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A NOVEL VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF BAMIFYLLINE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, accurate and precise Stability indicating RP-HPLC method for the estimation of Bamifylline hydrochloride in pure and pharmaceutical dosage form has been reported. Chromatography was performed with Shimadzu HPLC equipment comprising Enable C_{18} (25 × 2.6 × 5µ) column with photodiode array detector. A Rheodyne injector fitted with a 10µL loop was also used and data was recorded and evaluated using LC-20 solutions software. The mobile phase consists of 50% Methanol and 50% phosphate buffer solution and at a flow rate of 1 milliliter/minute. The Bamifylline hydrochloride was eluted at approximately 3.314 minutes. The wavelength was found to be 272nm. A linear response was observed in the concentration ranges of 20-120µg/ml with a regression coefficient of 0.999. Forced degradation studies were performed on pure sample of Bamifylline hydrochloride using acid (0.1 Normal (N) hydrochloric acid), base (0.1 N sodium hydroxide), peroxide (30% H_2O_2) and thermal (105°C) conditions. The developed method was validated with respect to specificity, precision (% RSD about 0.4%), linearity (linearity of range about 20-120 µg/mL), robustness, LOD and LOQ values were found to be 0.362 µg/ml and 1.096 µg/mL respectively.

Keywords: High performance liquid chromatography, Naloxegol.

INTRODUCTION

Bamifylline hydrochloride (BMFH) is a stimulant drug of the xanthine chemical class which acts as a selective adenosine A_1 receptor antagonist, used in the systemic treatment of obstructive airway diseases and asthma. BMFH is soluble in water, methanol, ethanol, HCl and NaOH. Chemically bamifylline is 8-benzyl-7-[2-[ethyl(2-hydroxyethyl)amino]ethyl]-1,3-dimethylpurine-2,6-dione.No official method for the estimation of BMFH is available in literature. Papadoyannis et al., reported reverse phase high performance liquid chromatographic (RP-HPLC) method for the simultaneous estimation of BMFH and major metabolite AC-119 [1]. Gerlo et al., developed RP-HPLC method for the determination of BMFH in human plasma of neonates [2]. Belliardo et al., developed reverse phase ion pair chromatography method for the determination of BMFH and its major metabolite in human plasma [3]. Nicot et al. reported HPLC for determination of BMFH and its three major metabolites in human plasma [4]. Carlucci et al., reported determination of BMFH impurities in bulk material and pharmaceutical forms using liquid chromatography with ultraviolet detection [5]. Patel et al., reported high performance thin layer chromatographic method for estimation of BMFH in bulk and tablet formulation [6]. Most of the methods reported were proposed for the analysis of biological samples and require tiresome procedures for sample pretreatment. Further, the lack of an official pharmacopoeia method for estimation of BMFH provoked the authors to develop stability indicating HPLC method for estimation of BMFH which is simple, rapid, less

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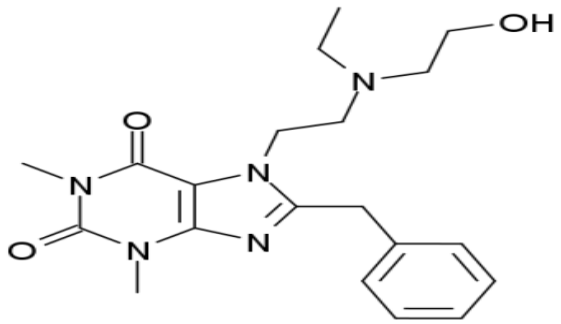
expensive and environment friendly. The current study describes the development and validation of a stability-indicating RP-HPLC method for estimation of BMFH in the presence of its degradation products according to ICH guideline [7]. The developed method is applied for routine analysis of BMFH in pharmaceutical tablet dosage form [8-13].



Structure of Bamifyline

MATERIALS AND METHODS

Methanol, Water, KH_2PO_4 , NaH_2PO_4, Phosphoric acid ,Hydrogen peroxide, Hydrochloric acid.

INSTRUMENTATION

Analysis was performed on a Shimadzu HPLC system, Model: 2010 A HT liquid chromatography Manufacturer: Shimadzu, Japan. Consisted of a system controller (SCL-10AVP), on-line degasser (DGU-14A), low pressure gradient flow control valve (FCV-10ALVP), solvent delivery module (LC-10ADVP), auto injector (SIL-10 ADVP), column oven (CTO-10AVP), UV – VIS and PDA detector (SPD-10AVP) and CLASS – VP software version 6.14 SP1 & Agilent HPLC system Model: 1200 series with Inertsil ODS3V C_{18} column (150 mm x 4.6 mm i.d., 5 µm) and PDA detector with Chemstation software version.

CHROMATOGRAPHIC CONDITIONS

Chromatographic separation achieved using an analytical column, Inertsil ODS3V C18 column (150 mm x 4.6 mm i.d., 5 µm. Mobile phase was consisted of Methanol : Phosphate buffer [50:50]. The elution was achieved isocratically at a flow rate of 1 mL/min with injection volume of 20 µL. Column temperature was maintained at 45°C and chromatograph was recorded at wavelength 272 nm.

PREPARATION OF SAMPLE

Preparation of standard stock solution:

50mg of Bamifylline Hydrochloride was weighed accurately and dissolved in 50ml of mobile phase to get the concentration of 1000µg/ml. Resultant solution was filtered through Whatman filter paper.

Preparation of working standard solution

Working standard solutions of Bamifylline Hydrochloride were prepared by accurately transferring the (0.2, 0.4, 0.6, 0.8. 1.0 and 1.2 mL) aliquots of the standard stock solution into a series of six 10 ml volumetric flasks. The volume was made upto mark with mobile phase to obtain concentration of 20 – 120 µg/mL.

Preparation of Sample Solution

Ten tablets were accurately weighed and finely powdered. A portion of the powder equivalent to about 10mg of Bamifylline Hydrochloride was weighed accurately and transferred into100mL volumetric flask and mixed thoroughly for 20 minutes for complete dissolution of Bamifylline Hydrochloride with mobile phase and then the sample solution was filtered and diluted to 100mL with mobile phase to get concentration of 100µg/mL and used for analysis.

RESULTS AND DISCUSSION

Method development and validation

Some important parameters like pH of the mobile phase, concentration of the acid or buffer solution, etc., were tested for a good chromatographic separation. Trials showed that mobile phase with reverse phase C_{18} column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation of Bamifylline as carried out by the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Bamifyllinel was recorded in Fig.1.

Now the sample solution was injected separately and chromatogram was recorded until the reproducibility of the peak areas were satisfactory.

Validation

HPLC method was validated according to the International Conference on Harmonization Guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as system suitability, linearity, precision, accuracy, and robustness.

Linearity

From the stock solutions of Bamifylline Hydrochloride 0.2 ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml and 1.2ml were taken in six different 10 ml volumetric flasks and diluted with the mobile phase to the give the concentrations from 20-120 µg/ml. These Solutions were injected into the chromatographic system and the response was recorded in Fig.2.

Precision

To study precision, six replicate standard solutions of Bamifylline Hydrochloride (100µg/ml) were prepared and analyzed using the proposed method. The

percent relative standard deviation (% RSD) for peak responses was shown in Tab. 1.

Accuracy

Accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed sample solution. The standard addition method was performed at 50%, 100% and 150% level of sample solution. The resulting solutions were analyzed in triplicate at each level as per the ICH guidelines. Good recoveries were obtained for each concentration, confirming that the method was accurate and shown in Tab.2.

Limit of Detection

Limit of Detection (LOD) is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. Limit of detection is determined by the analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected and it was found to be 2.4µg/ml of Bamifylline.

Limit of Quntification

Limit of quantification (LOQ) is the concentration that can be quantitated reliably with a specified level of accuracy and precision. LOQ was found to be 7.3 µg/ml of Bamifylline.

Robustness

Robustness of the developed method was demonstrated by purposely altering the experimental conditions. Robustness of method was carried out with variation of mobile phase ±0.2%, flow rate ±0.2 ml/min .It indicates that there was no effect on the results, hence the developed method is said to be more robust and shown in Tab. 3.

Specificity

Specificity is the ability of the analytical method to measure the analyte free from interference due to other components. Specificity was determined by comparing test results obtained from analyses of sample solution containing ingredients with that of test results those obtained from standard drug. Chromatograms for standard & samples were recorded and they represent no interference.

System Suitability

System suitability tests were carried out on freshly prepared standard stock solution of Bamifylline and it was calculated by determining the standard deviation of by injecting standard solutions in six replicates at frequent time interval.

Table 1. Results for Precision

S.NO	Bamifylline hydrochloride	
	Rt (min)	Peak Area (mV.sec)
1	3.313	2359.407
2	3.31	2338.546
3	3.333	2342.458
4	3.327	2340.143
5	3.328	2344.523
Mean	3.3202	2345.015
S.D	0.0082	8.3590
%RSD	0.2478	0.3564

Table 2. Accuracy

Name	Pre Analysed Sample Concentration (µg/ml)	Spiked amount (µg/ml)	Area (mV.sec)	Amount Recovered (µg/ml)	% Recovery	Average % Recovery ± SD
Bamifylline hydrochloride (Mean Standard Area = 2365.174 mv.Sec)	80	10	2123.052	89.76	99.7	99.66 ± 0.05
	80	10	2122.972	89.75	99.7	
	80	10	2122.030	89.71	99.6	
	100	10	2612.51	110.45	100.4	100.4 ± 0.05
	100	10	2612.745	110.46	100.5	
	100	10	2612.263	110.44	100.4	
	120	10	3077.549	130.11	100.1	100.16 ± 0.05
	120	10	3078.220	130.14	100.3	
	120	10	3077.381	130.12	100.1	

Table 3. Robustness

Flow rate	Bamifylline hydrochloride		% RSD
	Rt (min)	Area (mV.sec) n = 5	
Standard (1ml/min)	3.293	2248.266	-
0.9ml/min	3.927	2856.323	0.3463
1.1ml/min	3.220	2357.382	0.3779

Fig. 1. Chromatogram of standard

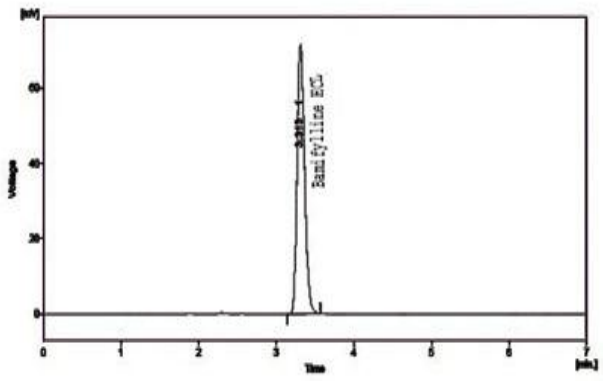


Fig.2. Chromatogram of sample

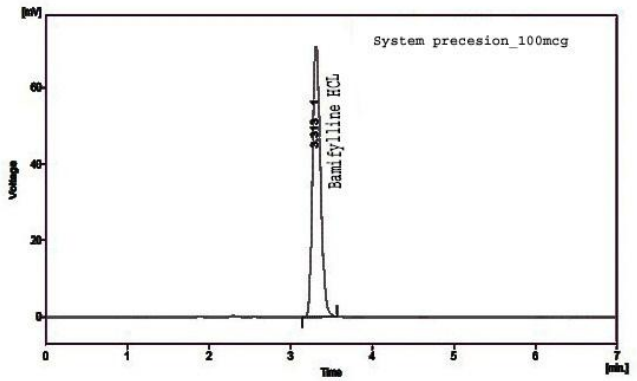
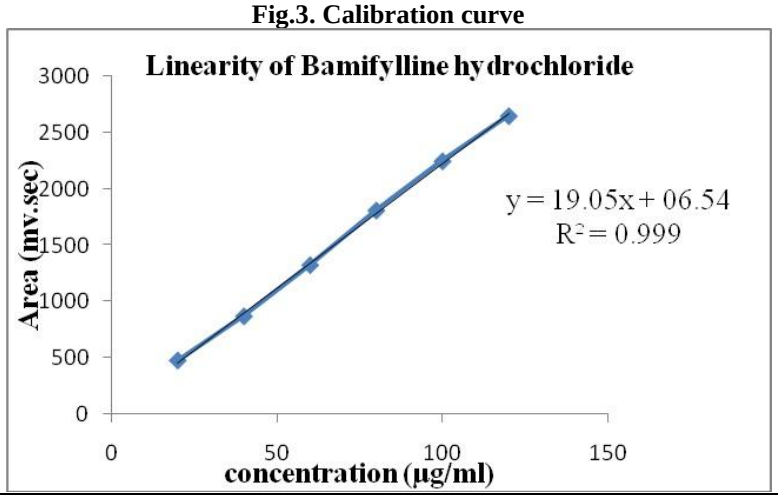


Fig.3. Calibration curve



CONCLUSION

The RP-LC method developed for the analysis of Bamifylline in their pharmaceutical preparations is simple, precise, and accurate. The method is useful for routine analysis due to short run time.

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REFERENCES

1. Jhon Wiley and Sons, Connors KA. A Textbook of Pharmaceutical Analysis. 3th Edn; 1999, 196-198.
2. Simon Dougles A Skoog. Analytical Chemistry, 8th Edition, Saunders College Publishers, Philadelphia, 1996, 1-15.
3. J. Garrett. CEM 333 Instrumental Analysis, 2nd Edition, McGraw Hill Book Company, 1998, 16.2.
4. Gurudeep R Chatwal. Instrumental Methods of Chemical Analysis, 5th Edition, Himalaya Publishing House, New Delhi, 2007, 624-630.
5. Anjaneyulu.Y, Marayyah. Quality Assurance & Quality Management in Pharmaceutical Industry. Pharma book publishers, Hyd, 2005, 78-108.
6. PD. High Performance Liquid Chromatography Quantitative Analysis of Pharmaceutical Formulations. CBS Publishers and Distributors, New Delhi, 1st Edn; 2001, 3-72, 116-120.
7. Mendham J, Denney RC, Barnes JD, Thomas M, Vogel's. Text Book of Quantitative Analysis.6th Edn; 2004, 1-10.

8. Ewing, GW. Instrumental methods of chemical analysis; 5th edition, Mcgraw-Hill,New Mexico, 1985, 1-558.
9. Williard HH, Merit LL, John A. Dean and Settle FA. Instrumental methods of analysis. 7th Edition, C.B.S. Publishers, New Delhi, 2002, 580-590.
10. Shulamit Levin. High Performance Liquid Chromatography in Pharmaceutical Analysis, Medtechnica Analytical Department, 2010.
11. http://www.drugbank.ca/drugs/DB00484.
12. http://drugs.webmd.boots.com/drugs/drug-59-Brimonidine-Tartrate.aspx.
13. http://www.drugs.com/monograph/brimonidine-tartrate.html.