Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2010, 2(1): 354-362
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Directly Suspended Droplet Three Liquid Phase Micro extraction of Ecstasy in Hair Combined with UV-Visible Spectroscopy / Diode array Detector

Zarrin Es'haghi[1]*, Mahin Hasanzade-Meidani[2], Maryam Mohtaji[2], Mahbobeh Masrournia[2]

[1]Department of Chemistry, Payame Noor University, P.O.Box. 26, Mashhad, Iran
[2]Department of Chemistry, Islamic Azad University, Mashhad Branch, Mashhad, Iran

---

Abstract

Directly suspended droplet liquid–liquid–liquid micro extraction (DSDME) has been used to determine residues of ecstasy (3,4-methylenedioxy-N-methylamphetamine) in hair samples. In this technique a free suspended droplet of an aqueous solvent is delivered to the top-center position of an immiscible organic solvent floating on the top of an aqueous sample while being agitated by a stirring bar placed on the bottom of the sample cell. In the present work, ecstasy was extracted from hair samples by LLLME and analyzed by UV-Vis Spectroscopy. Factors such as organic solvent, extraction and back extraction times, stirring rate and the pH of acceptor and donor phases were optimized. Enrichment factor and detection limit (LOD, n = 3) were 147.4 and 0.0057 $ng.ml^{-1}$. The linearity ranged from 0.010 to 15 $\mu g.ml^{-1}$ with a %RSD of 1.63 (n = 3). All experiments were carried out at room temperature (25 ± 0.5 ºC).

Introduction

The term" ecstasy" or "XTC" relates to the chemical family of amphetamine and its derivatives. The first report on the synthesis of amphetamine was published in Germany by Edelano in 1887, its stimulating properties were discovered almost 30 years later by an American chemist, Gordon Alles.

Amphetamine was first abused by soldiers in 1936 during the Spanish civil war. The term "designer drugs "is regularly used to describe chemical derivatives of amphetamine, and especially the 3,4-methylendioxy-substiuted phenyl- alkyl-amines such as MDA (3,4-methylene-dioxyamphetamine)

which was synthesized for the first time in Germany in1910, or MDMA(3,4-methylenedioxy-N-ethylamphetamine) which was synthesized and patented in1914 by Merck in Germany .At

that time these products reached significant popularity under the name ecstasy or XTC, and were associated with the "Techno and Rave culture " particularly in some British subcultures. Most of the other derivatives of MDA and MDMA(such as MDEA,MBDB or other ,less successful products such as BDB, MMDA, DOM, DOB and so on)are the result of targeted synthesis in underground laboratories during the last 20years.[1-11]

In order to avoid any misunderstanding in forensic descriptions, it will be important to use the chemical nomenclature of these substances or their accepted and correct abbreviations. It is imperative not to use general street names to describe a known chemical structure, for example "ecstasy "for MDMA or "speed' for methamphetamine.

Because of low concentration of drugs like MDMA in biological samples pre-treatment and a pre-concentration step is generally required for determination of trace amounts of drugs in the different matrixes. Recent research activities are oriented forward the development of efficient, economical, and miniaturized sample preparation methods for extraction and determination of drugs [12, 15].

Results and Discussion

2.1. Materials

Ecstasy tablets were gifts from the Ministry of Health and Cure of Iran, center of Khorasan Razavi. 1-octanol was obtained from Fluka. Toluene and all other chemicals were purchased from Merck(Darmstadt, Germany). Stock solution of MDMA was prepared by dissolving the 8.18 mg of tablets powder in 10ml methanol. The standard sample containing MDMA at 5.0 µg/ml was providing by dilution of stock solution in deionized water which was from Stamen Pharmacy (Mashhad, Iran).

2.2. Directly Suspended Droplet LLME Method

The sample solution (5ml, adjusted to PH7with HCL) was placed in a 6 ml glass vial. A stirring bar (3mm) was used to facilitate the mass transfer process. A heating-magnetic stirrer was used to stir the extraction mixture. A 25ml flat-cut HPLC microsyringe was used to introduce the acceptor phase and act as injection syringe. Sample solution was added to the glass vial and magnetic bar was placed into the vial. 350 µl of organic solvent was then added to the sample solution by a 1000 µl microsyringe. Then the mixture was agitated for 180 s at 1000 rpm. After this time the acceptor phase (10µl NaoH 0.1M, pH=12) was delivered to the top-center position of the immiscible organic solvent. The mixture was agitated at 600 rpm for 20min, the micro droplet was taken into a HPLC micosyringe and then neutralized by adding 3µl aqueous solution with pH=12. Finally the total amount was injected into the UV with detection λ=220nm. The experimental micro extraction setup is shown in Fig. 1.

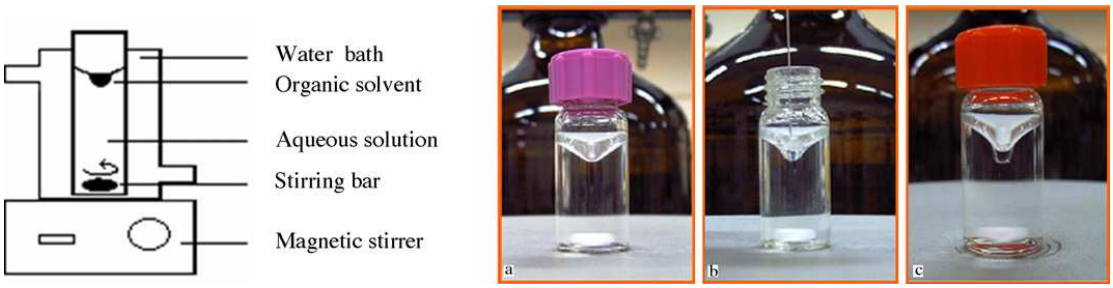

Figure 1. A photograph of the DSDME device.

2.3. Optimization of Directly Suspended Droplet Microextraction

The different parameters that influence on the extraction were optimized. The optimization was carried out on water solution of 5.0 $mg.l^{-1}$ ecstasy. Parameters such as kind of organic solvent, the extraction times, micro droplet volume, stirring rate and pH were considered and optimized.

2.3.1. Choice of Organic Solvent

The analyte in the sample solution (donor phase) should have high partition coefficient into the organic solvent. It should have high viscosity to hold the micro droplet and a lower density that water to lay it over the aqueous sample solution. In this work, 1-octanol was examined as the best solvent after tested some any other solvents like hexane, benzene, octane and benzyl alcohol.

2.3.2. Phases Volumes

The enrichment factor can be improved by the increasing the volume ratio of donor and acceptor phases [15–17]. The results indicated that the best extraction efficiency was obtained when the donor/ acceptor ratio was more than 100. Furthermore, the volume of the acceptor solution used for extraction may also be adjusted depending on the analytical technique coupled to LLLME. For example, in comparison with GC, injected sample volume in HPLC may be in the range of 10–25 µl. Therefore, the whole acceptor phase can be analyzed and a lower detection limits obtained [17].

In this manner, use of a larger drops results in an increase of the analytical response, but these large drops are not very stable especially with high stirring speed and may be fall into the sample solution (donor phase). Thus, a 10 µl droplet was chosen as the optimum volume of acceptor phase. On the other hand, because of the design of our extraction device, the volume of the organic phase was also important and needed to be optimized. The best volume of the organic solvent was found to be 350 µl. A smaller volume of organic solvent (i.e., less than 300 µl) is tended to cause instability of the aqueous drop during agitation, whereas the extraction efficiency is reduced if a larger volume of organic phase is used. Consequently, a 350 µl volume of organic solvent was chosen for subsequent work. (See Fig2)

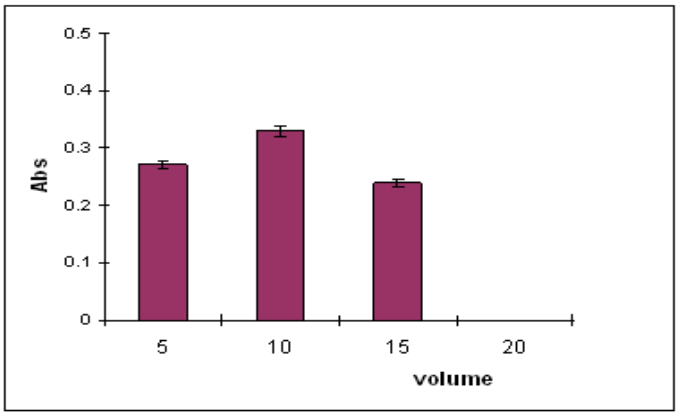

Figure 2. Effect of volume droplet on the extraction.

2.3.3. Extraction Time (T1)

The extraction of analyte from the water sample (p1) into organic phase by LLLME is a slow equilibrium process, and mass transfer is time-dependent [18].Because, solute molecules need enough time to pass the interface between the donor and organic phases the recovery depends

on the time that the analyte is in contact with the organic phase. Before addition of the suspended aqueous droplet, aqueous donor solution and organic solution was agitated at 1000rpm for 180s (T1) giving a cloudy mixture of sample solution and organic solvent. (See Fig3)

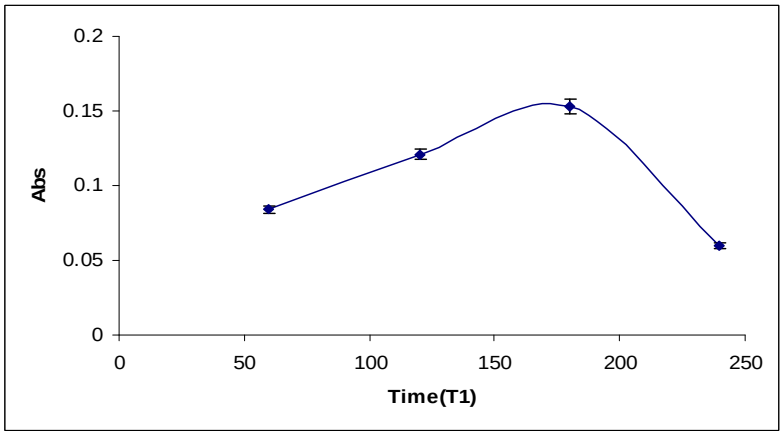

Figure 3. Effect of extraction time on the process.

2.3.4. Back-Extraction Time (T2)

Three-phase suspended droplet is not an exhaustive extraction technique. Although maximum efficiency is attained at equilibrium, complete equilibrium need not to be attained because of increasing of analysis time [18,19]. Droplet lifetime cannot be too long due to drop dissolution or loss. Therefore, the back extraction time (T2) from the organic solvent (1-octanol) into the aqueous acceptor phase (10ml NaoH 0.1M) should not be too long and 20min was chosen and enrichment factor did not increase significantly after 20min. (see Fig4)

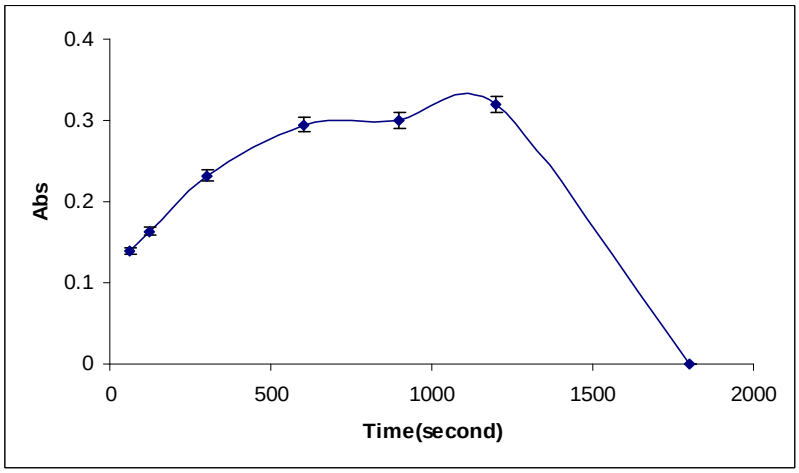

Figure 4. Effect of back-extraction time on the DSDME process.

2.3.5. The pH of Acceptor and Donor Phases

The compositions of both donor and acceptor phases are very important parameters that affect extraction efficiency in three phase liquid phase micro extraction.

For basic drugs, the donor phase should be strongly alkalized to effectively deionize the analyte and consequently reduce their solubility within the sample, while the acceptor phase should be acidized in order to promote dissolution of the basic analytes [20,21]. The effects of sample pH in the range of 3–12was investigated (Fig.5). As a result the best extraction efficiency was observed on pH 12.

Therefore, the pH of the sample solution (donor phase) and the aqueous micro droplet (Acceptor phase) was optimized. We used pH 7, for the donor and pH 12, for the acceptor phase respectively. After pH=12 the drug decomposed.

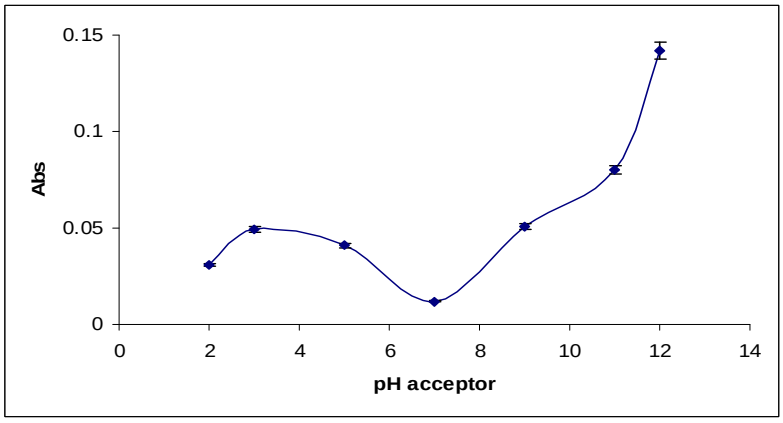

Figure 5. Effect of pH on the DSDME

2.3.6. Stirring Speed

Agitation of the sample solution is generally applied to facilitate the mass transfer process and accelerate the extraction kinetics. Increasing the stirring speed of the donor phase enhances the diffusion of analyte through the organic phase and improves the repeatability of the extraction [22,23]. Therefore, the stirring speed was also optimized for better extraction. Different stirring rates, i.e. 100, 300, 500 and 700 rpm were checked (see Fig .6). Higher speed of agitation increased extraction efficiency but the aqueous micro droplet become unstable at high speed of the magnetic stirrer. Thus, 600rpm was selected as the stirring speed.

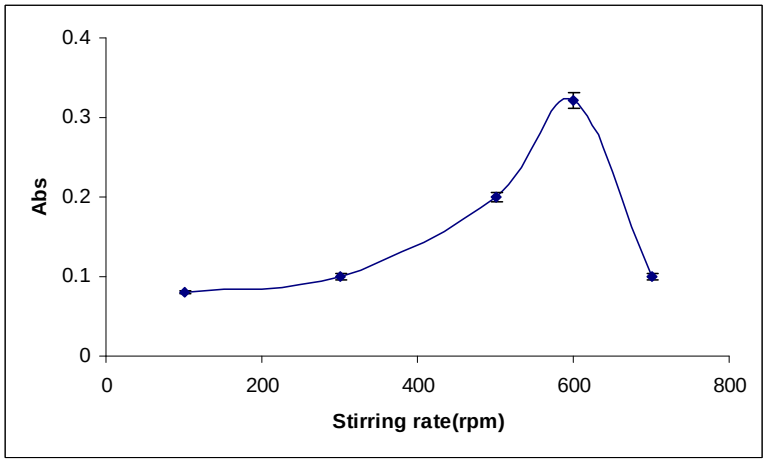

Figure 6. Effect of stirring rate on the extraction.

2.3.7. Nature and Concentration of Surfactant

The extraction efficiency of relative no polar organic compounds can reach to about 100% even when very low surfactant concentrations are used [24].we have carried out a comparative study of three different nonionic surfactants, Triton x-100,Brij 58,Brij 72,in the extraction and pre-concentration of abuse drugs, in the hair analysis. Surfactant concentration is an important parameter for effective extraction. The results obtained indicated that Triton x-100 has been shown better result than the others and the enrichment factors can be

increased as a function of the surfactant concentration till 20.0 ppm. Thus the Triton x-100 with concentration of 20.0 ppm was used as an optimal parameter.

2.4. Hair Treatment

2.4.1. Hair Samples

A bulk of blank hair is necessary for method development and validation. This blank sample was obtained from a men hairdresser's shop. The absence of opiate was verified in this blank sample. Hair samples of abusers were collected from 20 men ranging from 16 to 45 years old. They were captured by the police and for most of them, screening tests were positive for drug of abuse. Some of the addicted persons were under therapeutic treatment.

A standard of hair of about 5mm in diameter was cut from close to the scalp at the vertex posterior area, folded in aluminum foil, and the proximal and distal ends marked. The samples with 2–4 Cm long was selected for analysis.

2.4.2. Hair Washing

The hair was washed with different solvents as follow: 20 ml dichloromethane, 15 ml acetone, 15 ml methanol, 10 ml methanol, at room temperature for 5 min and then it was dried. The last washing solvent was tested with GC for checking residual content of opiates.

2.4.3. Digestion of Hair Matrix

The washed and dried hairs were finally cut into approximately 1mm pieces and digested by the following procedure; 2ml methanol as an extracting solvent was added to 50 mg of hair, in a 10ml screw-cap tube. The pH was adjusted to 7.4 by phosphate buffer solution. The samples were incubated at 50 ◦C For 5 h[25]. In case of a remaining solid matrix, extracts were filtered. The remaining was rinsed with 0.5 ml ethanol and both fractions were evaporated to dryness at 40 ◦C under a steam of nitrogen.

2.5. Calculation of Extraction Recoveries and Analyses Enrichments

The extraction recovery (*R*), was calculated by the following
Equation:

$$R = (\frac{n_{a,\,final}}{n_{s,initial}})100\% = (\frac{V_a C_{a,\,final}}{V_s C_{s,initial}})100\%$$

Where n_s, initial and n_a, final are the number of moles of analytes originally present in the sample and finally collected in the acceptor solution, respectively. V_a, is the volume of acceptor phase and V_s, the volume of sample, C_a, final, the final concentration of analyte in the acceptor phase, and C_s, initial, is the initial concentration of analyte within the sample. R=122.09 the analyte enrichment factor (EF) was calculated by the following equation:[26,27]

$$EF = \frac{C_{a,\,final}}{C_{s,initial}}$$

2.6. Analytical performance

Chargeable compounds can be successfully extracted into three-phase LLLME with expanded applicability range. As reported by previous researchers, LLLME may have shown good potential for the extraction of drugs from biological fluids [15,28,29]. Quantitative determination of drugs (ecstasy) in hair is strongly dependent on the method of digestion for hair sample and also on the blank hair matrix used for calibration. We have analysed the blank and sample hair from the examinants who were from the same aged category and sexuality.

The dynamic linear ranges, precisions and the limits of detection (LOD) have been evaluated in order to assess the performance of the microextraction method.

The calibration curves were linear in the range 10-15000 µg.ml^{-1} for ecstasy in hair, with correlation coefficient $r > 0.98$, so a direct proportional relationship between the extracted amount of compound and the initial concentration of the sample was demonstrated. Limits of detection were calculated as the minimum concentration providing chromatographic signals which is 3 times higher than background noise.

LOD was 5.7 µg.ml^{-1} for analyte which shows a good sensitivity of the method. The R.S.D. value obtained was satisfactory 1.63 % for ecstasy in hair and the enrichment factor was 147.4.

Thus the concentration of ecstasy in the final LLLME extract was directly proportional with the concentration of ecstasy in the hair sample and indicated that LLLME may be utilized for quantitative analysis of drugs in hair.

Calibration curve parameters for ecstasy were reported in Figure 7. The concentration of ecstasy in the hair of drug abusers was 43.0 µg ml^{-1}.

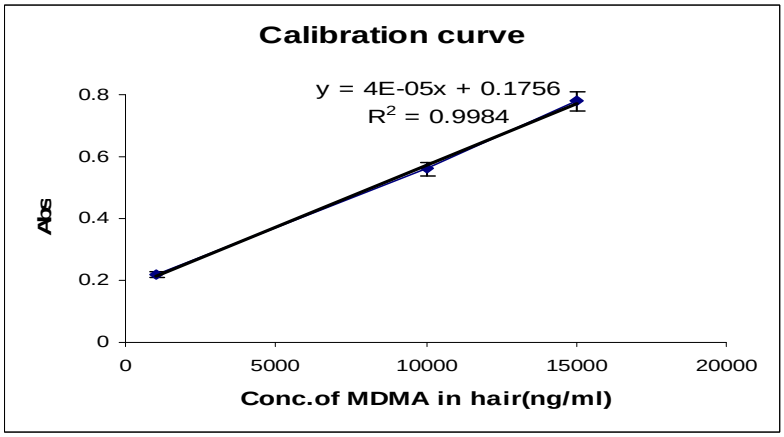

Figure 7. Calibration curve of ecstasy.

Conclusion

The aim of the present study was to develop and validate a rapid, sensitive, robust and reliable method for the quantitative determination of the drug abuse in human hair by UV-Vis spectrophotometery as a simple and common method and the results obtained with the method described above indicate that DSDME methodology is a good alternative extraction

technique for hydrophilic drugs in hair and offers highly interesting advantages from an analytical point of view, such as possibility of extracting and pre-concentrating the analytes of different polarities. The method was compared with many techniques which were used for determination of MDMA in the environmental and biological samples and the results were shown in Table.1.

Table 1. Comparison of the DSDME/UV-Vis with other related methods for determination of MDMA

Matrix	Method	Detection	LOD	LOQ	DLR[1]	RSD	r^2	Recovery
Blood	SPME	GC/MS	10ng/ml	$\mu g / ml$ 0.05-0.2	-	-	-	-
hair	SPME	GC/MS	0.01-0.17ng/mg	-	0.1-50ng/ml	-	0.998	-
hair	SPME	GC/MS	0.01-0.5ng/mg	-	0-10 ng/mg	-	-	-
Oral fluid	LLE	HPLC/Fluresance	2ng/ml	10ng/ml	-	-	-	--
urine	SPME	GC/MS	2.95ng/ml	9.85ng/mg	$\mu g / ml$ 0.5-5	-	-	91
urine	LLE	HPLC/Fluresance	15ng/ml	-	-	-	-	85-102
Blood	LLE	LC/MS	-	-	1-1000ng/ml	-	-	-
urine	LLE	HPLC/Fluresance	-	-	0.5-15ng/mg	-	0.997	-
Blood	LLE	LC/MS/MS	-	0.1ng/ml	0.1-50ng/mg	-	0.997	-
urine	LLE	LC(ESI)MS/MS	0.2ng/ml	1ng/ml	1-1000ng/mg	12.7%	0.99	80
urine	SPE	GC/MS	4-10ng/ml	12-34ng/ml	50-2000ng/ml	-	0.994	93.5
urine	SPME	GC	-	-	-	-	-	7-9.6
urine	SPME	GC/MS	-	-	250-2000ng/mg	-	-	-
urine	SPE	GC/MS	5ng/ml	50ng/ml	10-2000ng/mg	10%	-	-
hair	SPE	HPLC	0.02-0.16ng/ml	-	-	5-6.9%	0.99	-
hair	SPE	GC/MS	0.05-0.3ng/mg	-	-	5-16.1%	-	-

1-Dynamic Linear Range

Acknowledgements

The authors would like to express their thanks to Islamic Azad University for their financial support. Thanks are also extended to ministry of "Health and Cure" center of Khorasan-e-Razavi, Iran.

References

[1] G. Akram, *Int. J. Drug Policy*, **2000,** 11, 265.
[2] K. Bailey, H. D. Beck stead, D. Legault, D. Varner, *J. Assoc. Off. Anal. Chem.*, **1974,** 57, 1134.
[3] K. Bailey, A.W. By, D. Legault, D. Varner, *J. Assoc. Off. Anal. Chem.*, **1975**, 58, 62.
[4] C. Burgess, A. O'Donohoe, M. Gill, *Eur. Psychiatry.*, **2000**, 15, 287.
[5] M. Yegles, F. Mersch,R. Wennig, *Forensic Sci. Int.*,**1997**,84, 211.
[6] P.Kintz. *Forensic Sci. Int.*,**2004**, 142, 127.
[7] F. Pragst, M. Rothe , K.Spiegel, F. Sporkert, *Forensic Sci. Rev.*,**1998**,10, 81.
[8] M. Fendrich, M. E. Mackesy-Amiti, T.P. Johnson, A. Hubbell, J. S. Wislar, *Addict. Behav.*,**2005**, 30, 175.
[9] M.A. Jeannot, F. Cantwell. *Anal. Chem.*, **1996**,68, 2236.

[10] J. Å. Jönsson ,L. Mathiasson J. *Sep. Sci.*,**2001**, 24, 495.
[11] S. Pedersen-Bjergaard, K.E. Rasmussen, *Electrophoresis*., **2000**, 21, 579.
[12] R. Musiol, M. Sajewicz, *Der Pharm.Chemica* , **2009**, 1, 63.
[13] L. Sivasubramanian, K. S. Lakshmi, *Der Pharm. Chemica* , **2009**, 1, 37.
[14] G. Ouyang, W. Zhao, J. Pawliszyn, *Anal. Chem.*, **2005**, 77, 8122.
[15] Y. He, H.K. Lee, *Anal. Chem.*, **1997**, 69, 4634.
[16] E.Psillakis, N.Kalogerakis, J *Chromatogr. A*, **2001** , 938,113.
[17] G. Shen, H.K.Lee, *Anal Chem.*, **2002**, 74, 648.
[18] S. Pedersen-Bjergaard, K.E. Rasmussen, T.G. Halvorsen, *J Chromatogr A*, **2000**, 902, 91.
[19]S.Palmarsdottir, E.Thordarson, E. EdhomL, J.A. Jonsson, L. Mathiasson, *Anal Chem.*, **1997** 69, 1732.
[20] R.P. Frankewich, W. Hinze, *Anal. Chem.*, **1994**, 66, 944.
[21] A. Eiguren Fern´andez, Z. Sosa Fereera, J.J. Santana Rodriguez, *Anal. Chem. Acta*, **1998**, 358, 145.
[22] A. Sarafraz Yazdi, Z. Es'haghi, *Talanta*, **2005**, 66, 664.
[23] A. Sarafraz Yazdi, Z. Es'haghi, *J. Chromatogr. A.*, **2005**, 1082,136.
[24] S. Rubio, P´erez-Bendito, *Trends Anal. Chem.*, **2003,** 22,470.
[25] M.A. Bal´ıkov´a, V. Habrdov´a, *J. Chromatogr. B.*, **2003**, 789, 93.
[26] M. Montagna, C. Stramesi, C. Vignali, A. Groppi, A. Polettini, *Forensic Sci. Int.*, **2000**, 107 ,157.
[27] T.S. Ho, S. Pedersen-Bjergaard, K. Rasmussen, *J. Chromatogr. A.*, **2002**, 963, 3.
[28] K.A. Hadidi, J.K. Almasad, T. Al-Nsour, S. Abu-Raghebi, *Forensic Sci. Int.*, **2003,** 135, 129.