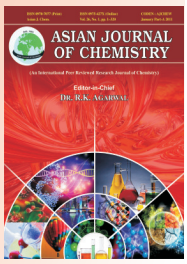
Asian Journal of Chemistry; Vol. 26, No. 19 (2014), 6650-6654

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17150



Determination of Prilocaine HCl in Pharmaceutical Preparations by GC-MS Method

ALPTUG ATILA and YUCEL KADIOGLU*

Department of Analytical Chemistry, Faculty of Pharmacy, Ataturk University, 25240 Erzurum, Turkey

*Corresponding author: Fax: +90 442 2360962; Tel: +90 442 2315212; E-mail: yucelkadi@yahoo.com

Received: 31 January 2014; *Accepted*: 30 April 2014; *Published online*: 16 September 2014; AJC-15980

The novel analytical method using gas chromatography with mass spectrometry detection (GC-MS) method for determination of prilocaine HCl in pharmaceutical preparations were developed and validated. The using lidocaine HCl as the internal standard (IS) was carried out on HP-5MS capillary column. The calibration curves were linear (R = 0.999) in concentration range of 40-1000 ng/mL of prilocaine HCl. The LOD and LOQ values were 15 and 31 ng/mL, respectively. The within- and between-day RSD and RE % values of proposed method were found to be ≤ 3.4 and ≤ 3.5 %, respectively. *o*-Toluidine was formed in stored on auto sampler during 72 h was determined by proposed method. The developed method can be directly and easily applied for determination of prilocaine HCl without derivatized in pharmaceutical preparations using internal standard methodology.

Keywords: GC-MS method, Prilocaine HCl, Pharmaceutical preparations, Validation.

INTRODUCTION

Local anesthetic drugs are mainly used to reversibly block nerve function in various local or regional treatments. Local anesthetics play an important role clinically in dentistry and minor surgery for temporary relief of pain[1,2]. Prilocaine (2-propilamino-N-*o*-tolil-propiyonamit hydrochloride) (Fig. 1-A) is one of local anesthetics with the amide type[3]. Prilocaine, unlike other amide anesthetics, is a secondary amino derivative of toludine. It produces less vasodilation and toxicity than lidocaine and is considered relatively free from an allergic reaction[4]. Prilocaine is extensively metabolized by the liver. Prilocaine's primary limiting factor clinically is the production of methemoglobinemia, a side effect caused by its metabolite *o*-toludine[5-7].

The literature shows that capillary electrophoresis[3], HPLC with different detection[8-19], Liquid chromatography-tandem mass spectrometry[20], sequential injection chromatography with Franz cell[21] and adsorptive square wave method[22] have been reported for determination of alone prilocaine and the binary mixture with other local anesthetics of prilocaine in biological samples (plasma and serum) and pharmaceutical preparations. Besides the determination of prilocaine and the mixtures with other local anesthetics of prilocaine in biological materials (plasma and urine) have been done with gas chromatography-mass spectrometry method[23,24] and gas chromatography method[25-31].

To our best of knowledge, no GC-MS method for the analysis of prilocaine HCl in any pharmaceutical preparations was available in the literature. The development and validated an effective method for analysis of drugs in pharmaceutical preparations and biological samples for understanding the therapeutic and toxic effects of drugs is critically important. The aim of the present work is to develop and validate a new GC-MS method for determination of alone prilocaine HCl with a simple sample preparation without derivatization in pharmaceutical preparations using internal standard methodology.

EXPERIMENTAL

Prilocaine HCl reference substance and lidocaine HCl used as internal standard (Fig. 1-B, IS) was a kindly supplied by Novartis Pharmaceutical Industry (Ankara, Turkey) and Doping Control Center of Hacettepe University (Ankara, Turkey). The high-purity all other reagents were purchased from Merck (Germany). All gases were supplied by Havas (Ankara, Turkey)

Pharmaceutical preparations: The following pharmaceutical preparations of prilocaine HCl were obtained from local sources in Erzurum (Turkey):

Emla® cream (5 % cream, Astra Zeneca A.S., Turkey) containing 25 mg prilocaine HCl, 25 mg lidocaine HCl, 55 mg poloxamer188 and 155 mg poloxomer 407.

Citanest® injection (2 % flacon, Astra Zeneca A.S., Turkey) containing prilocaine HCl 400 mg/20 mL, methyl *para*-hydroxybenzoate and sodium chloride.

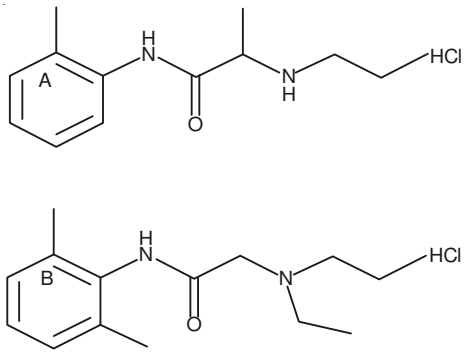


Fig. 1. Chemical structure of prilocaine HCl (A) and IS [lidocaine HCl (B)]

Determination was performed HP 6890 Series II gas chromatography equipped with a HP 7673 autosampler, electronic pressure control, Hewlett-Packard automatic injector (Model 7673) and an Agilent 5972 series mass selective detector with electron ionization. The chromatographic separation was performed on 5 % methyl phenyl silicone HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thicknesses) purchased from Hewlett-Packard (Waldbronn, Germany). The split mode (10:1) was used with helium carrier gas and the flow rate of carrier gas was kept constant during run at 1 mL/min. The injector volume was 1.5 µL. The injector and detector temperatures were set at 270 °C. The GC temperature gradient program was as follows: initial temperature 80 °C, held for 1.5 min; to finally temperature 260 °C at a rate 10 °C 1/min, held for 5 min. The MS detector parameters were: transfer line temperature 240 °C, solvent delay 2.5 min and electron energy 75 eV. The MS was run in the electron ionization (EI) mode with selected ion monitoring (SIM) with a 0.5 s/scan for quantitative analysis (*m/z* 86 for both prilocaine HCl and lidocaine HCL; *m/z* 106 for *o*-toluidine). The MS spectrums were given at Fig. 2 A-B.

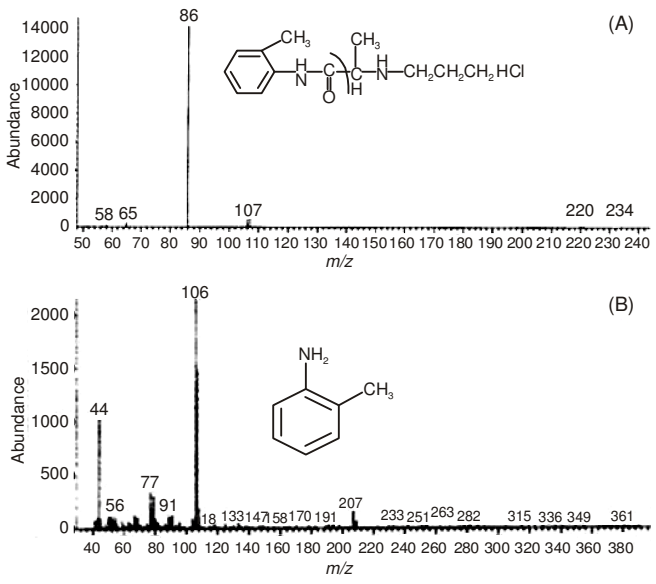


Fig. 2. MS spectra of prilocaine HCl (A) and *o*-toluidine (B)

Preparation of standard solution and quality control solutions: The standard working (SW) solutions (40, 100, 250, 500, 750 and 1000 ng/mL) and quality control (QC) solutions (100, 250 and 500 ng/mL) were daily prepared from stock solution (100 µg/mL) of prilocaine HCl in methanol and stored at -20 °C when not in use.

Internal standard (lidocaine HCl, IS) working solution was prepared at the final concentration of 100 ng/mL.

Procedure of pharmaceutical preparations

Citanest® injection (2 % flacon): The content of a flacon were mixed into flask and an aliquot of the solution equivalent to 20 mg prilocaine was quantitatively transferred to 50 mL-calibrated measuring flask and made up to the mark with methanol. The solution was filtered through a 0.22 µm millipore filter. The filtrate was adjusted to volume with methanol to obtain a 150 ng/mL concentration of prilocaine HCl for flacon.

Emla® cream (5 %): An amount of the cream equivalent to about 25 mg of prilocaine HCl, accurately weighed into a centrifuge tube, was mixed with methanol for 5 min in ultrasonic bath and centrifugated for 5 min 4000 rpm. The supernatant was filtered through a 0.22 mm millipore filter into calibrated flask. The filtrate was adjusted to volume with methanol to obtain a 150 ng/mL concentration of prilocaine HCl for cream sample.

About 100 ng/mL concentration of IS (*i.e.*, lidocaine) was added into the solutions prepared from cream and injections. The solutions were analyzed as described earlier. The content of prilocaine HCl in pharmaceutical preparations was quantified using regression equation.

Method validation: The validation of method was carried out by establishing specificity, linearity, recovery values, precision, accuracy and sensitivity according to international conference on harmonization guidelines[32] for validation of analytical procedures.

RESULTS AND DISCUSSION

Specificity: The specificity of method has been demonstrated by the representative chromatograms for prilocaine HCl and IS in standard solutions shown in Fig. 3. The retention time of prilocaine HCl and lidocaine HCl is 5.84 min and 6.58 min, respectively. Different temperature gradient programs were investigated for exception of matrix interference. At the end of this investigation, the best temperature program described earlier was selected for a good resolution.

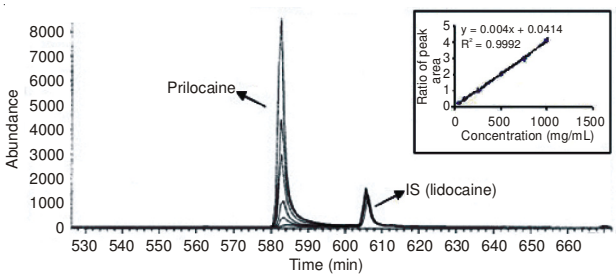


Fig. 3. Chromatograms of standard solutions (40, 100, 250, 500, 750 and 1000 ng/mL) of prilocaine and IS (100 ng/mL)

Linearity: Linearity of method was demonstrated over a linear range of A volume of 40 to 1000 ng/mL at six concentrations (40, 100, 250, 500, 750 and 1000 ng/mL) with 100 ng/mL concentration of IS (n = 6). The calibration curve was plotted by the ratio of the peak areas of prilocaine HCl and IS,

versus the concentrations of prilocaine HCl (Fig. 3). The linear regression equation was calculated by the least squares method using Microsoft Excel® program and summarized in Table-1.

TABLE-1
RESULTS OF REGRESSION ANALYSIS OF PRILOCAINE HCl

Parameters	GC-MS
Linearity (ng/mL)	40-1000
Regression equation[a]	y = 0.004x + 0.0414
Standard deviation of slope (Sa)	6.6×10^{-4}
Standard deviation of intercept (Sb)	6.4×10^{-4}
The standard deviation of the residuals, (Sy/x)	9.9×10^{-2}
Correlation coefficient	0.9992
Standard deviation of correlation coefficient	4.57×10^{-3}
Limit of detection (LOD, ng/mL)	15
Limit of quantification (LOQ, ng/mL)	31

[a]Average of six replicate determinations

Sensitivity: The limit of detection (LOD) is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The limit of quantitation (LOQ) is the lowest amount of analyte which can be quantitatively determined with suitable precision. The LOD and LOQ values were determined as 3:1 and 10:1 of the signal/noise ratio, respectively. LOD and LOQ values were given in Table-1. Both accuracy and precision of these values were well within the proposed criteria (RSD % < 20 %).

Precision and accuracy: The repeatability (intra-day) and intermediate precision (inter-day) were done in precision studies. The intra-day studies were evaluated by measuring QC samples, using six replicates at same concentrations and during the same day. The inter-day studies were done by comparing the assay the same samples on six different days. The precision of method were given as the relative standard deviation (RSD % = 100 × standard deviation/mean) and the accuracy were reported with percent relative error [RE % = (found concentration-known concentration) × 100/known concentration]. The RSD % values for intra-day and inter-day precision were found to be ≤ 3.4 %. The RE % for intra-day and inter-day accuracy were found to be ≤ 3.5 %. Precision and accuracy studies in pharmaceutical preparations showed acceptable RSD and RE % values as shown in Table-2.

Analytical recovery: To double check the accuracy of the proposed method, the standard addition technique was applied. The QC solutions were added to 150 ng/mL concentration of pharmaceutical preparations solutions and assayed with GC-MS method. The present analytical recovery of the added standard to the assay samples was calculated from followed equation:

$$\text{Analytical Recovery \%} = [(C_t - C_u)/C_a] \times 100$$

where C_t is total concentration of the analyte determined; C_u is the concentration of the pure analyte added to the preparation; C_a is the concentration of the present analyte in the preparation. The average per cent recoveries were quantitatively determined as 99.6 % for both preparations of method, indicating good accuracy of the method. No interference from the common excipients was observed. The RSD % values of recovery were found as ranged from 0.8 to 1.9 % (Table-3).

Interferences study: The effects of common excipients and additives were tested for their possible interferences in the assay of prilocaine HCl. In addition to the active ingredient, prilocaine HCl, flacon and cream content contains the following inactive ingredients: methyl *para*-hydroxybenzoate, sodium chloride, 55 mg poloxamer188 and 155 mg poloxomer 407. It has been determined any interference of these substances at the levels found in dosage forms.

Stability: Stability studies were performed with standard solutions of prilocaine HCl (250, 500 and 750 ng/mL) and these solutions were stored at 4 °C (refrigerator), room temperature and on auto sampler at[24,48] and 72 h time and then changes in concentration of solutions under conditions of the study were evaluated using the proposed method. One set of these solutions were assayed immediately and taken as standard (100 %). Stock solution of prilocaine HCl was found to be stable for three month. Standard solutions of prilocaine HCl were found to be stable for 48 h at room temperature and on auto sampler. After 72 h at room temperature and auto sampler, it was observed that prilocaine were converted to its metabolite

TABLE-2
PRECISION AND ACCURACY OF PROPOSED METHOD

Added (ng/mL)	Intra-day			Inter-day		
	Found ± SD	Precision RSD (%	Accuracy RE (%)	Found ± SD	Precision RSD (%	Accuracy RE (%)
100	96.5 ± 3.2	3.3	-3.5	101.5 ± 3.5	3.4	1.5
250	247.4 ± 6.3	2.5	-1.0	249.9 ± 6.6	2.6	-0.04
500	486.9 ± 8.6	1.8	-2.6	489.2 ± 6.4	1.3	-2.1

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, RE: Relative error

TABLE-3
ANALYTICAL RECOVERY VALUES BY STANDARD ADDITION METHOD (n = 6)

Pharmaceutical preparations	Amount taken (ng/mL)	Amount added (ng/mL)	Total amount found (ng/mL) (mean ± SD)	Recovery (%)	RSD (%)
Emla® cream (5 %)	150	100	247.9 ± 2.6	98.6	1.1
		250	398.9 ± 4.7	99.3	1.2
		500	649.2 ± 5.1	99.5	0.8
Citanest® injection (2 % flacon)	150	100	249.9 ± 4.9	99.9	1.9
		250	400.9 ± 5.2	100.6	1.3
		500	649.7 ± 7.6	99.8	1.2

(*o*-toluidine) (Fig. 4). Because of its chemical structure, prilocaine is readily hydrolyzed in alcohol medium. The formation of *o*-toluidine as a major degradation product in solutions of prilocaine HCl was checked by spiking of the standard solution of *o*-toluidine. *o*-Toluidine could be formed during degradation of prilocaine HCl was based on information in literature that amide anesthetics were degraded to *o*-toluidine in temperature change[16,17].

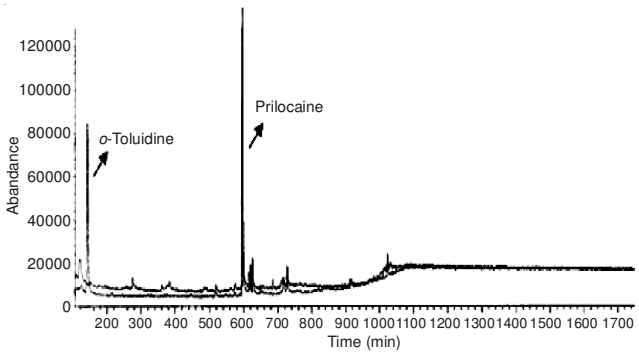


Fig. 4. Stability of prilocaine HCl on auto sampler for 72 h time

Application of the method for analysis of pharmaceutical preparations: The proposed method was evaluated in the assay of commercially available flacon containing prilocaine HCl 400 mg/20 mL and cream containing 25 mg prilocaine and 25 mg lidocaine. Evaluation was performed using the calibration curve method since no significant difference between the slopes of the calibration curves for standards and pharmaceutical preparations solution was observed. The results obtained are satisfactorily accurate and precise as indicated by the excellent % recovery (Table-4). Experiments showed that there was no interference from the additions and excipients (Fig. 5). The determination repeated for six times, final recovery of method for both preparations was obtained approximately to be 99.6 and 99.2 %, with an RSD % of 2.85 and 1.29 %.

In the present study, we report a highly selective gas chromatography (GC) combined mass spectrometry detection (MS) that enabled us to quantify the prilocaine HCl without derivatization in pharmaceutical preparations. Prilocaine HCl is one of local anesthetic substances. An important aspect of the implementing a new assay in routine quality control analysis is that it should be thoroughly evaluated before introduction for routine use. GC method with different detections can be considered to be a very appropriate method for analysis of local anesthetic substances that these are very volatile substances. A rapid and reliable GC-MS method were developed and validated for the determination of prilocaine HCl in pharmaceutical preparations in the form of cream and flacon. The proposed method has supplied all the requirements in terms of accuracy, linearity, recovery and precision that could

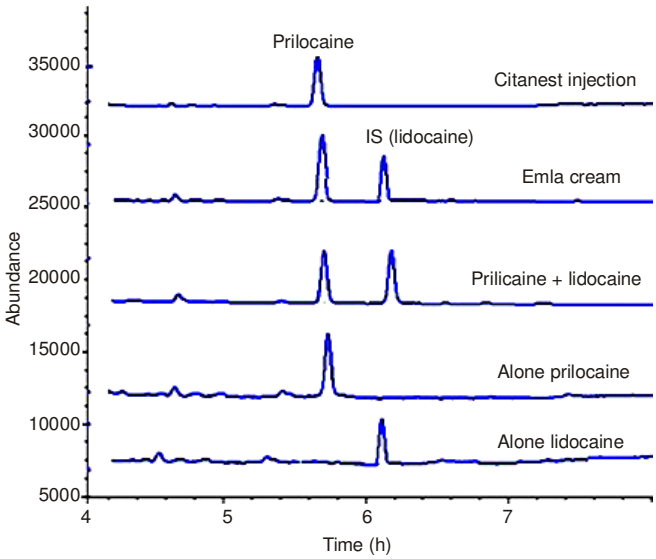


Fig. 5. Chromatograms of pharmaceutical preparations

be accepted as a reliable and applicable method. The precision of method were adequate, because the RSD % values were less than 3.4 %. Accuracy of method (RE %) was less than 3.5 %. There are several advantages of this method which are high specificity, good accuracy and precision values, short run time (6.3 min). In the chromatograms taken, the following peaks: prilocaine HCl with retention time approx. 5.82 min; lidocaine HCl used IS with retention time approx. 6.08 min and the degradation product *o*-toluidine with retention time of approx. 1.96 min (Fig. 3, 4). Under the described conditions (Fig. 3) a linear relationship between the peak-area ratio (y: prilocaine peak/IS peak) and analyte concentration (x) were obtained (Table-1). In addition to these, the analytical recovery percentage of proposed method is high.

There are many preparations for local anesthesia on the pharmaceutical market, in which prilocaine HCl and lidocaine HCl can occur as active substances. Both drugs were served as internal standard for each other. Prilocaine used in anesthetic practice is least toxic than lidocaine. *o*-Toluidine is a metabolite of prilocaine HCl. Prilocaine metabolizes to *o*-toluidine during biotransformation, which may oxidize hemoglobin to methemoglobin and also *o*-toluidine has been shown to be carcinogenic in laboratory animals in NTP studies. *o*-Toluidine can be potential technological impurities of medicinal products because they are used as substrates in the synthesis of pharmaceuticals. In addition, the hydrolysis of the amide linkage of prilocaine results in the formation of decomposition product *o*-toluidine during storage of drugs containing prilocaine[16,17,33]. After 72 h at room temperature and auto sampler, it was observed that prilocaine were converted to its metabolite (*o*-toluidine). After 72 h storage of solutions of prilocaine HCl, it was observed that has formed of *o*-toluidine in study. The

TABLE-4
DETERMINATION OF PRILOCAINE HCl IN PHARMACEUTICAL PREPARATIONS

Pharmaceutical preparations	[a]Found ± SD (mg)	Recovery (%)	RSD (%)	Confidence Interval
Emla®Cream (5 %) (25 mg prilocaine)	24.91 ± 0.71	99.6	2.85	98.0-101.0
Citanest® Injection (2 % flacon, prilocaine HCl 400 mg/20 mL)	396.8 ± 5.12	99.2	1.29	98.8-100.0

SD: Standard deviation, RSD: Relative standard derivation, [a]Average of six replicate determinations

content of *o*-toluidine in standard solutions chosen for this study was determined by the standard curve method.

Conclusion

In this study, new GC-MS method has been developed to provide a sensitive, simple and quantitative assay of prilocaine HCl in pure and pharmaceutical preparations. When the solutions of prilocaine HCl were stored at room temperature and on auto sampler during 72 h, it was observed that *o*-toluidine was formed. *o*-Toluidine was determined by proposed method. The preparation of samples and their analysis was performed in a relative short time. Therefore, it was suggested that the GC-MS method was equally applicable. Besides, the proposed method can be applicable in routine quality control and clinical laboratories.

ACKNOWLEDGEMENTS

The authors are grateful to Ataturk University for financial support of this works and Doping Control Centre of Hacettepe University for GC-MS equipment used.

REFERENCES

1. B. Rishiraj, J.B. Epstein, D. Fine, S. Nabi and N.K. Wade, *Int. J. Oral Maxillofac. Surg*., **34**, 220 (2005).
2. R.A. Cawson, D.R. Curson and D.R. Whittington, *Br. Dent. J.*, **154**, 253 (1983).
3. M. Siluveru and J.T. Stewart, *J. Chromatogr. B*, **693**, 205 (1997).
4. J.Y. Jastak, J.A. Yagela and D. Donaldson, In: Pharmacology of Local Anesthesia and Clinical Preparations and Drug Selection, Philadelphia: WB Saunders, p. 23 (1995).
5. R.E. Warren, T.B. Van de Mark and S. Weinberg, *Oral Surg. Oral Med. Oral Pathol*., **37**, 866 (1974).
6. E.J. Ariens, in ed.: A.M. Krstulovic, In: Chiral Separation in HPLC, Applications to Pharmaceutical Compounds, Ellis Harwood, W. Sussex, p. 31 (1989).
7. B. Rudlof, D. Durstewitz-Knierim, I. Ridderskamp, C. Scharenberg and L. Brandt, *Anaesthesist*, **44**, 445 (1995).
8. M. Siluveru and J.T. Stewart, *J. Pharm. Biomed. Anal*., **15**, 389 (1996).
9. A. Taddio, B. Stevens, K. Craig, P. Rastogi, S. BenDavid, A. Shennan, P. Mulligan and G. Koren, *N. Engl. J. Med*., **336**, 1197 (1997).
10. G.T. Tucker, L.E. Mather, M.S. Lennard and A. Gregory, *Br. J. Anaesth*., **65**, 333 (1990).
11. R. Whelpton, P. Dudson, H. Cannell and K. Webster, *J. Chromatogr. A*, **526**, 215 (1990).
12. K. Wiberg, M. Andersson, A. Hagman and S.P. Jacobsson, *J. Chromatogr. A*, **1029**, 13 (2004).
13. L.M. Storms and J.T. Stewart, *J. Pharm. Biomed. Anal*., **30**, 49 (2002).
14. K. Wiberg and S.P. Jacobsson, *Anal. Chim. Acta*, **514**, 203 (2004).
15. H.A. Adams, J. Biscoping, K. Ludof, A. Borgmann, B. Beckman-M and G. Hempelmann, *Reg. Anaesth*., **12**, 53 (1989).
16. Z. Fijalek, E. Baczynski, A. Piwonska and M. Warowna-Grzeskiewicz, *J. Pharm. Biomed. Anal*., **37**, 913 (2005).
17. E. Bacynski, A. Piwonska and Z. Fijalek, *Pol. Pharm*., **59**, 333 (2002).
18. J. Klein, D. Fernandes, M. Gazarian, G. Kent and G. Koren, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **655**, 83 (1994).
19. M.A.-A. Mohammad, *Chromatographia*, **70**, 563 (2009).
20. A. Koehler, R. Oertel and W. Kirch, *J. Chromatogr. A*, **1088**, 126 (2005).
21. J. Klimundova, D. Satinsky, H. Sklenarova and P. Solich, *Talanta*, **69**, 730 (2006).
22. P. Norouzi, M.R. Ganjali, R. Dinarvand, M.H. Eshraghi and H.A. Zamani, *Russ. J. Electrochem*., **46**, 999 (2010).
23. T. Watanabe, A. Namera, M. Yashiki, Y. Iwasaki and T. Kojima, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **709**, 225 (1998).
24. Y. Kadioglu and A. Atila, *Biomed. Chromatogr*., **21**, 1077 (2007).
25. A. Asada, *Osaka City Med. J*., **25**, 91 (1979).
26. M. Prat, *B. Clin. Chem*., **32**, 2098 (1986).
27. E.F. Salim and B. Örtenblad, *J. Pharm. Sci*., **56**, 1645 (1967).
28. J.D. Cameron, *Clin. Chim. Acta*, **56**, 307 (1974).
29. M. Björk, K.-J. Pettersson and G. Österlöf, *J. Chromatogr. A*, **533**, 229 (1990).
30. O.W. Lau, K. Chan and Y.C. Wong, *Reg. Anaesth*., **13**, 118 (1990).
31. Y. Kadioglu and A. Atila, *Chromatographia*, **67**, 755 (2008).
32. Validation of Analytical Procedures, Proceedings of the International Conference on Harmonisation (ICH), Commission of the European Communities (1996).
33. K. Gaber, U.A. Harreus, C. Matthias, N.H. Kleinsasser and E. Richter, *Toxicology*, **229**, 157 (2007).