Research Article

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Bamifylline Hydrochloride in Pharmaceutical Dosage Form

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ABSTRACT

A simple, precise, accurate, and rapid HPLC method has been established and authorised for simultaneous estimation of Bamifylline Hydrochloride in pharmaceutical dosage forms. Chromatographic separation was conducted on agilent technologies -1260 series with G1311C quarternary pump, eclipse XDB C18 column (4.6 mm i.d. x 250 mm, 5 µm particle sizes) and equipped with photo diode array detector G1315D. Mobile phase consisted of methanol and acetonitrile were mixed in the ratio of 70:30 v/v, was used at a flow rate of 1 mL/minute and detection wavelength was set at 264 nm. The bamifylline hydrochloride follows linearity in the concentration range of 2-10 µg /mL with good correlation coefficient value of 0.9997. The precision of the method was studied as an intra-day and inter-day studies. The % RSD value is less than two indicates that the method is precise. The % recovery was found to be in the range of 99.41 - 99.99%. Percentage assay of bamifylline hydrochloride tablets (Bamifix) got 98.83%. The Proposed spectrophotometric method was validated as per the ICH Q2 (R1) guidelines. The proposed UV method is precise, reproducible and accurate. Hence this rapid method can be feasible employed for the regular quality control analysis of bamifylline hydrochloride in pharmaceutical dosage form.

Keywords: Bamifylline hydrochloride, RP-HPLC, Validation, ICH guidelines.

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INTRODUCTION

The chemical name for bamifylline hydrochloride is 8-benzyl-7-[2-[ethyl (2-hydroxyethyl) amino] ethyl] -1,3- dimethylpurine-2,6-dione hydrochloride. Bamifylline is a stimulant drug of the xanthine chemical class which acts as a selective adenosine A_1 receptor antagonist[1]. It is used as a bronchodilator and cardiac asthama[2, 3]. It dilates the bronchi and also used in the treatment of reversible airways obstruction. Bamifylline hydrochloride is extensively absorbed from the GI tract, Distribution volume is 3 to 10 folds more rapidly than theophylline.

According to literature survey, it is revealed that the bamifylline hydrochloride has been estimated by High performance Liquid Chromatography[4-9], HPTLC[10, 11]. Majority of methods for determination of bamifylline hydrochloride in biological fluids and pharmaceutical dosage forms. So, we felt necessary to develop a simple, precise and rapid method for quantitative determination of bamifylline hydrochloride in tablet dosage form. The aim and objective of the present study was to develop and validate a precise, sensitive and simple cost-effective UV spectrophotometric method for bamifylline hydrochloride in its tablet dosage form. Figure 1 shows the chemical structure of bamifylline hydrochloride.

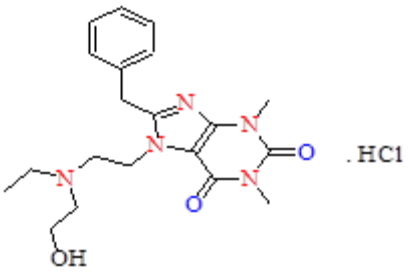

Figure 1: Chemical structure of bamifylline hydrochloride

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure drug of bamifylline hydrochloride was procured from Hetero Drugs Ltd., Hyderabad, Telangana, India. The Bamifix tablets containing 600 mg labelled claim of bamifylline hydrochloride procured from local market. Water, methanol was procured from E. Merck specialties, private Ltd., Mumbai, India.

Selection of solvent

Copious trials were performed to find out the suitable solvent system for dissolving the bamifylline hydrochloride. The solvents such as acetonitrile, dimethyl sulfoxide [DMSO] methanol and triple distilled water were tried based on the solubility of the drug. Bamifylline hydrochloride is soluble in solvents such as methanol & acetonitrile. So, methanol and acetonitrile were selected in the ratio of 70:30 v/v

Selection of detection wavelength:

To estimate the optimum λmax, bamifylline hydrochloride 10 mg /ml of the working standard solution was prepared and scanned in the UV wavelength range of 200 - 400 nm. It was observed that the drug showed maximum absorbance at 264 nm, which was chosen as the detection wavelength for the estimation of bamifylline hydrochloride.

Preparation of stock and working standard solution:

Bamifylline hydrochloride 10 µg/ ml standard stock solution was done by transferring precisely weighed 10 milligrams of standard bamifylline hydrochloride to ten milliliters calibrated flask and dissolved in water. The volume was filled up to the mark with triple distilled water (1000 µg/ml standard stock solution). From this solution one milliliter was precisely transferred into a hundred milliliter volumetric flask and volume was filled up to the mark with triple distilled water to get a concentration of 10 µg/ml which was treated as the working standard solution.

Preparation of Calibration curve:

From the above prepared bamifylline hydrochloride stock solution, appropriate dilutions were prepared to get the eventual concentration of 2, 4, 6, 8, and 10 µg/ ml and absorbance was taken at λ_{max} 264 nm. Average of such five sets of values were taken for standard calibration plot, and the calibration curve was plotted. Calibration curve was done by plotting bamifylline hydrochloride concentration on X-axis and their respective absorbance's on Y-axis. Calibration data are shown in table 1. The calibration curve is exhibited in figure 2.

Table 1: Calibration data of bamifylline hydrochloride

Concentration (µg/ml)	Absorbance
2	0.153
4	0.305
6	0.448
8	0.602
10	0.739

Table 2: Linear regression data

Parameter	Results
Detection wavelength (λ_{max})	264 nm
Beer's law limits (µg/ml)	2-10
Regression equation (Y = mx+ c): Slope (b)	y = 0.0741x + 0.0041
Standard error of slope (S_b)	0.9998
Intercept (a)	0.0041
Standard error of intercept (S_a)	0.003693
Standard error of estimate(S_e)	0.000601
Correlation coefficient (r^2)	0.9997

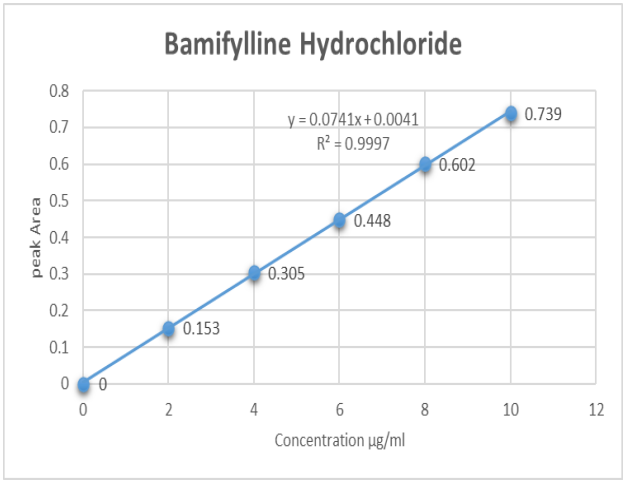

Figure 2: Calibration curve of bamifylline hydrochloride

Method development and validation:

A number of solvents were analyzed, including acetonitrile, dimethyl sulfoxide [DMSO] methanol and triple distilled water at 10 µg/ml concentrations. Nevertheless bamifylline hydrochloride was soluble and stable for minimum 6 hours at room temperature and slightly decreased absorbance after twenty-four hours in water. Hence methanol and acetonitrile were selected in the ratio of 70:30 v/v for the determination of detection wavelength and preparation of standard and working concentration. In order to check the proposed method to the pharmaceutical formulation, an assay of Bamifix 600 mg tablets was utilized at working concentration. Assay for working concentration of the sample at 264 nm. According to ICH Q2 (R1) [12,13] has provided guidelines for validation of analytical method which has defined this process as characteristic performance that is established by laboratory studies. A UV spectrophotometric method developed according to guidelines for validation of analytical procedures. The method was validated for parameters such linearity, specificity, precision, accuracy, robustness, ruggedness, LOD and LOQ.

Precision:

System precision

In system precision 10 µg/ml concentrations of six replicate recordings of absorbance at 264 nm were observed on the same day and corresponding responding responses were studied. The mean, SD and % RSD were calculated.

Method precision

Method precision was determined by preforming assay of the sample under the test of repeatability (intraday precision) and intermediate precision performed during two consecutive days by two different working concentrations. Eventually the mean, SD and % Relative standard deviation was counted. The intermediate precision results, i.e., inter-day and intra-day precision of bamifylline hydrochloride are tabulated in tables 3-5.

Table 3: Results of system precision

S. No	Absorbance
1	0.74
2	0.741
3	0.742
4	0.741
5	0.741
Mean	0.7412
Standard deviation	0.000849
% Relative Standard deviation	0.112968

Accuracy (recovery studies):

Recovery studies of bamifylline hydrochloride were carried out by utilizing standard addition method in which estimation of % mean recoery of smaple by % method at 3 different levels (80 %, 100 % and 120 % i.e., 4.8 µg/ml, 6 µg/ml, 7.2 µg/ml). These 80 to 120 levels of the sample solutions were prepared as per the procedure given in the methods from the dilutions used for linearity (6 µg/ml). At each level, 3 analyses were performed. % mean recovery was calculated as shown in table 6. The accepted limits of recovery are 98 % - 102 %. In fact, from the amount of bamifylline hydrochloride found, % recovery was estimated. The results are presented in Table 6.

Table 4: Results of method precision (Intraday precision)

Concentration (µg/ml)	Sample absorbance	Mean absorbance ± S. D	% RSD
4	0.301 0.302 0.300	0.301 ± 0.001	0.333426
6	0.452 0.455 0.451	0.452 ± 0.002	0.468867
8	0.604 0.601 0.603	0.602 ± 0.001	0.236461

Table 5: Results of method precision (Interday precision)

Concentration (µg/ml)	Sample absorbance	Mean absorbance ± S. D	% RSD (n=3)
4	0.302 0.303 0.305	0.3033 ± 0.001	0.50428
6	0.454 0.452 0.457	0.4543 ± 0.002	0.554813
8	0.602 0.606 0.603	0.6036 ± 0.002	0.343937

Table 6: Accuracy of results

Level (%)	Absorbance	% Recovery	Mean % Recovery	% RSD
80	0.366	99.72	99.90	0.836
80	0.362	100.82		
80	0.368	99.18		
100	0.455	98.78	99.52	0.553
100	0.457	99.34		
100	0.452	100.44		
120	0.546	99.45	99.99	0.482
120	0.542	100.18		
120	0.541	100.36		

Table 7: Result of assay of pharmaceutical formulation (Bamifix)

Concentration (µg/ml)	Mean Absorbance ± S. D	% RSD	% Recovery (Amount found)
Bamifix 6 µg/ml	0.452 ± 0.001	0.2217	99.83

*mean of 3 determinations

Analysis of marketed formulation

The validated method was applied to the estimation of bamifylline hydrochloride tablets. 20 tablets were assayed and the results are represented in table 9 which indicates that the amount of drug in the tablet sample was in good agreement with label claim of the formulation as indicated by percentage recovery 99.83%.

RESULTS AND DISCUSSION

The ultraviolet spectra of bamifylline hydrochloride were scanned in the region between 200-400 nm. The overlay spectra of bamifylline hydrochloride at different concentrations were absorbed maximum at 264 nm, which was selected as the detection wavelength. The response of the bamifylline hydrochloride was found to be linear in the concentration range of 2-10 µg/ml with a good correlation coefficient of r^2 = 0.9997 and the figure 2 shows the bamifylline hydrochloride linearity calibration curve and the table 1 shows the calibration data. Table 2 shows the Linear regression data of the proposed UV method. The system precision and method precision of the method with interday and intraday precision was found to be good with % RSD less than 2 which indicates that the method was precise and the results are presented in table 3 to table 5. Accuracy studies were carried out by a recovery study using a standard addition method at three different concentration levels (80%,100%,120%). The mean percentage recovery at each level should be 98.0-102.0%. All the results are well within the acceptance criteria (99.52-99.99) and results indicate that the method is accurate. Results of accuracy study represented in table 6. Ruggedness was performed to check the reproducibility which showed the % RSD less than 2 which indicates that the method was rugged (Table 7). Robustness were performed by changing two different wavelengths. Even though by changing the minor modifications, the % RSD got < 2 which shows that the method developed is robust (Table 8). The developed method was eventually applied for quantification of bamifylline hydrochloride in tablets. The mean % assay values were found to be 99.83. The amount of the drug in the tablet sample was in good agreement with label claim of the formulation. The assay results are shown in table 9. The developed method has good linearity, accuracy, and precision results indicate that the high quality of the method.

CONCLUSION

The UV Spectrophotometric method for the estimation of bamifylline hydrochloride in pharmaceutical dosage forms was found to be rapid, simple, accurate, sensitive and precise. Moreover, this UV method offers time saving, cost effective for an HPLC method of analysis for bamifylline hydrochloride from formulations. Hence the proposed method can be utilized for the routine analysis of bamifylline hydrochloride in its pharmaceutical dosage form.

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