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Liquid Determination of Methamphetamine in Heroin Narcotic Plant by UV-Chromatography Technique.

Shaker A. N. AL-Jadaan*

Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Basrah, Iraq.

ABSTRACT

A fast method was described for the determination of [(S)-N-Methyl-1-PhenylPropan-2-amine], methamphetamine (MMP) as microcrystalline standard dissolved in methanol comprehensive with extraction (MMP) from plants, this method using IC-HPLC, It was based on the spectrophotometric UV in max. wave length 275nm. The (MMP) spectra of this system were examined under the optimum conditions to obtained a good truly result for active material (MMP) from standard and extraction plants. The detection limit (S/n=3) is $5x10^{-2}$mg/l with relative standard deviation is 2.0% for all measurements at 0.1-0.9 gmL^{-1} (MMP) standard solution. The method has been successfully applied to the determination of (MMP) in standard (Microcrystalline) and plants extraction preparation with the recovery at 96.6% - 100.0 %, alsoillustrated sensitivity ,accuracy ,evaluation method and reproducibility by method standard addition .

Keywords: Fast method IC-HPLC, UV-ION chromatography(UVIC), Narcotic plant, methamphetamine, extraction.

Corresponding author

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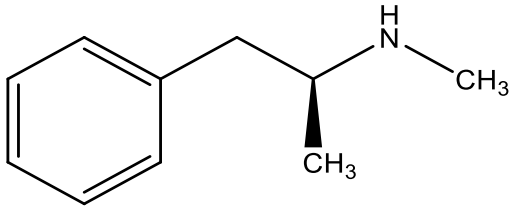
INTRODUCTION

One of the most important applications of UV chromatography (UVIC) was the determination of methamphetamine (MMP) compound in Microcrystalline and active material in Heroin plants. Approximately 10-25% of drugs were developed in salt forms certain physicochemical and biopharmaceutical properties of active pharmaceutical developed salt (APD) can be improved by pairing a basic or acidic drugs.[1,2], The drugs with high solubility, stable microcrystalline form, and good bioavailability. Chromatography techniques with UV detection plays an important role in the heroin plant at wave length 275nm and pressure 160 bar at (25-30)C^{o} with Ion Pac Arcos EP - C18 ; 5µm , 4.5×250 mm *(P/N* 11051194 L*)*also flow APPL spectrophotometer system PD 303UV and flow rate 1.2ml/Min, the Isocratic Eluent mixture [(acetonitrile, Methanol, DI, Water, Methane sulphonic acid. (MSA) (10%: 40%: 50%: 30mM)], the loop of this system 100µl flow in 18µl volume quartz cell and the separation time t_R (6-18min)[3,4].

The microcrystalline as standard and Heroin plants were an important traditional chine's medicine were used for the treatment such aliments as a cute fever, head ache,anti-emetic, anti-convulsing[5]. The major active components in Heroin plant are Methamphetamine and amphetamine with different high-performance liquid chromatography (HPLC)[6]. Two steps for this determination process, First step was extraction of nacrotic plants, Second was to determine the active compound (MMP_{EXT})as crystalline[7,8]. The chemical structure of active compound in heroin narcotic plants was shown in Figure (1).



(*S*)-*N*-methyl-1-phenylpropan-2-amine

Figure: 1 MMP Structure

To determine (MMP) in plant samples containing 5900 – 6000mg/l, The sample was evaporated to dryness and reconstitute in the initial sample volume with methanol as extraction solvent[9]. This HPLC method allows for the analysis of (MMP) samples according to flow PD-303UV system and has high sensitivity[10].

Accurate measurement for (MMP) in plant matrices required: (i) a robust (MMP) extraction method and (ii) a sensitive analytical method for (MMP) quantification. Analytical method that have been used for determination of (MMP_{EXT}) in plant matrices include: colorimetric based on catalytic reactions microscopic 8 MPX, IC with UV detection as absorption spectrometry[11] . A number of (MMP) extraction methods have also been developed for (MMP) in matrices plant products – microwave digestion in open or closed vessels with perchloric acid, tetra methyl ammonium hydroxide, Oxygen combustion, alkaline extraction, acid digestion (Hydrochloric acid, acetic acid)[12-14], precipitation with evaporation methanol acetonitrile and ultra-centrifugation, but the mean method was UV detection[15,16] .(UVIC) has the advantages at 1.2ml / min flow rates, low eluent consumption and available IC system without additional consumable replacement costs. The (UVIC) system is designed to run continuously, saving time spent on equilibrating and recalibration typically need after each start up[17-19].

Aim

To develop an efficient and comprehensive HPLC and quality control (QC) methods for the analysis of methamphetamine (MMP) in Heroin plant, this method must separate active motivate from plant. The application method to create an IC-HPLC based method for determination (MMP) from plant and shown to be fast, robust, and sensitive.

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EXPERIMENTAL

AnIon Pac Arcos EP - C18 ; 5µm , 4.5×250 mm *(P/N* 11051194 L*)* was chosen for this separation because its phenyl groups are structurally similar to the phenyl groups and aromatic structures contained in the compound at interest. The separation of (MMP) sample can be completed within Run time 18min using a 10% acetonitrile ,40% methanol , 50%, DI. Water with 30 mM methane sulphonic acid.

Equipments

A home-mad IC-UV system including

- LKB Bump 2150 –HPLC ,Broman
- Ion Pac column Arcos EP - C18 ; 5µm , 4.5×250 mm (*P/N* 11051194 L**)**
- Electric injection valve
  100 µL loop inject in system before flow cell quartz.
- Detector PD 303 UV
- Flow cell quartz-UV, 18 µL

Reagents and standards

- Deionized water (DI), 18.2Ω-Cm resistivity.
- Acetonitrile (CH_3CN), HPLC grade, BDH Chem.LTD 562240A
- Methanol (CH_3OH), HPLC grade ,BDH M/ 405/17 LTD 116967 Cass 67-56-1
- Methane sulfonic acid (MSA), analytical grade, Aldrg;51,684-8 LTD ;S 67573
- 20% $HC_2H_3O_3$ + AuCl, General color reagent grate for microscope 8 MPX for all narcotic material.
- di- meth in H_2PtCl_6, specific color reagent grade (MMP).
- Heroin narcotic plants
- Microcrystalline methamphetamine as standard.

All reagentsand standardscan be savedunderN_2 gas [20] .

Sample preparation

The (MMP) sample was supplier from Basrah Governorate police / Criminal Evidence Dep. /Criminal Lab. by document No.1032 in 18-1- 2015.

Table 1: method parameters

Parameters	Conditions
Description Column	Ion Pac Arcos EP-C18;5µm ,4.5×250mm (P/N11051194 L *)*
System Suitability Requirement	USP Tailing Factor @ 5 %Peak Height< 1.3 Resolution between any analyte and I.S. is > 1.4 Plates > 16000-16,810
Isocratic Mobil phase	10% acetonitrile ,40%methanol ,50% DI Water +30 mM methane sulfonic acid
Detection System	UV detection
Wave length Maximum	275 nm
Flow Rate	1.2 mL / min
Temperature	25- 30 °C
pressure Background	160 Bar
Run Time	(standard) 13 Min
Injection Volume	100µL

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Accurately weigh 5g of sample mass was placed in a 30 ml Volumetric flask, 30mL methanol/water (1:1 V/V) was added, the mixture were heated on water bath stirring for 48 hours, cool to room temperature filter through a 0.4µm membrane prior to injection[21].

The second step after plant extraction was HPLC method to develop an efficient and quality control method parameters. Table1.

All peaks were well resolved and the precision was acceptable. System suitability of the method was studied through method development by calculating theoretical plates, peak symmetry factor and tailing factor. Resolution and repeatability favourable for the system are summarized in Table 1.

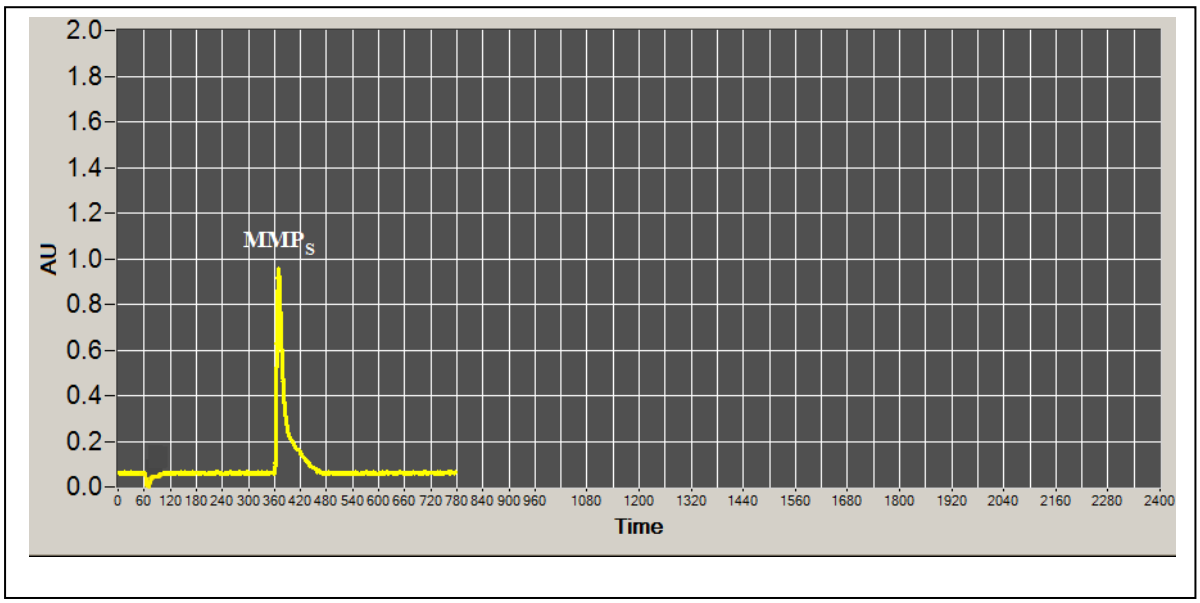

Figure 2A:peak of MMP_S

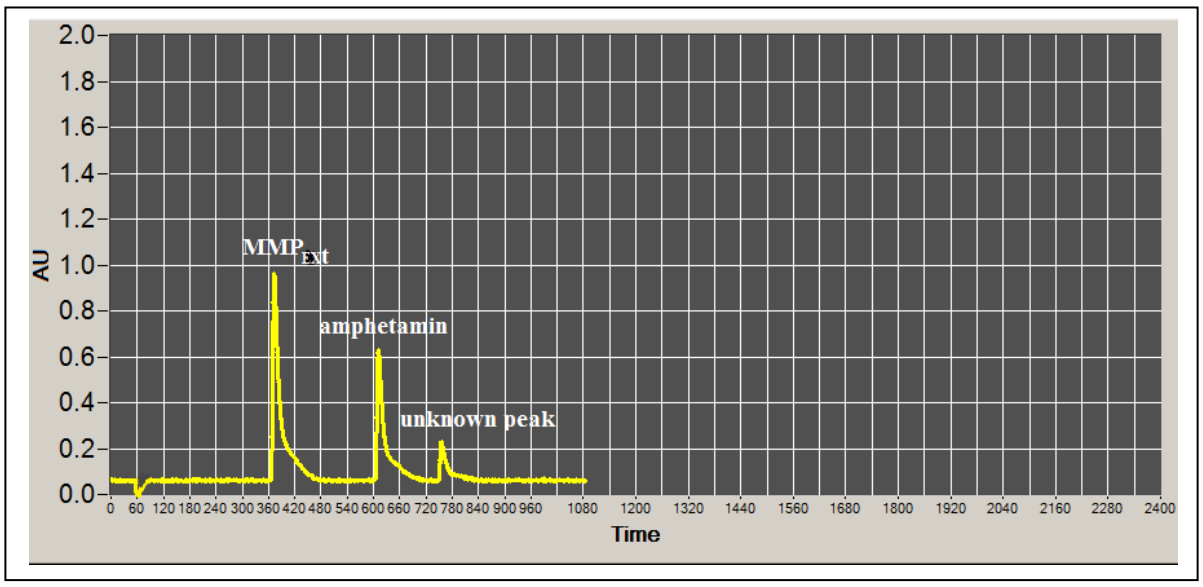

Figure 2B:peak of MMP_{EXT}

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RESULTS AND DISCUSSION

Effect of eluent on the separation and time

Figure 1 shows the separation of 0.5 mg/l (MMP) on a C_{18}column set using mixture eluent (10% CH_3CN, 40% CH_3OH,50% DI.H_2O + 30mM CH_3SO_3H). The (MMP) elutes in less than 5min. and is well resolved from the void volume. The baseline dip at approximately 1 min. is due to dissolved Oxygen from the previous injection[22] (elution time of approximately 13 and 18 min) and varies from column to column after installing a new column, ensure that 13 min is an appropriate cycle time.Three consecutive peaks appearance in chart ,the mean cause of separation is a properties column and type eluent ,first peak refers to (MMP_{EXT})Methamphetamine which is the mean study , second peak is amphetamine that is other compound extraction within mean peak ,third peak unknown didn't important now in this study.

Effected Column Temperature on the separation of(MMP) Active components

The effect column temperature was evaluated for the separation of (MMP) using C_{18} Column. The separation of standard on the column, with temperature changing from 25-45C^o in five degree steps. As expected, increasing the column temperature decreased retention time, and baseline separation of the standards were achieved in the temperature range, however, the investigation of samples that changing column temperature could be caused to some other compound to interfere with detection of the analysis, the good separation for the sample at a column temperature at 25-30C^o.

Method performance (Reproducibility, linearity and Detection Limits)

The method reproducibility was estimated by making consecutive injection of a (MMP) sample. Excellent RSD for retention time and peak height were obtained as shown in Table 2 and Figure 1.Lower limit of detection (LLOD) and quantitation (LLOQ) were the concentrations that give signal to noise ratio of 3:1 or 10:1 respectively, which can be detected and verified by the relation of standard deviation of response (SD) to the slope of calibration curves (S):

$$LLOD=3.3\ SD/S$$
$$LLOQ=10\ SD/S$$

LLOD and LLOQ[23]were calculated using the single-sided student's test method (at the 99% confidence limit). Using five consecutive injections of (MMP) sample and microcrystalline standard, the testing determined standard deviation values for calculating LLOD and LLOQ[24] , also reported in Table 2.

Table 2: Regression statistics of the proposed method with LOD, LOQ, Intercept and Slope for Microcrystalline (MMP_S) material as standard and (MMP_{EXT}) from extraction narcotic plant.

compound	Retention Time(min)	R^2	Std. Er[a]	Std.ErEst[b]	Intercept	Slope	LOD ng mL^{-1}	LOQ ng mL^{-1}
MMP_S	6	1.00	0.332	0.365	0.68254	8.17777	10.71985	32.4844
MMP_{EXT}	6	0.971	31.836	37.3160	77.836	60.761	10.677	32.354

[a]standard error, [b]standard error estimate

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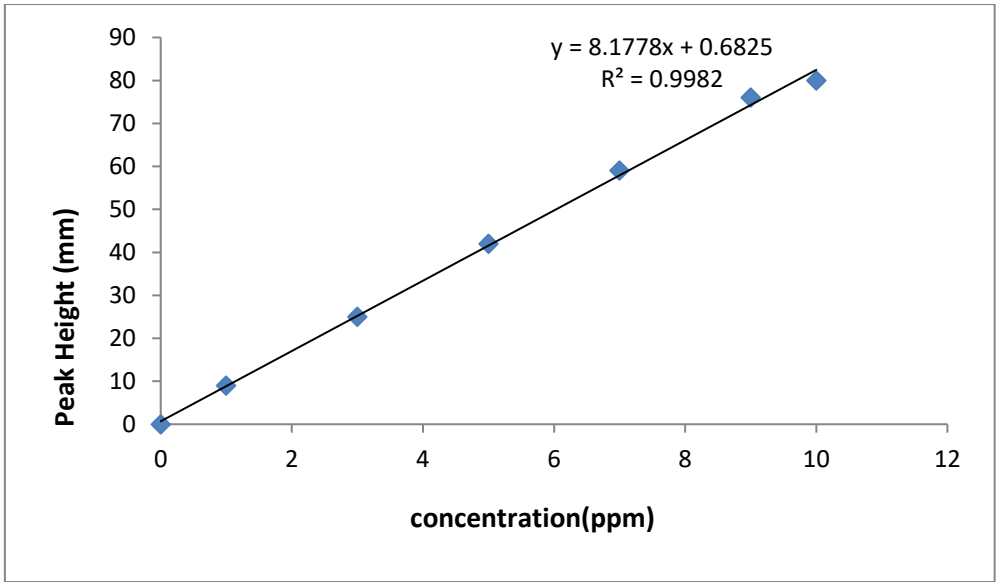

Figure 3: Linearity of MMP_S

Calibration linearity for the compound was investigated by making five consecutive injections of a standard prepared at five different concentrations,(0.1, 0.3 ,0.5 , 0.7 and 0.9) ppm and peak Height (9 ,25 ,42 ,59 and 76) mm Consecutively and 0.5 ppm the external standard method was used to establish the calibration curve components in sample. Table 3 reports data from the calibration as calculated by the SPSS CD software.

Sample Analysis

Chromatograms of a (MMP) sample as well as comparison of UV spectra and retention time allows the identification of (MMP), recoveries for standard sample ranged from 100% suggesting that the analysis method is accurate[25]. The results are shown in Table3.

Table 3: Method accuracy from recovery assay for the studied compounds analysis.

Concentration Range (µg mL^{-1})	% Recovery		Recovered conc. (µg mL^{-1})		%RSD	
	MMP_S	MMP_{EXT}	MMP_S	MMP_{EXT}	MMP_S	MMP_{EXT}
1	100.0	98.0	1.00	0.98	0.24	0.14
3	96.6	93.3	2.90	2.80	0.19	0.04
5	100.0	100.0	5.00	5.00	0.08	0.15
7	97.1	98.5	6.80	6.90	0.64	0.52
9	100.0	97.7	9.00	8.80	0.01	0.13

Standard addition method

In order to illustrate sensitivity ,accuracy ,evaluation method and reproducibility by standard addition method, unknown concentration were added to consecutive form from (MMP_S) to fixed that the method is successfully analysis method after find unknown concentration by intercept in X axes ,the unknown addition concentration is 0.25 ppm, also standard addition method refers to didn't get any interference among to mean standard microcrystalline concentration and other material, shown in figure 4. .

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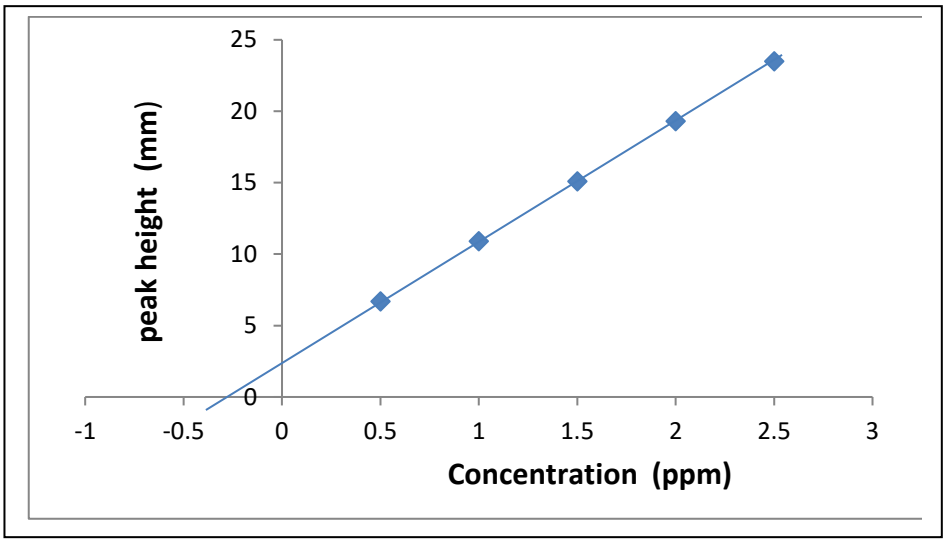

Figure 4:standard addition method

Accuracy

The accuracy of this method was verified by determining recoveries of micro crystalline standard and (MMP_{EXT}) sample over three consecutive days[26]. The amount of sample rang 0.1 – 0.9 mg/L .The samples were (MMP_{EXT}) with standard 0.5 mg/ l.

Recoveries were calculated from the difference in response between the standard and (MMP_{EXT}) sample. The average recovery of (MMP_{EXT}) ranged from 96.6-100 %. Recovery in samples that were standard after the sample preparation steps ranged from 93.3 – 100.0% Table 4. The suggests that the matrix does not inhibit standard detection after the sample preparation steps[27]. The above results indicate that this method could be used for accurate determination of (MMP).

Table 4: Intra and inter-day precision and accuracy of MMP_S analysts (n=5) .

Added (mg l^{-1})	Intra-day			Inter-day		
	Found	%Rec	%RSD	Found	%Rec	%RSD
1.0	1.00	100.0	0.24	0.98	97.8	0.25
3.0	2.99	96.6	0.19	2.89	93.3	0.04
5.0	5.00	100.0	0.08	5.00	99.8	0.09
7.0	6.70	97.1	0.64	6.75	99.0	0.65
9.0	9.00	100.0	0.01	8.90	97.5	0.01

CONCLUSION

This work describes an HPLC method that baseline separate one active components in heroin narcotic plants using extraction method before separation (MMP) using C_{18} column 4.6×250mm, 5µm and mixture eluent (10%CH_3CN + 40% CH_3OH + 50% DI. Water 18.2 Ωcm^{-1} + 30mM CH_3SO_3H) as mobile phase. This method can be used for the quality control of heroin plants a common medicinal plants in many states. It is superior to the HPLC method that measured only one purport active component with spirited method that required long separation time and have insufficient peaks.

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REFERENCES

[1] Thermo Fisher Scientific, Application Note 116, LPN 0924. Sunnyvale, CA, 2007.
[2] Law, L. MA pp Sc Thesis, Auckland University of Technology, New Zealand, 2010.
[3] Hess, G. BP and DuPont Plan. Chem. Eng. News 2006, 84, 9.
[4] Gou, Z.P.; Wan, D.G, Chin. Traditional Patent Med. 2006, 28, 408.
[5] Pharmacopoeia Commission of the Ministry of Health of the People's Republic of China. Pharmacopoeia of the People's Republic of China, 9th ed.; China Medical Science and Technology Press, 2010; Vol. 1, 179.
[6] Qian, Z.M.; Li, H.J.; Li, P.; Tang D.; Qin, S.J., Chin. J. Anal. Chem. 2007, 8, 1159–1163
[7] Qian, Z.M.; Li, H.J.; Li, P.; Chen, J.; Tang D., Biomed. Chromatogr. 2007, 21, 649–654.
[8] Li, H.J.; Qian, Z.M.; Li, P.; Ren, M.T.; Chen, J.; Xin, G.Z., Natural Product Communications. 2008, 3 (5), 655–659.
[9] U.S. FDA, Agency Response Letter to GRAS Notice No. GRN 000253, Dec 17, 2008.
[10] U.S. FDA, Agency Response Letter to GRAS Notice No. GRN 000252, Dec 17, 2008.
[11] Prakash, I.; Dubois, G.E.; Clos, J.F.; Wilkins, K.L.; Fosdick, L.E. Food and Chemical Toxicology, 2008, 46, S75–S82.
[12] Steviol Glycosides, Prepared at the 69th JECFA, 2008, published in FAOJECFA Monographs 5, 2008.
[13] Rebaudioside A, Food Chemicals Codex; Seventh Ed., Supplement 1, USP, Inc.: Washington DC, 2010.
[14] Thermo Fisher Scientific, Product Manual for Acclaim Trinity P1 Columns. Document No. 065306-02., Sunnyvale, CA.2010
[15] Hurum, D.; Rohrer, J. Application Update 184: Mogroside V, LPN2977,. Thermo Scientific: Documents, Applications.2011
[16] Tarcomnicu, I.; Silvestro, L.; Savu, S. R.; Gherase, A.; Dulea, C. J. Chromatogr., A2007, 1160 (1–2), 21–33.
[17] Zacharis, C.; Tzanavaras, P. J. Pharm. Biomed. Anal. 2008, 48 (3), 483–496.
[18] Dionex (now part of Thermo Fisher Scientific) Technical Note 90: LPN 2649, Sunnyvale, CA, 2011.
[19] Wang, J.; Schnute, W. C. 59thASMS Conference on Mass Spectrometry and Allied Topics, Denver, CO, 2011.
[20] Hara, S.; Saski, N.; Takasa, D.; Shiotsuka, S.; Ogata, K.; Futagami, K.; Tamura, K. Anal. Sci. 2007, 23, p 523.
[21] Restek Corporation. Applications Note 580006, Bellefonte, PA.2006
[22] Waters Corporation (EPA Method 549.2), Milford, MA. 2008
[23] Dionex Corporation (now part of Thermo Fisher Scientific), Application Note, LPN 2098, , Sunnyvale, CA.2008
[24] Pacquette, L.H.; Levenson, A.M.; Thompson, J.J. J. AOAC Int. 2012, 95 (1), 169–176.
[25] Dionex (now part of Thermo Scientific) Application Update 122: Sunnyvale, CA (accessed Dec 10, 2012).
[26] Yebra, M.C.; Bollain, M.H. Talanta 2010, 82 (2), 828–833.
[27] European Committee for Standardization (2007) EVS-EN 15111:2007, Brussels, Belgium 1–12[Online] www.evs.ee/preview/evs-en-15111-2007-en. pdf (accessed Dec 10, 2012).