichment is solid-phase extraction, however reported procedures usually concern mainly the extraction of atrazine from pure water samples. There are only a limited number of reports, which describe the extraction of those herbicides from more complicated matrices, particularly from fruit juices, for example such as grape juice (limit of detection 20 μg/l for simazine) [15] or orange juice (sample containing 1 μg/l of atrazine) [16]. In these cases, the main problems that are encountered during the extraction process result from the presence of the matrix interferences. Generally, this inconvenience is reduced by dilution of samples but it can lead to the increase of the measurement

errors. The other possibility is the application of immunoaffinity based solid-phase extraction (IASPE) (orange juice, 1 μg/l of atrazine) [16] or SPE with molecularly imprinted polymers (apple extract, 40 μg/l of atrazine) [17], but these methods require specially prepared sorbents and are significantly more expensive. Therefore, we aimed to compare the potency of extraction of atrazine from orange juice samples by SPE and SPE combined with SLM and application of relatively inexpensive and easily available materials.

In this report, the application of SLM extraction for the clean-up and enrichment of atrazine in juice samples prior to SPE preconcentration is presented. The main goal of this work was to emphasise the necessity of employing the SLM extraction in combination with SPE for determination of atrazine in this matrix at μg/l level. The application of SPE as the single clean-up and enrichment method was insufficient due to the significant number of interfering substances, that adsorbed on the sorbent and co-eluted with atrazine. This made it impossible to perform the quantitative analysis of atrazine by capillary electrophoresis with UV detection. The application of the proposed combination of those two extraction methods enabled the determination of atrazine in various fruit juices at the required level.

2. Experimental

2.1. Chemicals and materials

Atrazine, dihexyl ether (DHE), sodium dodecyl sulphate (SDS) and background electrolyte salts were obtained from Sigma, Poland. Water was purified with MilliQ system (Millipore, Bedford, MA, USA). Methanol used for conditioning SPE disc and for elution as well as other chemicals was obtained from POCh, Gliwice, Poland.

All fruit juices are commercially available and are produced by Hortex Holding S.A., Poland.

2.2. SLM and SPE enrichment

The membrane unit used in SLM experiments were described previously [13] and consisted of two circular PTFE (polytetrafluoroethylene) blocks (diameter=12 cm, thickness=8 mm) with grooves arranged as an Archimedes' spiral (depth=0.25 mm, width=1.5 mm, length=2.5 m) each with total volume of 0.95 ml. The liquid membrane was prepared by immersing the membrane support, Fluoropore FG, average pore size: 0.2 μm, porosity: 0.70 total thickness: 175 μm of which 115 μm is polyethylene backing provided by Millipore, Bedford, MA, USA – in dihexyl ether for 30 min. The prepared membrane was clamped tightly and evenly between the surfaces of PTFE blocks by eight screws. Aluminium blocks (6 mm thick) were used on both sides of the PTFE blocks to stabilise the construction.

The juice samples were pumped using PVC acid resistant tubing (Elkay Products, MA, USA) with a Gilson Minipuls 3 (Gilson S.A., France) peristaltic pump.

Then, 100 ml of juice sample spiked with the proper volume of a stock solution of atrazine (0.1 mg/ml in methanol) was centrifuged (15 000 rev./min, 20 °C, 10 min). After that, the sample was pumped through the donor channel of the SLM unit at 0.2 ml/min. The acceptor phase consisting of 10 ml 0.5 *M* H_2SO_4 was circulating in acceptor channel with the same flow-rate during extraction time. Both phases were pumped at 0.2 ml/min. After the extraction, 10 ml of acceptor phase was neutralised with NaOH on a Bakerbond Speedisk C_{18} XF disc (J.T. Baker Inc.). Prior to enrichment step, the disc was conditioned by washing with 10 ml of methanol and 10 ml of water. After elution of the acceptor phase, the disc was washed with 10 ml of water and vacuum dried to remove the remaining water. Subsequently, the analytes were eluted with 10 ml of methanol. The eluted solution were filtered and evaporated to the dryness on a rotary evaporator, diluted with 1 ml of background electrolyte and analysed by capillary electrophoresis.

2.3. Atrazine determination with capillary electrophoresis

For the determination of the atrazine, micellar electrokinetic chromatography was used with a background electrolyte consisting of 10 m*M* phosphate buffer, 60 m*M* SDS, 20% methanol solution adjusted to pH 9.2. This method was adapted from the

literature [6], but the borate buffer was substituted with phosphate buffer. The analysis was performed with a Beckman P/ACE 5000 system with UV detection at 214 nm (Beckman, Palo Alto, CA, USA); voltage: 20 kV; 10 s of pressure injection time.

3. Results and discussion

3.1. Calibration of capillary electrophoresis analysis

Calibration for atrazine was carried out by injection of standard solutions of atrazine concentration within 1–25 mg/l range. Linear calibration curve was found in the tested range with the correlation factor 0.996. The limit of detection was 0.3 mg/l, calculated as three times the signal-to-noise (S/N) ratio. This is worse than the results described in the literature [6] (for comparison: calibration range 50 μg/l–2 mg/l, detection limit around 30 μg/l). The observed differences can be a result of different equipment used as well as application of a different buffer. Thus, under our experimental conditions a preconcentration factor of 100 is required to determine atrazine in the tolerance limit concentration for food according to EU and EPA directives.

3.2. SPE extraction of atrazine from fruit juices

The experiments were initially performed with only solid-phase extraction as the clean-up and enrichment method. The extraction was performed on spiked samples of four commercially available fruit juices, orange, apple, blackcurrant and grapefruit. The representative electropherograms of the samples after SPE demonstrated the presence of substantial interferences (Fig. 1). The best clean-up of the sample was obtained for the grapefruit juice (Fig. 1a), for which the estimated limit of quantification was around 250 μg/l. It seems that either the amount of the interfering substances is lower or the SPE is more efficient than for other juices. However, the measured concentration still is far away from the tolerance limit concentration for food according to EPA directives. An estimated limit of the quantification of atrazine in orange or blackcurrant juice is around 1000 μg/l. In case of apple juice even an approximate quantitative estimation was impossible. The results show that a single application of SPE for sample pretreatment is insufficient as a method for the clean-up of juice samples for the capillary electrophoresis determination of atrazine with UV detection.

3.3. SLM–SPE extraction of atrazine from fruit juices

In order to improve the detection limit of atrazine, SLM extraction was applied prior to SPE extraction. The conditions were taken from the work of Chimuka et al. [13]. Atrazine occurs in two forms in the water solution depending on the pH. With a pK_a at 1.65, atrazine in solutions of lower pH that this value is present in a cationic form, while above this pK_a value it appears as an uncharged species. Therefore, the pH of donor (source) phase has to be higher than 1.65 to ensure the presence of atrazine predominantly as an uncharged species. This is a basic condition for transfer of the analyte from water solution into the organic, immobilised liquid phase. In order to trap the atrazine in the acceptor (receiving) phase, this phase must be strongly acidic so that the analyte is to be converted into a charged, protonated form. The trapping prevents the analyte from being transported back into the donor phase. The pH of juice samples as the donor phases varied from 2.80 (for orange juice) to 3.45 (for blackcurrant), hence the additional adjustment of pH was unnecessary because at this pH condition, atrazine is present mostly as an uncharged compound. The acceptor phase usually consisted of 0.5 M H_2SO_4 solution (pH around 1), in which atrazine is converted into charged form. The acceptor phase was than neutralised for the extraction on the SPE disc.

The influence of the SLM–SPE combined extraction on the enrichment of atrazine from four different juices was studied (Fig. 2). By using of the combination of these two extraction techniques a significant improvement in the clean-up and enrichment was achieved. The limit of quantification was lowered from 1000 μg/l (for SPE only) to 50 μg/l. Additionally, the extract was significantly cleaner for all juices and almost all interfering substances from

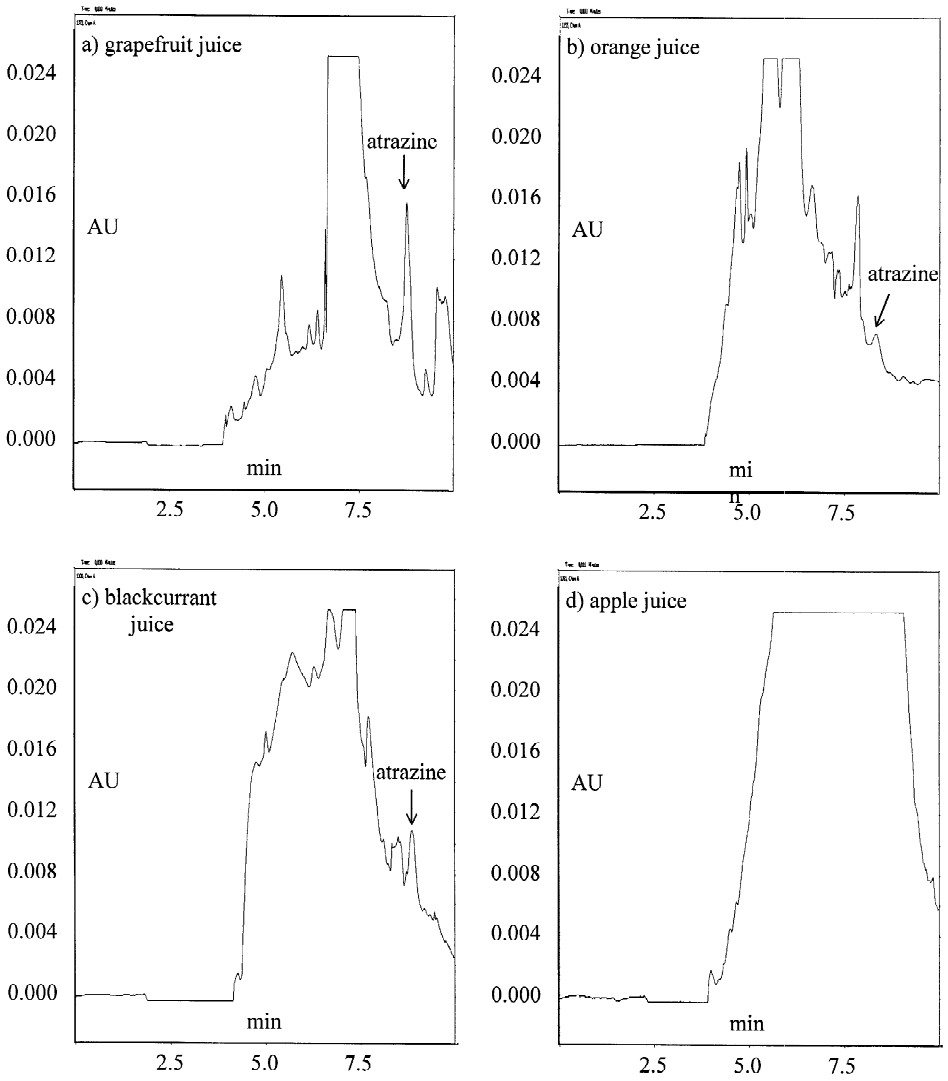

Fig. 1. Representative electropherograms of atrazine (1000 μg/l) in 100 ml samples of different juices after SPE extraction.

the juice were removed. Even in the case of grapefruit juice, where some interferences are still present in the sample after SLM–SPE extraction, it was possible to determine the atrazine without significant errors (Fig. 2a). In case of orange juice, a 100 ml of sample volume was insufficient to determine atrazine at 50 μg/l due to the uncertainty of the identification of atrazine peak. This can be caused by adsorption of the atrazine on the fruit particles in the orange juice or/and the presence of interferences co-migrating with atrazine during capillary electrophoresis measurement. This renders impossible the atrazine determination at the required level. In order to improve the determination of atrazine in orange juice, the sample volume was increased to 200 ml leaving the same volume of acceptor phase (Fig. 2b) and thus doubling the amount of atrazine present in the sample available for SLM extraction. As a result, it was possible to determine this herbicide at required level. For remaining two juices (Fig. 2c, d) the extracts were clean and the obtained electropherograms are of satisfactory quality. Therefore, it can be

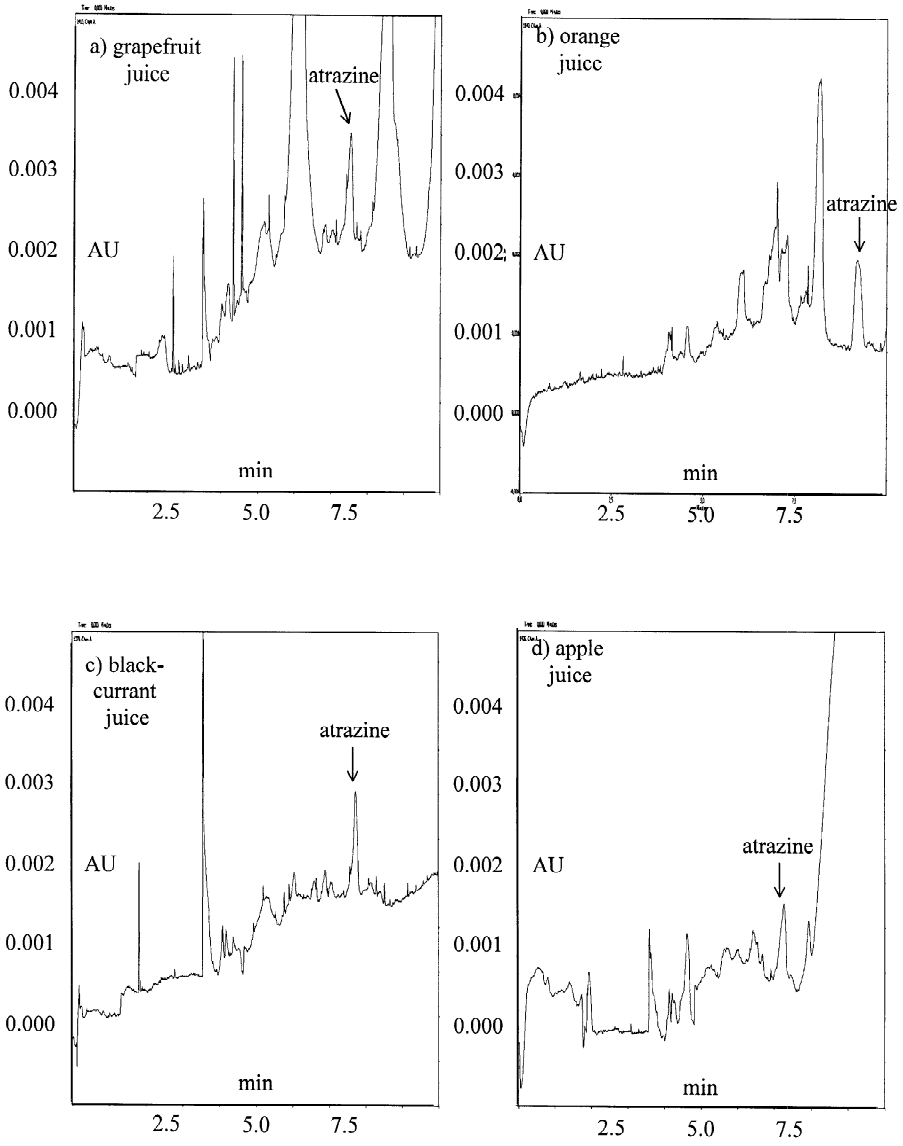

Fig. 2. Representative electropherograms of atrazine (50 μg/l) in samples of different juices after SLM–SPE extraction: orange juice, 200 ml; other juices, 100 ml (for explanation see in text).

concluded that SLM extraction is more selective than SPE with C_{18} sorbent alone. Moreover, tuning the selectivity is much easier to achieve, than in the case of solid-phase extraction, where the selectivity towards atrazine is obtained in most cases by means of special preparation of sorbents. The application of SLM–SPE possesses other advantages in comparison with SPE during pretreating of juice samples. Firstly, an enrichment of the sample takes place during all extraction process because the sample is concentrated both during SLM and SPE and no dilution occurs at any stage of the process. Secondly, the presence of the solution with high ionic strength in acceptor phase (after SLM extraction the acidic solution is neutralised with NaOH) is beneficial for SPE in respect of improvement of recovery. This is

Table 1
The influence of donor phase flow-rate on the atrazine (50 μg/l) SLM–SPE extraction from orange juice (200 ml)

Flow rate (ml/min)	Concentration of atrazine after enrichment (mg/l)	Recovery (%)
0.20	4.48	44.8
0.34	4.79	47.9
0.72	6.58	65.8

Acceptor phase for SLM 10 ml of 0.5 *M* H_2SO_4 pumped with the flow-rate 0.2 ml/min. SPE performed as in Experimental.

probably a result of the salting out effect, which has been found as a factor improving of the extraction of many types of organic compounds by SPE [18].

3.4. The influence of donor phase flow-rate

The flow-rate of the pumped donor phase (sample) through the membrane module is an important parameter for enrichment of analyte. As it was established by Jönsson et al. [19], the enrichment factor (defined as ratio of the concentration of analyte in the acceptor phase to that in donor) is related to extraction efficiency and to the flow-rate by equation: $E_e = EV_D/V_A = E\,F_D t/V_A$, where V_D is a volume of extracted sample, V_A volume of acceptor phase, F_D flow-rate of sample and t enrichment time. E is an extraction efficiency and can be expressed by equation: $E = n_A/n_D = c_A V_A/c_D V_D$, where n_A and n_D are the amount of moles of analyte in donor and acceptor phase, respectively, c_A and c_D are concentrations of analyte. As it can be seen from the first equation the enrichment factor increases with an increase in flow-rate. However, the increase of enrichment factor also depends on that how the extraction efficiency depends on flow-rate. This is determined by the partition coefficient $\log K_{ow}$ (hydrophobicity) of the analyte. For polar compounds with low $\log K_{ow}$ values, the dissolution into the membrane limits the extraction efficiency and by increasing the flow-rate the enrichment decreases. Therefore, in this case low flow-rates are preferable.

In Table 1 the influence of donor phase flow-rate on the recovery of atrazine from orange juice samples after SLM–SPE extraction is shown. In this case the recovery is defined as the ratio of the concentration of atrazine after whole analysis procedure to the theoretically calculated concentration of herbicide. As it can be seen, the recovery of atrazine after SLM–SPE also rises up with an increased donor flow-rate. It is in an accordance with the results obtained by Chimuka and co-workers [13] and can be attributed to hydrophobicity of atrazine ($\log K_{ow} = 2.7$). Therefore it seems reasonable to perform SLM extraction with high flow-rates. However, in this work a flow-rate of 0.2 ml/min was chosen because it enabled a comparison of the SLM extraction of atrazine with experiments on the extraction of other organic compounds performed in our laboratory [20–23].

3.5. Recovery studies

In Table 2 the recoveries after SLM–SPE extraction are also shown. They vary with the kind of juice, but with some exceptions it can be said that generally a recovery for SLM–SPE ranges from 40 to 60%. This is an acceptable value when compared with other reported results [8] and additionally taking into account that during enrichment two extraction

Table 2
SLM–SPE extraction of atrazine from samples of four fruit juices (100 ml)

Initial concentration of atrazine in sample	Concentration of atrazine after SLM-SPE enrichment (mg/l) and recovery (in parenthesis) (%)			
(μg/l)	Orange juice	Apple juice	Blackcurrant juice	Grapefruit juice
50	4.48[a] (44.8)	1.99 (39.8)	2.34 (46.9)	2.45 (49.0)
100	5.02 (50.2)	6.58 (65.8)	7.59 (75.9)	5.25 (52.5)
250	9.00 (36.2)	14.14 (68.5)	13.09 (52.4)	11.59 (46.3)
500	23.00 (46.3)	np	np	np

Acceptor phase for SLM 10 ml of 0.5 *M* H_2SO_4 pumped with the flow-rate 0.2 ml/min. SPE performed as in Experimental.
np, experiment not performed.
[a] 200 ml of juice sample.

steps are carried out. The recovery of the SPE step was estimated, by extraction of 10 ml solution of 0.4 mg/l atrazine concentration in 0.5 *M* H_2SO_4 neutralised with NaOH. This concentration corresponds to the complete extraction of atrazine by SLM in the juice sample equal to 40 μg/l. The atrazine concentration after SPE extraction was 2.87 mg/l, and this corresponds to the SPE recovery around 70%. Therefore, assuming that for combined SLM–SPE extraction the recoveries were 40–60% (Table 2), the extraction recovery in SLM step can be set as 60–80%. The difference of recoveries found for different juices could be a result of varied constitution of matrix. For example the recovery for orange juice samples is generally lower than for blackcurrant juice, which might be a result of accumulation of atrazine by the solid particles present in the orange juice, whereas blackcurrant juice is clear and does not contain any solid particles.

3.6. Linearity and limit of detection

The performance of the SLM–SPE extraction was evaluated by using the 100 ml orange juice samples spiked with atrazine and 10 ml of acceptor phase (circulating with flow-rate 0.2 ml/min). Linear calibration curve was obtained in the atrazine concentration range between 50 and 500 μg/l with the correlation factor of 0.970 and the average RSD value 15% ($n=3$). The limit of detection of atrazine for the orange juice was 30 μg/l and the limit of quantification 50 μg/l. It can be also noticed that the similar limit of detection can be reached for other examined juices. This limit can be significantly improved if the volume of used samples is higher. In case of SLM, where preconcentration depends on the volume ratio of donor to acceptor phases, it might be possible to decrease the limit of detection even to 1 μg/l or less, by increasing the donor phase volume. It can be achieved on the expense of time however, the increase of flow-rate can diminish this disadvantage. In order to demonstrate this possibility the SLM–SPE extraction of atrazine at concentration 10 μg/l in 500 ml of blackcurrant juice sample was carried out (Fig. 3). As it can be seen, the electropherogram is very similar to the electropherogram obtained for standard enrichment of 50 μg/l (Fig. 2c) sample. The recovery is also in the same range and the same level of clarity and interferences can be observed.

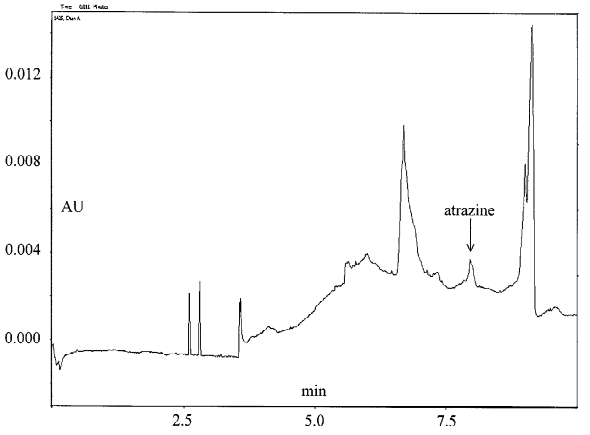

Fig. 3. Representative electropherograms of atrazine (10 μg/l) in 500 ml sample of blackcurrant juice after SLM–SPE extraction.

4. Conclusions

It has been demonstrated that it is possible to improve significantly the extraction of atrazine from juice samples by means of combination of SLM and SPE extractions. The results suggest that the level of extraction of atrazine is comparable for various juices and the enrichment as a well as recoveries are similar. In comparison to the standard SPE procedure of atrazine extraction the introduction of the SLM extraction before SPE allows the removal of the analyte from interferences in the samples. As a consequence, a considerably cleaner final extract can be obtained and it is possible to decrease significantly the limit of detection for UV detection. It was also shown that by varying the parameters of SLM extraction mainly, flow-rate of donor phase and sample volume, it could be possible to obtain even better enrichment of the sample as well as to lower the limit of detection.

Acknowledgements

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