

## Preconcentration and Determination of Cyproheptadine by using Liquid Phase Microextraction and Solvent Bar in Biological Fluids in Trace Level

Mina Minaii, Mahnaz Qomi\*, Seyed Sajjad Hoseini and Azade Sadri

Department of Medical Chemistry (Pharmaceutical Sciences Research Center),  
Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.

DOI: <http://dx.doi.org/10.13005/bbra/1693>

(Received: 09 February 2015; accepted: 20 March 2015)

Solvent bar microextraction combined with HPLC-UV was used for the detection and determination of Cyproheptadine in biological samples. Cyproheptadine is used to treat allergic reactions. Extraction parameters including organic solvent, pH of sample solution and acceptor phase, extraction time, stirring rate, salt addition and sample solution temperature were optimized. The target analyte were extracted from a 10 mL aqueous sample solution at pH 10.5 through a membrane organic solvent immobilized in polypropylene hollow fiber pores to 10  $\mu$ L acceptor phase at pH 3.0 in the lumen of hollow fiber. After a 50 min extraction, the acceptor phase was directly injected into an HPLC system for analysis. Under the optimized conditions, cyproheptadine could be determined within the linearity range with a good correlation coefficient ( $r^2 > 0.9885$ ). The limit of detection for cyproheptadine was  $15 \mu\text{g L}^{-1}$ , and the intra- and inter-day relative standard deviations were no more than 3.15% and 3.57%, respectively. This procedure afforded a simple, sensitive and inexpensive method with high extraction efficiency for the detection and determination of cyproheptadine. Finally, proposed method was applied to determination and quantification of cyproheptadine in human plasma and urine samples.

**Key words:** Cyproheptadine; High performance liquid chromatography; Solvent bar microextraction; Microextraction.

Cyproheptadine is used to treat allergic reactions<sup>1-3</sup>. The chemical structure, IUPAC name, the octanol-water distribution coefficient, and acidity constant of the target drug are classified in Table 1<sup>4</sup>. Cyproheptadine is an antihistamine used to relieve allergy symptoms such as watery eyes, runny nose, itching eyes and/or nose, sneezing, hives, and itching. It works by blocking a certain natural substance such as histamine that the human body makes during an allergic reaction. This medication also blocks another natural substance in the body, such as serotonin<sup>5-7</sup>. This medication should not be used in newborn or premature

infants. Along with their useful effects, most medicines can cause unwanted side-effects, although not everyone experiences them<sup>8,9</sup>. Blurred vision, constipation, dizziness, drowsiness, dry mouth, throat, or nose, excitability, nausea and nervousness may occur the first few days as you took the medication.

Several methods have been presented for detection and determination of cyproheptadine until now. Numerous articles have been published on the detection and determination of cyproheptadine in different matrices. Most of them concern with high-performance liquid chromatography (HPLC)<sup>10-15</sup>, Liquid chromatography-mass spectrometry<sup>16</sup>, gas chromatography (GC)<sup>17, 18</sup>, chemiluminescence system<sup>19</sup> and electrochemistry techniques<sup>20-23</sup>.

\* To whom all correspondence should be addressed.

Today, there is a constant need for the development of simple, fast, sensitive and more selective sample preparation systems. To best of our knowledge, only one microextraction method has been presented for extraction and enrichment of cyproheptadine from biological fluids<sup>10</sup>. The major aim of this study is the development of a simple, sensitive, selective and fast analysis method, which allows the analysis of cyproheptadine in biological fluids. For the first time, in current study, solvent barmicroextraction (SBME) technique combined with HPLC with ultraviolet (UV) detection was optimized and validated for detection and determination of cyproheptadine in biological samples.

A new technique of LPME, SBME was reported by Jiang and Lee in 2004 [24]. In SBME, target analytes are extracted from an aqueous sample, into the membrane organic solvent immobilized as a SLM in hollow fiber wall pores, and into the acceptor solution placed inside the lumen of the hollow fiber<sup>25</sup>. Generally, SBME presents better extraction efficiency, sensitivity, selectivity and extraction time compared to other LPME methods. Today, SBME has two modes. Both of the membrane organic solvent immobilized in the fiber pores and the acceptor phase are same in two-phase SBME [26-28] but, in three-phase SBME, the analytes are extracted from the aqueous sample solution to the organic solvent immobilized in the fiber pores, and then into another aqueous phase inside the lumen of hollow fiber [29-34]. Compared with two-phase SBME, three-phase extraction has a much cleanup and selectivity.

In this study, we describe a one-step microextraction method for detection, extraction, and preconcentration of cyproheptadine in plasma samples. The factors, which influenced the extraction, such as membrane organic solvent, sample solution and acceptor phase pH, extraction time, stirring rate, and salt addition effect, are discussed and optimized to the determination of the target analyte in real sample.

## EXPERIMENTAL

### Reagents and reference standards

Cyproheptadine standard, kindly donated by Drug and Food Administration (Tehran,

Iran). Reagent grade 1-Octanol, *dodecanol*, *isobutylmethyl ketone*, *n-hexane*, and *n-heptane* were purchased from Merck (Darmstadt, Germany). HPLC grade acetonitrile and Methanol were from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide, sodium chloride, and orthophosphoric acid were purchased from Sigma-Aldrich. Ultra pure water was purified with a MilliQ apparatus by Millipore (Madrid, Spain), and used in the HPLC mobile phase and preparation of all sample solutions.

PPQ3/2 polypropylene hollow fiber was used in the extraction process from Membrana (Wuppertal, Germany) with the inner diameter of 0.6 mm, wall thickness of 200  $\mu\text{m}$ , and pore size of 0.2  $\mu\text{m}$ .

A 100 mg L<sup>-1</sup> stock solution of cyproheptadine was prepared in the mobile phase. The stock solutions were stable for at least three months when stored at -4 °C. Daily standard solutions were prepared by diluting the stock solutions in pure water.

### HPLC conditions

The HPLC system consisted of a Younglin YL9100 HPLC (Seoul, Korea) equipped with a Quaternary 9110 HPLC pump (Seoul, Korea), a 4-channel mixing valve with a 10  $\mu\text{L}$  sample loop, YL9101 vacuum degasser and a YL 9120 UV-Vis detector. Chromatographic data were recorded and analyzed using Younglin Auto Chro 3000 software. An ODS-3 column (150 mm  $\times$  4.6 mm, with particle size of 5  $\mu\text{m}$ ) from MZ-Analysentechnik (Mainz, Germany) was used for separation. The mobile phase was a 20 mM sodium dihydrogen phosphate-acetonitrile solution (40:60). The flow rate of the mobile phase was set at 1.0 mL min<sup>-1</sup> under isocratic condition. Total analysis time was 14 min. The injection volume was 10  $\mu\text{L}$  for all of the samples and UV detection wavelength was set at 230 nm.

Sample solutions were stirred using a MR Hei-standard magnetic stirrer from Heidolph (Schwabach, Germany). Sample solution and acceptor phase pH were adjusted by means of GPHR 1400 digital pH meter from Greisinger (Regenstauf, Germany).

### Extraction procedure

Extractions were performed according to the following procedure: A new 40 mm length of hollow fiber was cut and washed with acetone in

an ultrasonic bath for 10 min and dried at room temperature. Then, 100  $\mu$ L Hamilton syringe stuffed full of acceptor solution and the tip of the Hamilton syringe was inserted into the lumen of the treated hollow fiber from one end. The fiber was then dipped in the membrane organic solvent (1-octanol) for 10 s to impregnate the pores of the hollow fiber wall, and then inserted the hollow fiber into the water for 3 s to wash the excess organic solvent from the hollow fiber walls. Then, the acceptor solution from the Hamilton syringe was filled into the lumen of the hollow fiber. First, 40  $\mu$ L of acceptor solution was flushed out of the fiber in order to remove any organic solvent remaining inside the lumen of hollow fiber, and then 10  $\mu$ L acceptor solution was remained in the lumen of hollow fiber. The upper and the lower end of the hollow fiber were mechanically sealed with two-piece of aluminum foil. For each experiment, 10 mL of the donor solution was transferred into the 50 mL sample vial, followed by a stirring bar. The fiber was dipped into the sample solution and extraction was performed for a 50 min. During extraction, the solution was stirred at 500 rpm. After extraction, the 10  $\mu$ L acceptor phase was withdrawn into the microsyringe and collected into the micro-vial and finally, acceptor solution was injected into the HPLC instrument.

#### Real sample analysis

Drug-free human plasma was obtained from the Iranian Blood Transfusion Organization (Tehran, Iran). Urine samples were collected from healthy young volunteer. The samples were stored at  $-4^{\circ}\text{C}$  until required. The frozen urine sample was thawed and shaken at room temperature before use.

#### Calculation of preconcentration factor, and relative recovery

The analyte preconcentration factor ( $PF$ ) is calculated by the following formula:

$$PF = \frac{C_{f,s}}{C_{i,s}} \quad \dots(1)$$

where  $C_{s, \text{final}}$  is the final analyte concentration in the acceptor phase, and  $C_{o, \text{initial}}$  is the initial analyte concentration within the sample. Relative recovery ( $RR$ ) was calculated by the following equation:

$$RR = \frac{C_{\text{found}} - C_{\text{real}}}{C_{\text{added}}} \times 100 \quad \dots(2)$$

where  $C_{\text{found}}$ ,  $C_{\text{real}}$ , and  $C_{\text{added}}$  are the concentrations of the analytes after addition of known amounts of the standard into the real sample, the concentration of analyte in real sample, and the concentration of known amounts of the standard which was spiked into the real sample, respectively.

## RESULTS AND DISCUSSION

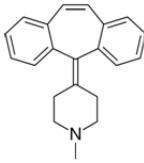
### Choice of the organic solvent

The nature of membrane organic solvent immobilized in the pores of the hollow fiber is a substantial role for extraction and preconcentration of target analyte [35]. In this work, five types of membrane organic solvent including 1-octanol, 1-dodecanol, isobutyl methyl ketone, *n*-hexane, and *n*-heptane were investigated for use in SBME. As shown in Fig. 1A, the highest extraction efficiency was obtained with 1-octanol. Therefore, 1-octanol was selected as the immobilization membrane organic solvent for subsequent experiments.

### Composition of sample solution and acceptor phase for SBME

In the three phases SBME, the pH of the sample solution and the acceptor phase play very important roles. Generally, in three-phase LPME, analytes are extracted from an aqueous sample solution, through the membrane organic solvent, and into an aqueous acceptor phase. For

**Table 1.** Chemical structures,  $pK_a$  and  $\log P$  of cyproheptadine.

Name	Chemical structure	IUPAC name	$pK_a$	$\log P$
Cyproheptadine		4-(5H-dibenzo [a,d]cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride	8.05	4.38

the extraction of basic compounds, the sample solution pH was adjusted into the basic region to suppress analyte solubility, whereas the pH in the acceptor solution was adjusted into the acidic region to promote analyte solubility. In the three-phase SBME, a protonation process was applied to increment extraction. In the basic sample solution, the target analytes were deionized to their neutral form, which caused their solubility to decrease in the sample solution. In the acidic acceptor phase, the target analytes were in its ionic form, which caused its solubility to increase in the acceptor phase. Therefore, experiments were conducted to optimize the pH of both the sample solution and the acceptor phase. For this reason, donor sample pH was studied in the basic pH from 9 to 12. As shown in Fig. 1B, the highest extraction efficiency for cyproheptadine was obtained using pH=10.5. Therefore, pH=10.5 was used for all further experiments. The pH of acceptor phase was studied from 2 to 5. As shown in Fig. 1C, the highest

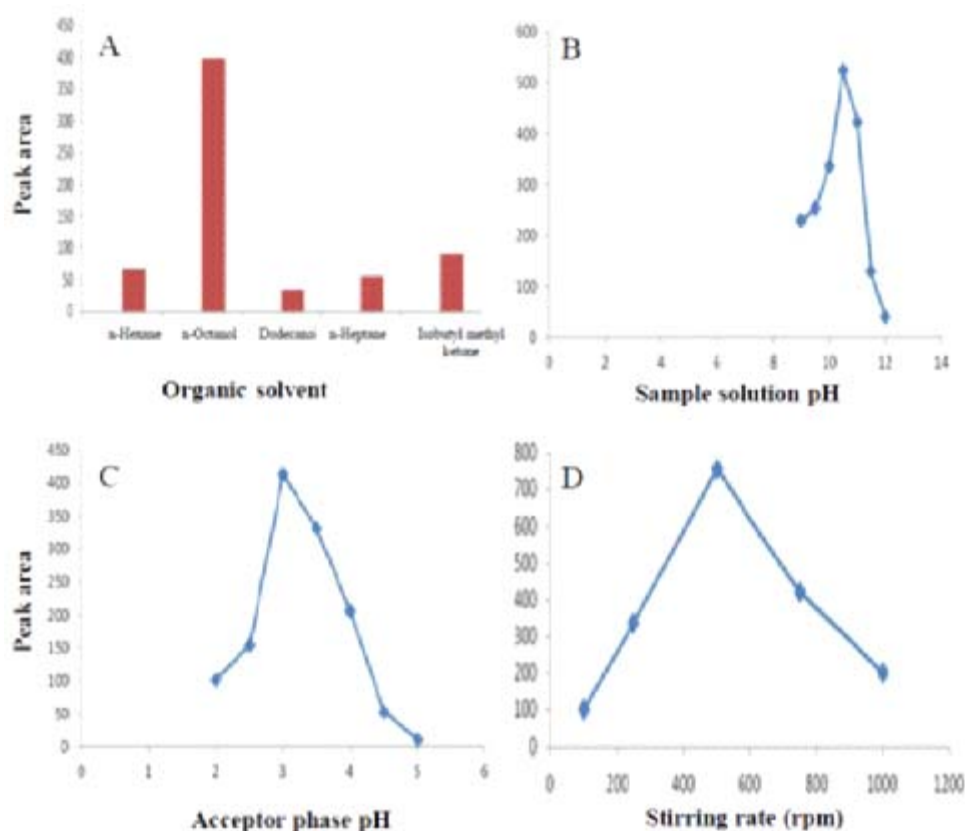
extraction efficiency was obtained at pH=3.0. Therefore, pH=3.0 was used for all further experiments.

#### Effect of stirring rate

Sample solution stirring enhances extraction efficiency and reduces the extraction time required to reach equilibrium. Sample solution stirring also facilitates the transfer of target analyte from donor solution to acceptor phase and improves the extraction efficiency. In this study, the stirring rates ranging from 100 to 1000 rpm were investigated. The extraction efficiency was highest at 500 rpm (Fig. 1D). Hence, 500 rpm was selected as a sample solution stirring rate for further optimization. At higher stirring rate, extraction efficiency decreased because the immobilized membrane organic solvent was removed from the hollow fiber wall pores.

#### Effect of salt addition to the sample solution

Salt addition could enhance the extraction efficiency due to salting-out effect. In some



**Fig. 1.** Optimization of (A) organic solvent, (B) sample solution pH, (C) acceptor phase pH and (D) stirring rate for extraction of cyproheptadine

instances, the high concentration of salt changed the physical properties of the Nernst diffusion film and increased the aqueous solution viscosity, which lead to difficult mass transfer and decrease extraction efficiency. Thus, NaCl was added at concentration of 0% to 20% (w/v) of the sample solution and results are shown in Fig. 2A. The extraction efficiency, decreased when the NaCl concentration was increased from 0 to 20% (w/v). Therefore, extraction without salt addition was chosen as optimal condition, and used for all further experiments.

#### Effect of extraction time

Thus, the effect of the extraction time was studied from 10 to 60 min. The result in Fig. 2B showed that up to 50 min, the extraction efficiency rapidly increased. The mass transfer is a time-dependent process. Thus, extraction efficiency of

target analyte increased with extraction time. However, when the extraction time was longer than 50 min, the extraction efficiency slightly decreased due to membrane organic solvent instability and dissolution in the sample solution. Therefore, an extraction time of 50 min was selected as the extraction time in all further experiments.

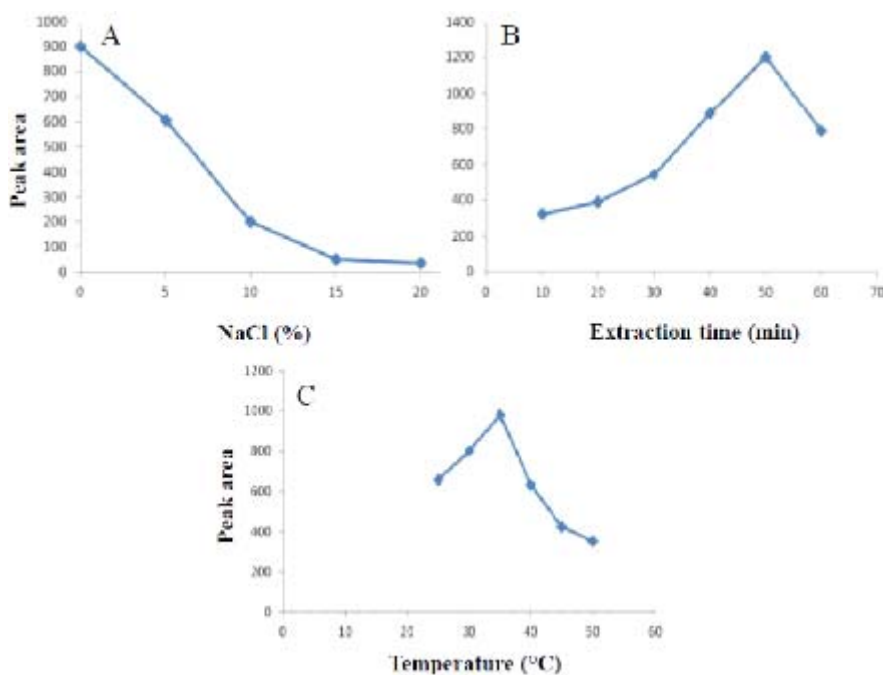
#### Effect of extraction temperature

The sample solution temperature plays very important roles in the three phases SBME. The effect of temperature on the extraction efficiency of cyproheptadine was examined over a temperature range of 25 to 50 °C and result is shown in Fig. 2C. Increasing of temperature from 25 to 35 °C increase extraction efficiency. Increasing extraction temperature accelerates the mass transfer rates of analytes and increase extraction efficiency. But when the extraction temperature increased over

**Table 2.** Figures of merit of SBME in drug-free distilled water sample.

LOD (ngmL <sup>-1</sup> )	LOQ (ngmL <sup>-1</sup> )	Linearity (ngmL <sup>-1</sup> )	R <sup>2</sup>	PF	RSD% <sup>a</sup> (n=4)	
					Within day	Between day
15.0	45.0	45.0-5000.0	0.9885	105	3.15	3.57

<sup>a</sup> Within day and between day RSDs% were obtained by four replications.



**Fig. 2.** Optimization of (A) salt addition effect, (B) extraction time (C) temperature for extraction of cyproheptadine

than 35 °C, significant decrease in extraction efficiency was observed. Therefore, all further experiments were performed in 35 °C as a sample solution temperature.

#### Method evaluation

Under the optimized conditions, a series of experiments were performed to determine the linear ranges, preconcentration factor, and LODs of the proposed method. As shown in Table 2, target analyte exhibited good linearity in the range of 45.0–5000  $\mu\text{g L}^{-1}$  with correlation coefficient  $r^2 > 0.9885$ . The limits of the detections were less than 15.0  $\mu\text{g L}^{-1}$ . The preconcentration factor were 105-fold and the relative standard deviations (RSDs)

were less than 3.15% and 3.57% for intraday and interday experiment, respectively.

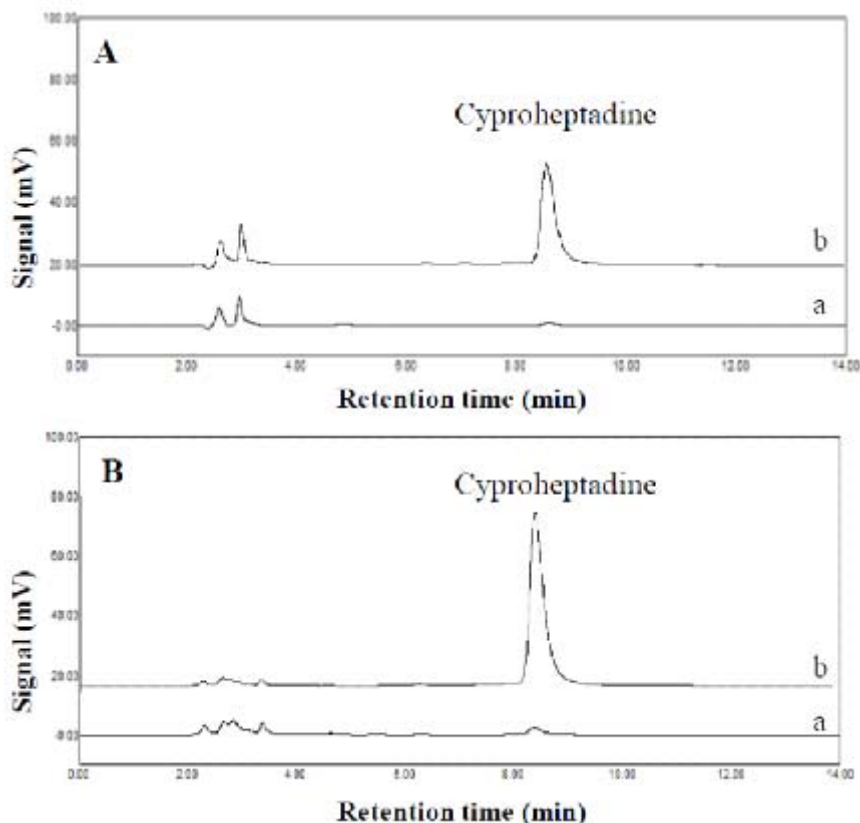
#### Real samples analysis

The applicability of the proposed method was evaluated by analysis of two biological real samples, human urine and human plasma. In order to reduce matrix effects, calibration curves were plotted in drug free biological samples. Drug-free human urine and plasma samples was a diluted before SBME (1:4) with ultra pure water and added the proper amount of NaOH solution to achieve pH of sample solution to 10.5. The analytical results were shown in Table 3. In both of the two samples, target analyte was below their detection limits, so

**Table 3.** Determination of cyproheptadine in urine sample

Sample	$C_{real}$ (ngmL <sup>-1</sup> )	$C_{added}$ (ng mL <sup>-1</sup> )	$C_{found}$ (ng mL <sup>-1</sup> )	RSD% (n = 4)	RR%
Plasma	nd <sup>a</sup>	200.0	175.0	3.54	87.5
Urine	nd	200.0	183.0	4.3	91.5

<sup>a</sup>Not detected



**Fig. 3.** Chromatograms obtained after SBME extraction of (A) plasma and (B) urine samples ((a) non-spiked sample and (b) spiked sample at a concentration level of 0.2 mg L<sup>-1</sup> for plasma and urine samples).



**Table 4.** Comparison of the SBME with other analytical techniques for determination of cyproheptadine

Analytical method	Sample preparation method	Sample	LOD (ngmL <sup>-1</sup> )	Linearity (ngmL <sup>-1</sup> )	RSD%	Ref.
HPLC	SBME	Plasma	15.0	50.0-5000.0	2.7	This work
LC-MS-MS	SPE	Bovine urine	-	150.0-10000.0	9.4	[36]
HPLC-DAD	DLLME	Human urine	13.1	20.0-4500.0	4.9	[37]
HPLC-MS-MS	LLE	Plasma	50.0	50.0-10000.0	-	[38]
HPLC	SPE	Urine	15.0	-	-	[39]
HPLC	Extraction	Dog plasma/ human urine	50.0	200.0-3000.0	-	[13]
HPLC	Solvent extraction	Human plasma/ Human milk	15.0	50.0-739.0	-	[40]

plasma and urine samples were spiked with the target analyte at a concentration of 0.2 mg L<sup>-1</sup>. The relative recoveries were calculated by the concentration found to that spiked. Table 3 showed that the relative recoveries were in the range of 87.5 and 91.5%. The typical chromatograms were shown in Fig. 3.

#### Comparison of the proposed method with other methods

Comparison of the proposed method with various extraction methods for extraction and determination of cyproheptadine is summarized in Table 4. It is shown that, the proposed method provided good linearity range, high extraction efficiency, and a suitable sensitivity.

#### Concluding remarks

In this work, a new and fast analytical method based on SBME coupled with high performance liquid chromatography has been developed. Application of this method to the detection and determination of cyproheptadine in urine and plasma samples. An effective sample cleanup with a high preconcentration factor and extraction efficiency were obtained, as well as good linearity. The advantages of this method will allow its facility application to the analysis of low concentration levels of cyproheptadine in urine and plasma samples.

#### REFERENCES

1. K.G. Wu, T.H. Li, T.Y. Wang, C.L. Hsu, C.J. Chen, A comparative study of loratadine syrup and cyproheptadine HCL solution for treating perennial allergic rhinitis in taiwanese children aged 2-12 years, *International Journal of Immunopathology and Pharmacology*, 25 (2012) 231-237.
2. F.J. Waxman, J.M. Taguiam, C.C. Whitacre, Modification of the clinical and histopathologic expression of experimental allergic encephalomyelitis by the vasoactive amine antagonist cyproheptadine, *Cellular Immunology*, 85 (1984) 82-93.
3. C.S. Baraf, Treatment of pruritis in allergic dermatoses: an evaluation of the relative efficacy of cyproheptadine and hydroxyzine, *Current Therapeutic Research - Clinical and Experimental*, 19 (1976) 32-38.
4. F.a.D. Administration, *Guidance for Industry; Bioanalytical Method Validation*, , Rockville, U.S. Department of Health and Human Services, 2001.
5. S.W.J. Lamberts, T. Verleun, R. Oosterom, The mechanism of action of cyproheptadine on prolactin release by cultured anterior pituitary cells, *Life Sciences*, 36 (1985) 2257-2262.
6. P. Donatsch, D.A. Lowe, B.P. Richardson, P. Taylor, Mechanism by which cyproheptadine inhibits insulin secretion, *British Journal of Pharmacology*, 70 (1980) 355-362.
7. J.N. Stiel, G.W. Liddle, W.W. Lacy, Studies of mechanism of cyproheptadine-induced weight gain in human subjects, *Metabolism*, 19 (1970) 192-200.
8. J. Wortsman, N.G. Soler, J. Hirschowitz, Side effects of cyproheptadine, *British Medical Journal*, 1 (1978) 1217.
9. K.E. Von Muehlendahl, E.G. Krienke, Toxicity of cyproheptadine side effects and accidental overdose, *Monatsschrift fur Kinderheilkunde*,

- 126 (1978) 123-126.
10. M. Maham, V. Kiarostami, S. Waqif-Husain, P. Abroomand-Azar, M. Saber-Tehrani, M.K. Sharifabadi, H. Afrouzi, M. Shapouri, R. Karami-Osboo, Extraction and determination of cyproheptadine in human urine by DLLME-HPLC method, *Iranian Journal of Pharmaceutical Research*, 12 (2013) 311-318.
  11. A. El-Gindy, F. El-Yazby, A. Mostafa, M.M. Maher, HPLC and chemometric methods for the simultaneous determination of cyproheptadine hydrochloride, multivitamins, and sorbic acid, *Journal of Pharmaceutical and Biomedical Analysis*, 35 (2004) 703-713.
  12. J.E. Koountourellis, K.O. Ebete, Reversed-phase high performance liquid chromatographic determination of cyproheptadine from urine by solid-phase extraction, *Journal of Chromatography B: Biomedical Applications*, 664 (1995) 468-471.
  13. N.H. Foda, H.W. Jun, J.W. McCall, Quantitation of Cyproheptadine in Plasma and Urine by HPLC, *Journal of Liquid Chromatography*, 9 (1986) 817-830.
  14. E.A. Novak, M. Stanley, I.M. McIntyre, L.M. Hryhorczuk, High-performance liquid chromatographic method for quantification of cyproheptadine in serum or plasma, *Journal of Chromatography B: Biomedical Sciences and Applications*, 339 (1985) 457-461.
  15. G.W. Burrows, C.L. Alliger, High-performance liquid chromatographic determination of cyproheptadine hydrochloride in tablet formulations, *Journal of Pharmaceutical Sciences*, 72 (1983) 1212-1213.
  16. X. Feás, L. Ye, S.V. Hosseini, C.A. Fente, A. Cepeda, Development and validation of LC-MS/MS method for the determination of cyproheptadine in several pharmaceutical syrup formulations, *Journal of Pharmaceutical and Biomedical Analysis*, 50 (2009) 1044-1049.
  17. H.B. Hucker, J.E. Hutt, Determination of cyproheptadine in plasma and urine by GLC with a nitrogen-sensitive detector, *Journal of Pharmaceutical Sciences*, 72 (1983) 1069-1070.
  18. R.T. Sane, P.P. Karkhanis, P.G. Anaokar, Gas chromatographic estimation of cyproheptadine in pharmaceuticals, *Indian Journal of Pharmaceutical Sciences*, 43 (1981) 111-112.
  19. R.X. Liu, J.R. Lü, Determination of cyproheptadine hydrochloride with riboflavin chemiluminescence system, *Fenxi Huaxue/Chinese Journal of Analytical Chemistry*, 37 (2009) 267-270.
  20. M. Arvand, A. Pourhabib, Adsorptive stripping differential pulse voltammetric determination of risperidone with a multi-walled carbon nanotube-ionic liquid paste modified glassy carbon electrode, *Journal of the Chinese Chemical Society*, 60 (2013) 63-72.
  21. I.H. Tasdemir, O. Çakirer, N. Erkb, E. Kiliçç, Square-wave cathodic adsorptive stripping voltammetry of risperidone, *Collection of Czechoslovak Chemical Communications*, 76 (2011) 159-176.
  22. O. Cakirer, I.H. Tasdemir, Electrochemical and spectrophotometric methods to assay of risperidone an antipsychotic drug in pharmaceutical and biological samples, *Asian Journal of Chemistry*, 22 (2010) 6353-6365.
  23. M.S. Hazem, S.M. Saadeh, Chemically modified carbon paste electrode for potentiometric analysis of cyproheptadine hydrochloride in serum and urine, *Canadian Journal of Analytical Sciences and Spectroscopy*, 52 (2007) 225-232.
  24. X. Jiang, H.K. Lee, Solvent Bar Microextraction, *Analytical Chemistry*, 76 (2004) 5591-5596.
  25. M. Ghambarian, Y. Yamini, A. Esrafil, Developments in hollow fiber based liquid-phase microextraction: principles and applications, *Microchim Acta*, 177 (2012) 271-294.
  26. J. Zhou, Q. Zhang, J.B. Sun, X.L. Sun, P. Zeng, Two-phase hollow fiber liquid phase microextraction based on magnetofluid for simultaneous determination of Echinacoside, Tubuloside B, Acteoside and Isoacteoside in rat plasma after oral administration of Cistanche salsa extract by high performance liquid chromatography, *Journal of Pharmaceutical and Biomedical Analysis*, 94 (2014) 30-35.
  27. J. Zhou, P. Zeng, J.B. Sun, F.Q. Wang, Q. Zhang, Application of two-phase hollow fiber liquid phase microextraction coupled with high-performance liquid chromatography for the study of the echinacoside pharmacokinetics in Parkinson's disease rat plasma, *Journal of Pharmaceutical and Biomedical Analysis*, 81-82 (2013) 27-33.
  28. Y. Tao, J.-F. Liu, T. Wang, G.-B. Jiang, Simultaneous conduction of two- and three-phase hollow-fiber-based liquid-phase microextraction for the determination of aromatic amines in environmental water samples, *Journal of Chromatography A*, 1216 (2009) 756-762.
  29. M. Hadjmohammadi, H. Ghambari, Three-phase hollow fiber liquid phase microextraction of warfarin from human plasma and its determination by high-performance liquid chromatography, *Journal of Pharmaceutical and Biomedical Analysis*, 61 (2012) 44-49.
  30. M. Mirzaei, H. Dinpanah, Three phases hollow fiber LPME combined with HPLC-UV for



- extraction, preconcentration and determination of valerianic acid in *Valeriana officinalis*, *Journal of Chromatography B*, 879 (2011) 1870-1874.
31. M. Ghambarian, Y. Yamini, A. Esrafil, Three-phase hollow fiber liquid-phase microextraction based on two immiscible organic solvents for determination of tramadol in urine and plasma samples, *Journal of Pharmaceutical and Biomedical Analysis*, 56 (2011) 1041-1045.
  32. F. Piroozi, E. Ghasemi, M. Qomi, R. Rezaee, F. Hashemian, Hollow fiber liquid phase microextraction combined with high performance liquid chromatography for preconcentration and determination of cabergoline in biological samples, *Journal of Liquid Chromatography & Related Technologies*, 37 (2013) 760-771.
  33. F. Shamshiri, M. Qomi, S. N. Miraei, A. Shamshiri, Hollow fiber based liquid phase microextraction of trace amounts of citalopram from biological fluids followed by high performance liquid chromatography, *International journal of current life sciences*, 4 (2014) 12261-12267.
  34. S. N. Miraei, M. Qomi, F. Shamshiri, P. Raoufi, Hollow-Fiber liquid-phase Microextraction Followed by High Performance Liquid Chromatography for the Determination of Trace Amounts of Methylphenidate Hydrochloride in Biological Fluids, *Biomedical & Pharmacology Journal*, 4 (2014) 715-725.
  35. K.E. Rasmussen, S. Pedersen-Bjergaard, Developments in hollow fibre-based, liquid-phase microextraction, *TrAC Trends in Analytical Chemistry*, 23 (2004) 1-10.
  36. C.A. Fente, P. Regal, B.I. Vázquez, X. Feás, C.M. Franco, A. Cepeda, Development and Validation of an LC-MS/MS Confirmatory Method for Residue Analysis of Cyproheptadine in Urine of Food-Producing Animals, *Journal of Agricultural and Food Chemistry*, 57 (2009) 2595-2598.
  37. M. Maham, V. Kiarostami, S. Waqif-Husain, P. Abroomand-Azar, M.S. Tehrani, M. Khoeini Sharifabadi, H. Afrouzi, M. Shapouri, R. Karami-Osboo, Extraction and Determination of Cyproheptadine in Human Urine by DLLME-HPLC Method, *Iranian Journal of Pharmaceutical Research : IJPR*, 12 (2013) 311-318.
  38. G.D. Mendes, A. Arruda, L.S. Chen, J.C. de Almeida Magalhães, K.M. Alkharfy, G. De Nucci, Quantification of cyproheptadine in human plasma by high-performance liquid chromatography coupled to electrospray tandem mass spectrometry in a bioequivalence study, *Biomedical Chromatography*, 26 (2012) 129-136.
  39. J.E. Kountourellis, K.O. Obete, Reversed-phase high performance liquid chromatographic determination of cyproheptadine from urine by solid-phase extraction, *Journal of Chromatography B: Biomedical Sciences and Applications*, 664 (1995) 468-471.
  40. J.E. Kountourellis, K.O. Obete, E.T. Malliou, Isocratic high performance liquid chromatographic method for the quantitation of cyproheptadine in human milk and plasma using solvent and solid phase extraction techniques, *Journal of Liquid Chromatography & Related Technologies*, 22 (1999) 603-614.
  41. S.N. Miraei, M. Qomi, F. Shamshiri, P. Raoufi, Hollow-Fiber liquid-phase Microextraction Followed By High Performance Liquid Chromatography For The Determination Of Trace Amounts Of Methylphenidate Hydrochloride in Biological Fluids, *Biomedical & Pharmacology Journal*, Vol. 7(2), (2014) 715-725.
  42. F. Shamshiri, M. Qomi, S.N. Miraei, A. Shamshiri, Hollow Fiber Based Liquid Phase Microextraction Of Trace Amounts Of Citalopram From Biological Fluids Followed By High Performance Liquid Chromatography, *International Journal of Current Life Sciences - Vol.4, Issue, 12*, pp. 12261-12267, December, 2014.