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Development and validation of UV Spectrophotometric method for the determination of Bamifylline hydrochloride in pharmaceutical dosage form

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ABSTRACT

A simple, precise, cost effective, accurate UV- spectrophotometric method has been developed for the estimation of bamifylline hydrochloride in the Pharmaceutical dosage form. Bamifylline hydrochloride shows highest λmax at 263 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 99.52 - 99.99%. Percentage assay of bamifylline hydrochloride tablets (Bamifix) got 99.83%. 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.

1. 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 far to our present knowledge, no UV spectrophotometric method has been reported for the determination of bamifylline hydrochloride in pharmaceutical dosage form hitherto. 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 (chemical structure from pubchem).

2. MATERIALS AND METHODS

Instrument: A double beam ELICO SL 210 Ultraviolet -Visible spectrophotometer consisting of 2 matched quartz cells with one cm light path was taken for measuring of absorbance of bamifylline hydrochloride. Essaevibra AJ (0.1 mg sensitivity) balance was utilized for weighing. Ultra sonicator bath Model no - 91250, PCI Ltd., Mumbai were utilized in this study.

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 triple distilled water, methanol and

DMSO so triple distilled water were selected right through the experiment.

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 263 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} 263 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.

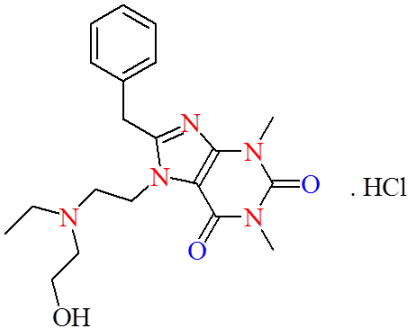

Figure.1.Chemical structure of bamifylline hydrochloride

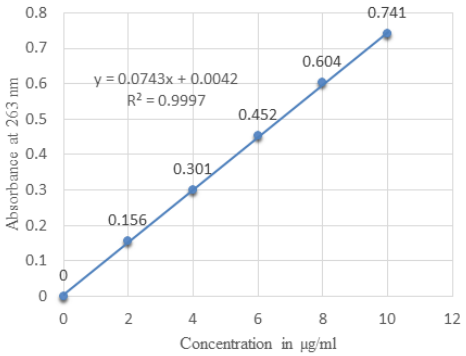

Figure.2. Calibration curve of bamifylline hydrochloride

Table.1.Calibration data of bamifylline hydrochloride

Concentration (µg/ml)	Absorbance
2	0.156
4	0.301
6	0.452
8	0.604
10	0.741

Table.2. Linear regression data

Parameter	Results
Detection wavelength (λ_{max})	263 nm
Beer's law limits (µg/ml)	2-10
Molar absorptivity (L. mole^{-1} cm^{-1})	753.333
Sandell's sensitivity (µg /cm^2/0.001 absorbance unit)	0.0132
Regression equation (Y = mx+ c): Slope (b)	0.0743x + 0.004
Standard error of slope (S_b)	0.9996
Intercept (a)	0.004
Standard error of intercept (S_a)	0.00361
Standard error of estimate(S_e)	0.00499
Correlation coefficient (r^2)	0.9997

Method development and validation: A number of solvents were analyzed, including ethanol, DMSO, and methanol 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 water solvent was used 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 263 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 263 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 were 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	0.741
2	0.74
3	0.741
4	0.742
5	0.742
Mean	0.7412
Standard deviation	0.000837
% Relative Standard deviation	0.112879

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	0.332226
6	0.452 0.455 0.451	0.452 ± 0.002	0.459867
8	0.604 0.601 0.603	0.602 ± 0.001	0.253461

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	0.50358
6	0.454 0.452 0.457	0.4543 ± 0.002	0.553913
8	0.602 0.606 0.603	0.6036 ± 0.002	0.344837

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 %. Infact, from the amount of bamifylline hydrochloride found, % recovery was estimated. The results are presented in Table 6.

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		

Ruggedness: Ruggedness is done by performing proposed method on different instruments. In addition to that this method is carried out by two different analysts and performing the method on different days to check the reproducibility. The results obtained are shown in table 7.

Table.7. Results of ruggedness

Analyst	Sample Absorbance	Mean Absorbance ± S. D	% RSD
Analyst 1	0.742 0.745 0.748	0.745 ± 0.003	0.402
Analyst 2	0.745 0.741 0.746	0.744 ± 0.002	0.355

Robustness: Robustness were estimated by performing the same proposed method on (263 +/- 2 nm wavelengths). The analysis showed % RSD less than 2 which indicates the proposed method is robust. The Robustness of bamifylline hydrochloride are tabulated in 8.

Table.8. Results of robustness

Wavelength (nm)	Sample Absorbance	Mean Absorbance ± S. D	% RSD
263 nm	0.747 0.745 0.746	0.746 ± 0.004	0.536
265 nm	0.743 0.744 0.744	0.743 ± 0.003	0.403

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%.

Table.9. 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

3. 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 263 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 frormulation. 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.

4. 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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