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RP-HPLC METHOD FOR THE ESTIMATION OF BUPIVACAINE HCl IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple and precise RP-HPLC method was developed and validated for the determination of bupivacaine hydrochloride in pharmaceutical dosage forms. Chromatography was carried out using Waters RP- C_{18} 150 x 4.6 mm, 3.5 μ, pH 6.5 buffer : acetonitrile (50 : 50) as the mobile phase at a flow rate 1.0 mL/min. The analyte was monitored using PDA detector at 220 nm. The retention time of the drug was 5.46 min for bupivacaine hydrochloride. The proposed method was found to have linearity in the concentration range of 25 – 150 μg/mL with correlation coefficient of $r^2 = 0.9999$. The developed method has been statistically validated and found simple and accurate. The mean recoveries obtained for bupivacaine hydrochloride were in the range 100.06-101.9%. Due to its simplicity, rapidness, high precision and accuracy of the proposed method, it may be used for determining bupivacaine hydrochloride in bulk and dosage forms.

Key words: Bupivacaine hydrochloride, RP-HPLC.

INTRODUCTION

Bupivacaine hydrochloride (BPCH) is 2-piperidinecarboxamide[1], 1-butyl-N-(2,6-dimethylphenyl)-, monohydrochloride, monohydrate, a white crystalline powder that is freely soluble in 95 percent ethanol, soluble in water, and slightly soluble in chloroform or acetone[2]. Bupivacaine is related chemically and pharmacologically[3] to the aminoacyl[4], local anesthetics[5]. It is a homologue of mepivacaine[6] and is chemically related to lidocaine[7]. All three of these anesthetics contain an amide linkage between the aromatic nucleus and the amino, or piperidine group[8]. They differ in this respect from the procaine-type[9] local anesthetics, which have an ester linkage[10-12].

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Only very few HPLC methods have been reported in the literature for the estimation of BPCH present in biological fluids and pharmaceutical dosage forms. Hence, the authors have made an attempt to develop a new HPLC method for the determination of BPCH in pharmaceutical formulations.

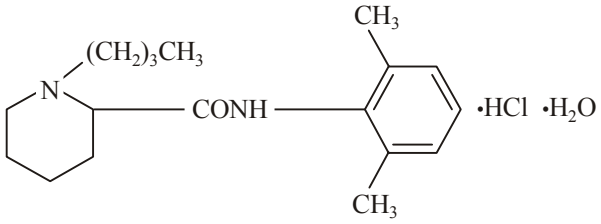

Fig. 1

EXPERIMENTAL

Materials and methods

Instrumentation

High performance liquid chromatograph (Shimadzu HPLC,Model:SPD- M20A) prominence with high pressure gradient system with photo diode array detector was used.

Chemicals and reagents

Bupivacaine hydrochloride was obtained as a gift sample from Pharma Zell R & D Centre, India Pvt. Ltd. water (HPLC grade), acetonitrile (HPLC grade) and methanol (HPLC grade) were used.

Chromatographic conditions

Mobile phase consists of pH 6.5 buffer : acetonitrile (50 : 50). Buffer was prepared by weighing accurately 6.8 g of potassium dihydrogen phosphate and dissolving it in 1000 mL of Milli-Q water. 5 mL of triethylamine solution was added and the pH was adjusted to 6.5 with dilute orthophosphoric acid and filtered through 0.45 µm nylon membrane filter. The mobile phase was pumped from the solvent reservoir to the column at a flow rate 1.0 mL/min. The column was maintained at 45°C and the volume of each injection was 10 µL. Prior to injection of the solutions, column was equilibrated for atleast 10 min with mobile phase flowing through the system. The eluents were monitored at 220 nm.

Diluent : Milli-Q water : Methanol (50 : 50 v/v)

Table 1: System suitability, precision and accuracy of the proposed methods for BPCH

Parameter	Results
Retention time (Rt) (min)	5.43
Theoretical plates (n)	12111
Peak asymmetry	1.04
Linearity range (µg/mL)	62.5 – 375
Limit of detection (µg/mL)	5.064
Limit of quantification (µg/mL)	50.64
Regression equation (Y = a + bc)	
Slope (b)	9268.9
Standard deviation of slope (S_b)	0.392
Intercept (a)	-9129.3
Standard deviation of intercept (S_a)	0.348
Standard error of estimation (S_e)	6.648
Correlation coefficient (r)	0.9999
(%) Relative standard deviation *	
Retention time	0.3003
Peak area	0.0692
% Range of error* (Confidence limits)	
0.05 level	0.068
0.01 level	0.101

* Average of six determinations
** Average of three determinations

Standard preparation

25.0 mg of BPCH was weighed accurately for working standard and transferred into a 100 mL clean dry volumetric flask. About 70 mL of diluent was added, sonicated for 5 minutes, and it was diluted to desired volume with diluents.

Table 2: Accuracy (Recovery) data for bupivacaine HCl

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical analysis of % recovery	
50% S - 1	125	126.5	101.2	Mean	101.19
50% S - 2	125	126.47	101.17	SD	0.0173
50% S - 3	125	126.5	101.2	% RSD	0.0168
100 % S - 1	250	253.94	101.58	Mean	101.57
100% S - 2	250	253.92	101.57	SD	0.0057
100% S - 3	250	253.92	101.57	% RSD	0.0056
150% S - 1	375	381.22	101.66	Mean	101.66
150% S - 2	375	381.21	101.66	SD	0.0057
150% S - 3	375	381.23	101.67	% RSD	0.0056

Sample preparation

5 mL of sample solution was taken in 50 mL clean dry volumetric flask, and made up to volume 50 mL with diluent.

RESULTS AND DISCUSSION

Several systematic trials were performed to optimize the chromatographic conditions for developing a sensitive, precise and accurate RP-HPLC method for the analysis of bupivacaine hydrochloride in pharmaceutical dosage forms. The present method contains mobile phase pH 6.5 buffer : acetonitrile (50 : 50), which was found to be the most suitable as the peak obtained with good peak shape and symmetry. Hence, this method was finalized for the estimation of BPCH.

Table 3: Assay and recovery* results of BPCH in pharmaceutical formulations

Method	Pharmaceutical formulation	Labeled amount (mg)	Amount found** ± S.D.	Reference method	% Recovery ± R.S.D.
RP-HPLC	Injection	2.5 mg/mL	75.02 ± 0.023t =1.47, F = 3.06	75.05 ± 0.016	100.1 ± 0.025

* Average of five determinations

**Average ± standard deviation of eight determinations

The t and F – values refer to comparison of the proposed method with reference method. Theoretical values at 95 % confidence limits t = 2.365 and F = 4.88

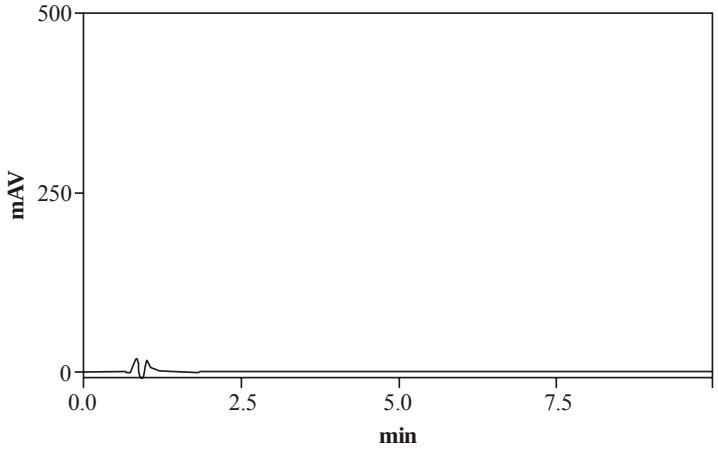

Fig. 2: Chromatogram of blank

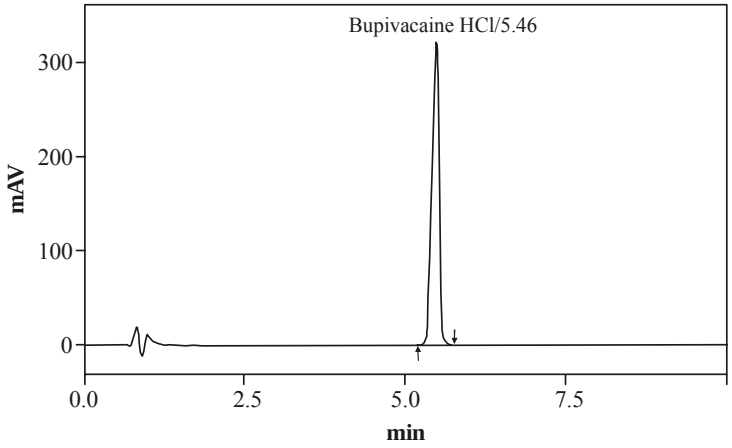

Fig. 3: Chromatogram of standard

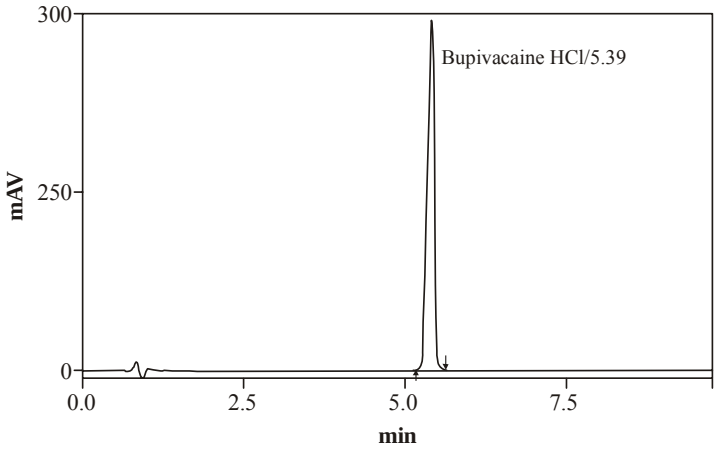

Fig. 4: Chromatogram of sample

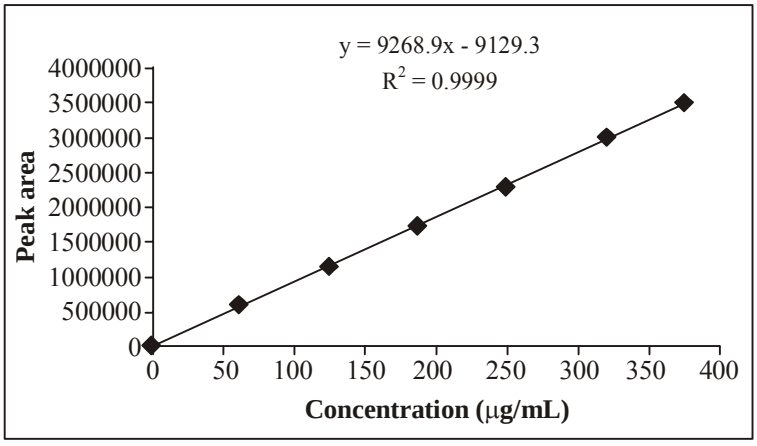

Fig. 5: Linearity curve for bupivacaine HCl

Linearity

A series of dilutions were prepared using BPCH working standard (500 µg/mL) at concentration levels from 25% to 150% of target concentration (25%, 50%, 75%, 100%, 125% and 150%). The peak area response of solutions was measured.

Accuracy

A study of accuracy was conducted. Drug assay was performed in triplicate as per test method with equivalent amount of BPCH into each volumetric flask for each spike level to get the concentration of BPCH equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of BPCH was calculated.

Precision

The system precision was performed by analyzing a standard solution of bupivacaine HCl at working concentration level for 6 times.

Robustness

Robustness of the proposed methods was evaluated by making small changes in flow rate, buffer concentration, pH of the buffer solution, organic modifier concentration and temperature. The results were found to be not affected by these small alterations.

CONCLUSION

From these results, it can be concluded that the proposed method is quite precise and accurate. The absence of additional peaks in the chromatogram indicated that there is no interference of the common excipients used in the tablets. The proposed HPLC method is sensitive and reproducible for the analysis of bupivacaine hydrochloride in tablet dosage forms. The method was duly validated by using required statistical parameters.

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