RESEARCH ARTICLE OPEN ACCESS

The Estimation of Mepivacaine Drug in Mepivacaine Hydrochloride Injection by Reverse Phase High Performance Liquid Chromatography

Dr.C. Hazarathaiah yadav[1] ,Mr. Mallibabu Alahari[2]
(Department of Chemistry, Vel Tech Rangarajan Dr.Sagunthala R&D institute of Science and Technology, Avadi, Chennai-600062, India)

Abstract:

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Mepivacaine drug in Mepivacaine Hydrochloride Injection. Develosil ODS-MG-5, (150 x 4.6 mm), 5µm with mobile phase consisting of Potassium dihydrogen phosphate buffer and acetonitrile were mixed in the ratio 65:35 (v/v) respectively and degassed for about 10 minutes. Isocratic mode of chromatography technique was used. The flow rate was 1.5 mL/min and the eluents were monitored at 220 nm. The retention time of the Mepivacaine peak is about 1.6 minutes. The detector response is linear in the concentration of 10.060 to 30.180 mcg/mL, Y-intercept is -726.11468, Residual sum of square is 72329401.8155and slop is 54607.2822.The percentage assay of Mepivacaine is 99.9 %. The method is validated by determining its accuracy, precision and linearity.

***Keywords* —** Mepivacaine Hydrochloride Injection, RP-HPLC method and Isocratic mode of chromatography.

I. BACKGROUND

Mepivacainehydrochloride is 2-Piperidinecarboxamide, N-(2,6-dimethylphenyl)-1-methyl-, monohydrochloride and has the following structural formula.

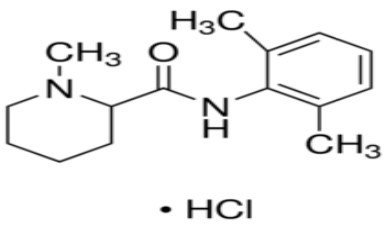

Fig 1: Structure of Mepivacaine Hydrochloride
The molecular formula C 15 H 22 N 2 O • HCl

Mepivacaine hydrochloride is a local anesthetic available as sterile isotonic solutions (clear, colorless) in concentrations of 1%, 1.5% and 2% for injection via local infiltration, peripheral nerve block, and caudal and lumbar epidural blocks.
Mepivacaine hydrochloride is related chemically and pharmacologically to the amide-type localanaesthetics. It contains an amide linkage between the aromatic nucleus and the amino group

Composition of Available Solutions In Water For Injection					
Dose Details	**1% Single Dose 30 mL Vial mg/mL**	**1% Multiple Dose 50 mL Vial mg/mL**	**1.5% Single Dose 30 mL Vial mg/mL**	**2% Single Dose 20 mL Vial mg/mL**	**2% Multiple Dose 50 mL Vial mg/mL**
Mepivacaine hydrochloride	**10**	**10**	**15**	**20**	**20**
Sodium chloride	**6.6**	**7**	**5.6**	**4.6**	**5**
Potassium chloride	**0.3**	**Nil**	**0.3**	**0.3**	**Nil**
Calcium chloride	**0.33**	**Nil**	**0.33**	**0.33**	**Nil**
Methylparaben	**Nil**	**1**	**Nil**	**Nil**	**1**
***In Water for Injection**					

Table 1: Available Water for Injections

The pH of the solution is adjusted between 4.5 and 6.8 with sodium hydroxide or hydrochloric acid.

In the literature, several analytical techniques like HPLC method, LCMS methods, Voltammetry, GC and UV spectrophotometric methods have been reported for its determination in biological fluids and formulations. The main purpose of the present study was to establish relatively simple, sensitive and validated liquid chromatographic methods for the determination of Mepivacaine in Mepivacaine Hydrochloride Injection. The method was validated by determining its accuracy, precision and linearity as per ICH guidelines, Q2R1 [5-9]

II. OBJECTIVES

The main objective of this study is to develop a simple, precise, linear and accurate reverse phase HPLC method for estimation of Mepivacainedrug in Mepivacaine Hydrochloride Injection.

A. Materials and Requirements

Commercially available Mepivacaine Hydrochloride Injection of POLOCAINE-MPF. Acetonitrile, Potassium dihydrogen phosphate anhydrous and HPLC grade water (Qualigens) were procured from market. Mepivacaine Hydrochloride Working standard was procured from the Centaur Laboratories Pvt. Ltd as a gift material

B. Instruments

HPLC, Waters Alliance system with equipped Diode Array Detector and automatic injector with injection volume 50 µl. The HPLC data was analyzed with Empower-3 Software, SARTOURIUS Analytical balance with model of MSA225P-100-DA and FISHER SCIENTIFIC pH METER with model of XL15.

III. METHODS

The contents of mobile phase were mixture of 0.02M Potassium Hydrogen Phosphate in Water and Acetonitrile in the ratio of 65:35 (v/v) respectively and degassed for about 10 minutes. The run time was set about 5 minutes and the column temperature was 40ºC. Prior to injection of Blank solution and drug solution, the column was equilibrated for at least 30 minutes with the mobile phase flowing through the system. The eluents were monitored at 220 nm

A. ***Preparation of Diluent:***

Water used as diluent for entire experiment

B. ***Preparationof Standard solution:*** *(20 mcg/mL)*

A standard stock solution of the drug was prepared by 20.0 mg of Mepivacaine Hydrochloride working standard was weighed and transferred into a 100 mL volumetric flask and diluted to volume with the diluent and mixed well. 10.0 mL of the above standard stock solution was pipetted into 100 mL volumetric flask, diluted to volume with diluent and mixed well.

C. ***Preparationof Sample solution:*** *(20 mcg/mL)*

5.0 mL of the sample solution (1% of POLOCAINE-MPF solution) was taken and diluted with diluent up to 100 mL and mixed well. 4.0 mL of this solution was pipetted into 100 mL volumetric flask, diluted to volume with diluent and mixed well

D. ***Linearity:***

Linearity of Detector response was established by plotting a graph of concentration (in mcg/mL) versus peak area and determining correlation coefficient, y-intercept, slope, %y-intercept and residual sum of squares. Mepivacaine Hydrochloride linearity solutions were prepared in the concentration range from about 50% (10 mcg/mL) to about 150 % (30 mcg/mL) level of the target concentration (20 mcg/mL) and injected into the HPLC system. The detector response was found to be linear and the results were found to be within limit, refer table 2 and 3.

% of Linearity Level	Concentration (mcg/mL)	Peak Area(AU)
50	10.060	549906
75	15.090	821778
100	20.120	1100875
125	25.351	1376991
150	30.180	1651299
Correlation coefficient, NLT 0.997	0.999	
Y-intercept	-726.11468	
Slope	54607.2822	
Residual sum of square	72329401.8155	

Table 2: Linearity data

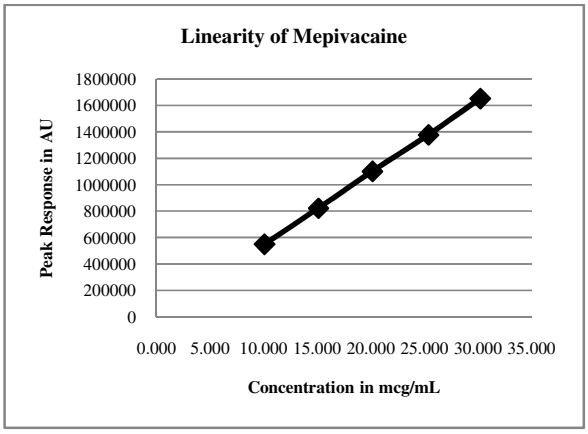

Table 3: Linear Regression of Calibration curve

E. ***Precision:***

The precision of test method was evaluated by preparing six samples and analyzed as per the test procedure. 50 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 1.6 minutes. The amount of drug present per each sample preparation was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in table 4. Typical chromatograms of Blank solution and Mepivacaine Hydrochloride sample solution as shown in fig 2&3.

Sample Preparations	% Assay of Ketorolac Tromethamine	
	Analyst-1	Analyst-2
Preparation – 1	99.8	100.6
Preparation – 2	100.2	100.1
Preparation – 3	99.5	100.2
Preparation – 4	99.9	100.8
Preparation – 5	99.6	100.5
Preparation – 6	100.5	100.1
Individual Average	99.9	100.4
Individual (% RSD), NMT 2	0.4	0.3
Overall Average	100.2	
Overall % RSD, NMT 2	0.4	

Table 4: Precision data

F. ***Accuracy:***

Accuracy was determined by recovery studies of Mepivacaine Hydrochloride, known amount of standard was added to the pre-analysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in table 4. The study was done from about 50% (10 mcg/mL) to about 150 % (30 mcg/mL) level of the test concentration (20 mcg/mL) at three different sample preparations for each level.

Recovery Level	Preparations	% RECOVERY			
		Injection-1	Injection-2	Mean (NLT 95.0% & NMT 105.0%)	Average (NLT 95.0% & NMT 105.0%)
50% Level	1	100.2	100.5	99.7	100.0
	2	99.8	100.8	99.1	
	3	100.5	99.3	100.3	
100% Level	1	100.0	100.9	100.7	100.5
	2	100.6	100.5	100.1	
	3	100.3	100.8	100.6	
150% Level	1	100.4	99.8	99.2	100.1
	2	100.1	100.8	100.6	
	3	100.6	100.5	99.3	

Table 5: Accuracy data

G. ***System Suitability:***

The system suitability tests were carried out on freshly prepared standard solution of Mepivacaine Hydrochloride. The parameters studied to evaluate the suitability of the system are given in table 6

System suitability parameters	Observed Values	
	Analyst- 1	Analyst-2
USP Tailing factor for Mepivacaine peak in standard solution (NMT 2.0)	1.0	1.1
Relative Standard Deviation for peak areas of Mepivacaine from five replicate injections of standard solution (NMT 2.0%)	0.4	0.2
USP Plate count for Mepivacaine peak in standard solution (NLT 2000)	13000	11000

Table 6: System suitability data

IV. RESULTS

The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Mepivacaine drug in Mepivacaine Hydrochloride Injection. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r2=0.999) was observed between the concentration range of 10.060 mcg/mL to 30.180 mcg/mL. Low values of standard deviation are indicative of the high precision of the method. The assay of Mepivacaine Hydrochloride Injection was found to be 99.9 %. From the recovery studies it was found that the Mepivacaine was recovered which indicates high accuracy of the method. The absence of additional unknown peaks in the chromatogram indicates non-interference of the common excipients used in formulation

V. CONCLUSIONS

The developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control testing of this dosage form of Mepivacaine Hydrochloride Injection within a short analysis time

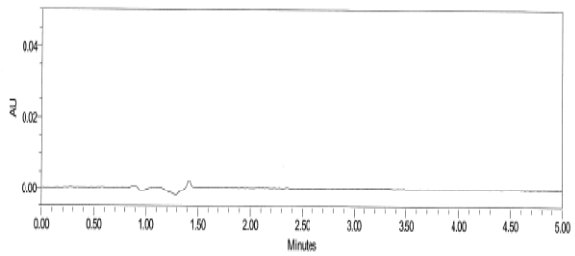

Fig.2 Blank Chromatogram

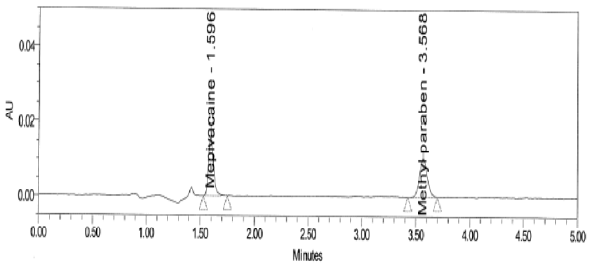

Fig.3Sample Chromatogram

ACKNOWLEDGMENT

The author is grateful to the Management of VelTech University for their encouragement and providing the necessary facilities to carry out the research work.

REFERENCES

1. *Mepivacaine Hydrochloride injection http://www.drugs.com/monograph/Mepivacine-.html*
2. *JEF Reynolds; Martindale, The extra pharmacopoeia., Pharmaceutical press, London, **2009,** 36, 74.*
3. *S. N. Razzq; I.Mariam; I. U. Khan; I.Ashaq; J.Liq.chrom.rel.Tech., **2012**, 35, 651-661.*
4. *J. Squella; I. Lemus; J. Sturm; L. Nunezvergara, Analytical letters., **1997**, 30, 553-564.*
5. *ElzbietaHuzar; AlicjaWodnicka; Acta PoloniaePharmaceutica- research., **2013**, 70, 41-49.*
6. *Praveen Kumar Baliyan; RP Singh; Surabh Arora; Asian journal of chemistry., **2009**, 21, 1739-1746.*
7. *FDA, Food and drug administration, Centre of drug evaluation and research, **1994**.*
8. *ICH, International conference on harmonization, Harmonised tripartite guideline, Text and methodology on validation of analytical procedures Q2(R1).*
9. *United states pharmacopoeia (USP), **2006**, 1218*
10. *C.HazarathaiahYadav; PeriyasamyMurugan; International Journal of ChemTech Research, CODEN (USA): IJCRGG ISSN: 0974-4290 , Vol.8, No.2, **2015**, page No. 860-868,*
11. *C. Hazarathaiah Yadav; D.Munirajasekhar ; Journal of Chemical and Pharmaceutical Research, **2014**, 6(7):109-116,*
12. *C. Hazarathaiah Yadav; Journal of Chemical and Pharmaceutical Research, **2015**, 7(6):562-567*
13. *Mallibabu; P. Janakipathi; N. Appala Raju ; International Journal of Chemistry and Pharmaceutical Sciences IJCTPR, **2014**, Vol. 2(3): 468-472*
14. *Mallibabu; P. Janakipathi; N. Appala Raju; International Journal of Chemistry and Pharmaceutical Sciences IJCPS, **2014**, Vol. 2(6): 878-882*

*15.C. Hazarathaiah Yadav; D.Miuniraja sekharJournal of Chemical and Pharmaceutical Research, **2014**, 6(7):109-116*