



Received on 13 December, 2011; received in revised form 12 January, 2012; accepted 29 March, 2012

DETERMINATION OF PRILOCAINE HCl IN PHARMACEUTICAL PREPARATION AND HUMAN PLASMA WITH SPECTROPHOTOMETRY

Alptug Atila* and Yucel Kadioglu

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

ABSTRACT

Keywords:

Prilocaine HCl,
Spectrophotometry,
Validation

Correspondence to Author:

Alptug Atila

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

A simple and sensitive spectrophotometric method was developed for the determination of prilocaine HCl in pharmaceutical preparation and human plasma. The quantitative analysis of prilocaine HCl was carried out using of wavelength at 230 nm. The method was linear in concentration range of 3-15 $\mu\text{g/mL}$ for standard solution and 4-15 $\mu\text{g/mL}$ for human plasma. Linearity was determined by calculating correlation coefficient. These values were found as a 0.9999 and 0.9967 in standard solution and human plasma, respectively. Developed spectrophotometric method was found suitable in terms of accuracy, sensitivity, precision, reproducibility. In addition to these, this method could be easily and directly applied to both human plasma and pharmaceutical preparation.

INTRODUCTION: The reason of using local anesthetics is to block the conduction of impulses in nerve fibers which cause anesthesia. They are most commonly used in dentistry and minor surgery in order to provide temporary relief of pain. There are two application types of local anesthetics which are topical and parenteral applications that reversibly block the nerve conductances.

Overdose of local anesthetics are toxic, causing cardiovascular and central-nervous disorders, and because of that reason therapeutic blood-plasma/serum concentrations should be kept in a certain concentration range.

Similar to many other drugs, local anesthetics bind to plasma proteins to some extent. α_1 -acid glycoprotein and albumin¹⁻². Prilocaine HCl, 2-propilamino-N-o-tolil-propionamit hydrochloride, is a drug. The chemical structure of prilocaine HCl is shown in **Figure 1**.

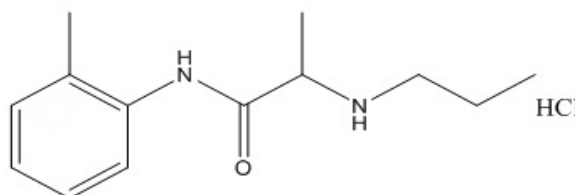


FIG. 1: CHEMICAL STRUCTURE OF PRILOCAINE HCl

Prilocaine is metabolized in liver. Prilocaine is hydrolyzed to hydrolyse to *o*-toluidine (OT) and N-propylalanine by the help of amidases enzymes. The main reason of methaemoglobinemia is taking overdose of prilocaine (exceeding 600 mg in adults or 8 mg kg^{-1} in children) which increases the concentration of *o*-toluidine³⁻⁵. In newborn infants, this enzyme, methaemoglobin, is not complete its development. Therefore, determination of prilocaine is clinically important for those patients. In these patients, less vasodilatation is clinically observed which is similar to other amide local anesthetics in terms of relative freedom from allergic reactions. For cardiac patients, It received short procedures which is administered in a plain solution⁶.

In order to determine prilocaine, many studies have been reported in literature. Several chromatographic and spectroscopic techniques were developed in order to determine prilocaine concentrations in both pharmaceutical preparations and human plasma⁷⁻²¹.

There are many advantages of using modern methods of analysis (HPLC, GC, Mass) for drug assay which are their simplicity, speed, good specificity, excellent precision and accuracy. However, they involve complex equipments, which could not be available in most of the laboratories. Therefore, the aim of the present work is to develop a simple, sensitive and accurate spectrophotometric method for the determination of prilocaine HCl in pharmaceutical formulations and human plasma. The developed method was validated according to International Conference on Harmonization guidelines²².

MATERIALS AND METHODS:

Chemicals and reagents: Prilocaine HCl reference standard was kindly supplied from Novagenix Company (Ankara, Turkey). The purity of prilocaine HCl was tested by checking its melting point, UV, H NMR, C NMR, infrared spectra no impurities were found. The plasma samples were obtained from Atatürk University-Arastirma Hosbital in Erzurum, Turkey. Methanol and all other reagents were obtained from Merck (Germany). Citanest flacone was provided from the pharmacy (Erzurum, Turkey).

Instrumentation: Thermospectronic double beam UV-Vis spectrophotometer (HELIOSβ, Thermospectronic, Cambridge, UK) with Vision 32 Software was used. All of the measurements was made in 1-cm quartz cells at a scan speed of 600 nm min⁻¹ and scan range of 215-275 nm, fixed slit width of 2 nm.

Wavelength Selection: Scanning of standard solutions of prilocaine HCl (10 µg/mL) was achieved in the region of 200 nm to 400 nm against solvent blank using SCAN mode by UV/Visible spectrophotometer to get normal (zero order) spectrum.

Preparation of the Standard and Quality Control Solutions: An accurately weighed quantity of prilocaine HCl (10 mg) was transferred into 100 ml volumetric flask. 50 ml of methanol was added and the mixture was sonicated for 5 min. Volume was made up

with methanol to obtain 100 µg/ml of stock solution. The stock standard solution of prilocaine HCl was prepared and stored at -20°C under refrigeration. The six standard solutions (3, 5, 8, 10, 12, 15 µg/mL) in methanol were prepared by doing required dilutions from stock solution. Three quality control (QC) samples at the concentrations of 5, 8 and 10 µg/mL were prepared from the stock standard solution.

Procedure for Pharmaceutical Preparation: Stock solution (100 mg/mL) is prepared with methanol from citanest flakon that contains 20 mg/mL prilocaine HCl. All required dilutions are made with methanol which is in the working range of spectrophotometer.

Preparation of Plasma Standard and Quality Control Solutions:

Different concentrations of prilocaine HCl were spiked to plasma (0.5 mL) in order to make final concentrations between 4 and 15 µg/mL (4, 6, 8, 10, 12, 15 µg/mL) and QC samples at three different levels (5, 8, 12 µg/mL). Then, 1 mL of 1 M NaOH was added and vortexed for 30 s. The samples were extracted with 5 ml of diethylether and mixed for 2 min. The organic layer was separated after centrifugation at 4000×g for 8 min. The separated organic layer was evaporated to dryness at room temperature under nitrogen gas. The residue was reconstituted in 5 ml of methanol and vortex-mixed for 30 s.

RESULTS AND DISCUSSION:

Linearity: For each concentration of prilocaine HCl standard solution, six samples were prepared and read at 230 nm. A calibration curve was linear in concentration range of 3-15 µg/mL for standard solution and 4-15 µg/mL for human plasma. The correlation coefficients of the linearity for standard solution and human plasma were found to be 0.9999 and 0.9967, respectively. The regressions equations slope (Sb) and intercept (Sa) were obtained by least-square regression method for proposed method. The results are presented in **Table 1**.

Limit of Detection and Quantification: In order to determine LOD and LOQ parameters, a series of prilocaine HCl was prepared and concentration of prilocaine HCl was lowered until there is no response from the detector of the method. LOD was determined when RSD is lower than 20%. LOD values for spectrophotometric method were found as 1.5 µg/mL

for standard solution and this value is 2 $\mu\text{g}/\text{mL}$ for plasma solution. LOQ was accepted as RSD of the lowest concentration of measured value is lower than 10 %. LOQ values for spectrophotometric method were found as 3 $\mu\text{g}/\text{mL}$ for standard solution and 4 $\mu\text{g}/\text{mL}$ for plasma sample.

Precision and Accuracy: The precision was determined by the ratio of the standard deviation to the mean (percent relative standard deviation, RSD %) both

TABLE 1 REGRESSION ANALYSIS OF PRILOCAINE HCl (n=6)

Material	Range ($\mu\text{g}/\text{mL}$)	LR ^a	Sa	Sb	R
Standard	3-15	$A_{230\text{ nm}}=0.0283x + 0.0059$	2.4×10^{-3}	8.3×10^{-2}	0.9999
Plasma	4-15	$A_{230\text{ nm}}=0.0313x - 0.0442$	8.3×10^{-4}	7.3×10^{-3}	0.9967

A:Wavelength ^a Based on six calibration curves, LR: linear regression, Sa: Standard deviation of intercept of regression line Sb: Standard deviation of slope of regression line, R: Coefficient of correlation, x: prilocaïne concentration ($\mu\text{g}/\text{mL}$), A:Absorbance

TABLE 2 PRECISION AND ACCURACY OF PROPOSED METHOD FOR DETERMINATION OF PRILOCAINE HCl (n=6)

Material	Added ($\mu\text{g}/\text{mL}$)	Intra-day			Inter-day		
		Found \pm SD ($\mu\text{g}/\text{mL}$)	Accuracy	Precision RSD%	Found \pm SD ($\mu\text{g}/\text{mL}$)	Accuracy	Precision RSD%
Standard	5	5.03 \pm 0.25	0.60	4.97	5.01 \pm 0.34	0.20	6.80
	8	7.95 \pm 0.36	-0.63	4.52	7.87 \pm 0.37	-1.63	4.70
	10	10.36 \pm 0.59	3.60	5.69	10.32 \pm 0.56	3.20	5.42
Plasma	6	6.54 \pm 0.45	9.00	6.88	6.01 \pm 0.07	0.17	1.16
	8	9.05 \pm 0.34	13.13	3.75	8.24 \pm 0.30	3.00	3.60
	10	10.86 \pm 0.33	8.60	3.03	10.55 \pm 0.49	5.50	4.64

Specificity and Selectivity: No interference was observed coming from standard, plasma and excipients (methyl parahydroxybenzoate, sodium chloride) of pharmaceutical preparation at 230 nm. The spectrums of the prilocaïne HCl in standard solutions and human plasma are shown in **Figure 2 and 3**.

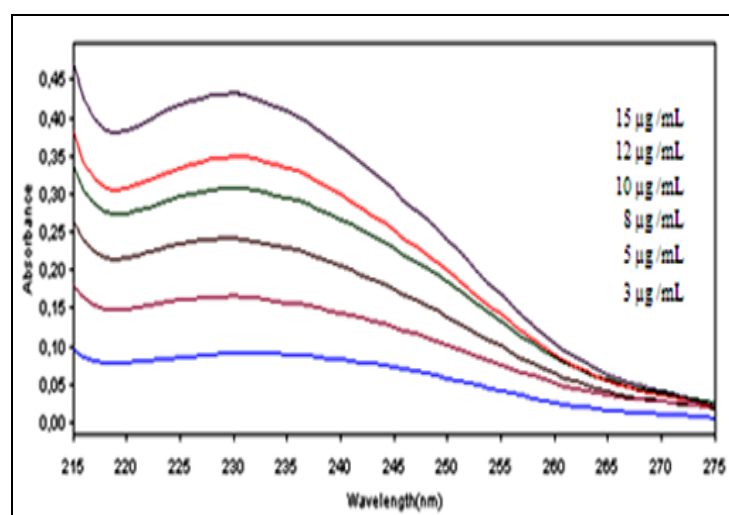


FIG. 2: UV SPECTRUM OF STANDARD SOLUTIONS

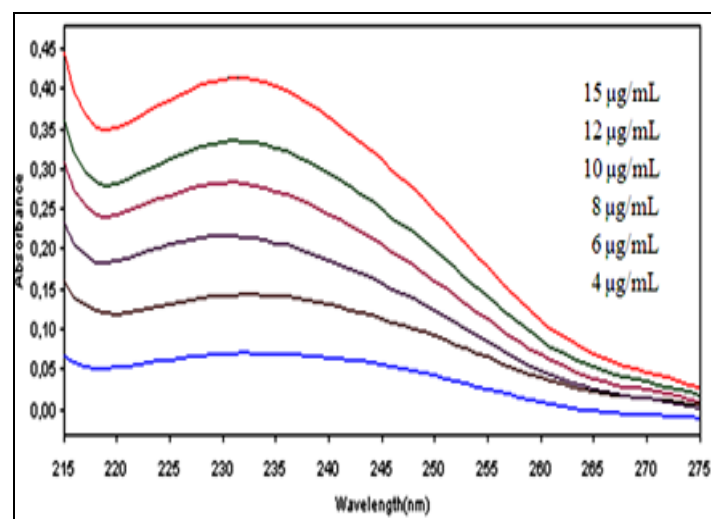


FIG. 3: UV SPECTRUM OF PLASMA SOLUTIONS

standard solutions and plasma samples. The values were calculated for both standard solutions and plasma samples with percent relative error (RE%). The intra-day and inter-day values for accuracy and precision were determined by replicate analysis of six sets of samples with three different concentrations of prilocaïne HCl (5, 8 and 10 $\mu\text{g}/\text{mL}$) for standard solution and plasma (6, 8 and 10 $\mu\text{g}/\text{mL}$) within 1 day or on 5 consecutive days (**Table 2**).

Recovery: Analytical recovery is calculated as follows first of all, known amount of pure drugs are added to pre-analyzed samples of commercial dosage forms. Then recovery of plasma samples were determined by comparing spiked human plasma data with standard prilocaïne HCl solutions at the same concentration levels in order to measure analytical recovery value.

The obtained recovery values of pharmaceutical preparation and human plasma were found as 100.4 and 102.6, 91.63 and 94.0, respectively.

CONCLUSIONS: Spectrophotometry method is widely used with respect to GC, HPLC and mass spectrometry procedures⁶⁻²⁰, in terms of its simplicity and cheapness. The reagent that was used in this method could be available in many analytical laboratories. Another advantage of this method is short analysis time. Therefore, this paper describes a new, simple and sensitive spectrophotometric method for the determination of prilocaine HCl both pharmaceutical preparation and human plasma. The liquid-liquid extraction method used in human plasma has been simplicity used and also successfully applied to GC-MS, GC-NPD method^{17, 18}. The method was validated by using sensitivity, specificity, linearity, accuracy and precision according to ICH²². The analytical method is can be utilized for the routine analysis and clinical laboratories.

REFERENCES:

1. Catterall W, Mackie K, Hardman J, Limbird L, Molinoff P, Ruddon R and Gilman A: The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, 1996:331-347.
2. Arthur GR, Wildsmith JAW, Tucker GT, Wildsmith JAW and Armitage EN: Principles and Practice of Regional Anesthesia. Churchill Livingstone, New York, 1993:29-44
3. Wildsmith JAW, Tucker GT, Cooper S, Scott DB and Covino BG: Plasma concentrations of local anaesthetics interscalene brachial plexus block. British Journal of Anaesthesia 1977, 49, 461-466.
4. Cano JP, Charbit-Bues M and Rochaix T: Pharmacokinetics of local anesthetics of amide type. Cahiers D'Anesthesiologie 1985,33, 501-507.
5. Gaber K, Harréus UA, Matthias C, Kleinsasser NH and Richter E: Hemoglobin adducts of the human bladder carcinogen *o*-toluidine after treatment with the local anesthetic prilocaine. Toxicology 2007, 229,157-164.
6. Rishiraj B, Epstein JB, Fine D, Nabi S and Wade NK: Permanent vision loss in one eye following administration of local anesthesia for a dental extraction. International Journal of Oral and Maxillofacial Surgery 2005, 34, 220-223.
7. Siluveru M and Stewart JT: Stereoselective determination of R(-)- and S-(+) - prilocaine in human serum by capillary electrophoresis using a derivatized cyclodextrin and ultraviolet detection. Journal of Chromatography B1997, 693,205-210.
8. Klein J, Fernandes D, Gazarian M, Kent G and Koren G: Simultaneous determination of lidocaine, prilocaine and the prilocaine metabolite *o*-toluidine in plasma by high-performance liquid chromatography. Journal of Chromatography B 1994, 655,83-88.
9. Wiberg K and Jacobsson SP, Parallel factor analysis of HPLC-DAD data for binary mixtures of lidocaine and prilocaine with different levels of chromatographic separation. Analytica Chimica Acta 2004, 514, 203-209.
10. Wiberg K, Andersson M, Hagman A and Jacobsson SP, Peak purity determination with principal component analysis of high-performance liquid chromatography-diode array detection data. Journal of Chromatography A 2004, 1029, 13-20.
11. Storms ML and Stewart JT: Stability-indicating HPLC assays for the determination of prilocaine and procaine drug combinations. Journal of Pharmaceutical and Biomedical Analysis 2002, 30, 49-58.
12. Mohammad MAA: LC Determination of Lidocaine and Prilocaine Containing Potential Risky Impurities and Application to Pharmaceuticals. Chromatographia 2009, 70, 563-568.
13. Klimundová J; Šatinský, D; H, Sklenářová and Solich P: Automation of simultaneous release tests of two substances by sequential injection chromatography coupled with Franz cell. Talanta 2006, 69 (3), 730-735.
14. Fijałek Z, Baczyński E, Piwońska A and Warowna-Grzeškiewicz M: Determination of local anaesthetics and their impurities in pharmaceutical preparations using HPLC method with amperometric detection. Journal of Pharmaceutical and Biomedical Analysis 2005, 37, 913-918.
15. Koehler A, Oertel R and Kirch W: Simultaneous determination of bupivacaine, mepivacain, prilocaine and ropivacain in human serum by liquid chromatography-tandem mass spectrometry. Journal of Chromatography A 2005 ;1088:126-130.
16. Watanabe T, Namera A, Yashiki M, Iwasaki Y and Kojima T: Simple analysis of local anaesthetics in human blood using headspace solid-phase microextraction and gas chromatography-mass spectrometry-electron impact ionization selected ion monitoring. Journal of Chromatography B 1998,709, 225-232.
17. Kadioglu Y and Atila A: Development and validation of gas chromatography-mass spectroscopy method for determination of prilocaine HCl in human plasma using internal standard methodology. Biomedical Chromatography 2007,21, 1077-1082.
18. Kadioglu Y and Atila A: GC determination of prilocaine HCl in human plasma: Analytical application to real samples. Chromatographia 2008, 67,754-759.
19. Yang Y, Zhang W and Ye L: Simultaneous determination of prilocaine and lidocaine in transdermal receiving fluid using gas chromatography-mass spectrometry. Chinese Journal of Chromatography. 2009, 27,74.
20. Taddio A, Stevens B, Craig K, Rastogi P, Ben-David S, Shennan A, Mulligan P and Gideon KG: Efficacy and safety of lidocaine-prilocaine cream for pain during circumcision The New England Journal of Medicine 1997, 336, 1197-1201.
21. Badran IM, An-Najah: National University, *Nablus*, 2000 (in Palestine)
22. ICH Validation of analytical procedures. In Proceedings of the International Conference on Harmonization. Commission of the European Communities, Brussels, 1996.
