

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF BAMIFYLLINE HYDROCHLORIDE IN TABLET DOSAGE FORM

PANCHUMARTHY RAVISANKAR^{1*}, SHAHEEM SULTHANA¹, INTURI MARY THANUJA¹, A. DIHITHA CHOWDARY¹, J. VYSHNAVI¹

Vignan Pharmacy College, Vadlamudi, Guntur, Andhra Pradesh, India
Email: banuman35@gmail.com

Received: 27 Apr 2017, Revised and Accepted: 14 Jun 2017

ABSTRACT

Objective: The objective of the current study was to develop and validate a novel RP-HPLC method for determination of bamifylline hydrochloride in pharmaceutical dosage form.

Methods: Chromatographic separation was conducted on Agilent technologies-1260 series with the G1311C quaternary pump, eclipse XDB C₁₈ column (4.6 mm i.d. X 250 mm, 5 µm particle sizes) and equipped with photodiode array detector G1315D. Mobile phase consisted of methanol and acetonitrile were mixed in the ratio of 90:10 v/v, was used at a flow rate of 1 ml/min and detection wavelength was set at 263 nm.

Results: The retention time for bamifylline hydrochloride was found to be 2.913 min. The calibration was linear ($r^2 = 0.9996$) in the concentration range of 2-10 µg/ml. The limit of detection and the limit of quantitation were found to be 0.4825 µg/ml and 1.4621 µg/ml respectively. Recovery of bamifylline hydrochloride in tablet formulation was observed in the range of 99.6-99.8 %. Percentage assay of bamifylline hydrochloride (Bamifix) was found to be 99.4 % w/w.

Conclusion: Thus the novel proposed method for bamifylline hydrochloride was found to be feasible for the estimation of bamifylline hydrochloride in bulk as well as a pharmaceutical dosage form.

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

© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)
DOI: <http://dx.doi.org/10.22159/ijap.2017v9i4.19438>

INTRODUCTION

The chemical name of bamifylline hydrochloride is 8-Benzyl-7-[2-[N-ethyl-N-2-hydroxyethyl amino] ethyl] theophylline hydrochloride (fig. 1). Bamifylline hydrochloride is a theophylline derivative and is used as Anti-asthmatic agent [1], bronchodilator [2], a Non-selective phosphodiesterase inhibitor and chronic obstructive pulmonary disease (COPD). Mechanism of action of bamifylline hydrochloride is an adenosine A₁ receptor antagonist [3]. Generally, bamifylline hydrochloride is not converted to theophylline in the body. Bamifylline and theophylline drugs are bronchodilators and their synthetic origin and belong to Xanthine. Theophylline and the physical mixtures TA-NSD [4] and compatibility studies with different polymers [5] were investigated by using a polarizing microscope, FTIR, DSC, PXRD and KFT. A thorough literature survey of bamifylline hydrochloride revealed that very few analytical methods have been reported for estimation of bamifylline hydrochloride hitherto. The majority of methods for determination of bamifylline hydrochloride in biological fluids and pharmaceutical dosage forms include liquid chromatography with ultraviolet detection [6] HPLC [7-11], HPTLC [12, 13]. This novel proposed method contributes quick estimation, correct peak shape, precise, simple, and quick, use of smaller sample volumes and utilizing methanol as a mobile phase which is economical when compared with other existing methods. So it is necessary to develop a simple, precise and rapid RP-HPLC method for quantitative determination of bamifylline hydrochloride. This work describes the validation parameters stated by the International Conference on Harmonization [ICH] guidelines Q2 (R1) [14, 15].

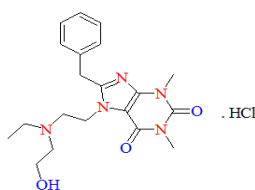


Fig. 1: Chemical structure of bamifylline hydrochloride from pubchem

MATERIALS AND METHODS

Chemicals and reagents

The above said the standard drug was gifted from Hetero Labs Ltd., Hyderabad, India. All the chemicals used in this method were of high-grade purity and purchased from Merck Chemical Division Ltd., Mumbai. HPLC grade acetonitrile, water, methanol and triethylamine were obtained from Merck Pharmaceuticals private Ltd., Mumbai, India. Commercial tablets of the above said formulation was obtained from a local pharmacy.

Instrumentation and conditions

The high pressure liquid chromatographic system utilized was an Agilent high-pressure liquid chromatograph 1260 series with the G1311C quaternary pump, eclipse XDB-C₁₈ column (5 µm particle size x 4.6 x 250 mm) (made in USA) and a diode array detector G1315D was utilized. Ezchrome elite software was used for chromatography data acquisition, processing and control of HPLC chromatograph. Digital pH meter (systronics model-802), an electronic balance (Shimadzu TX223L), a sonicator (spectral lab, model UCB 40) and UV-Visible spectrophotometer (systronics model-2203) were used in this study.

Preparation of mobile phase

To prepare mobile phase HPLC grade methanol and acetonitrile were mixed in the ratio of 90:10 % v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Preparation of stock and working standard solutions

Accurately 10 mg of pure bamifylline hydrochloride was weighed and transferred in 10 ml clean volumetric flask and 5 ml mobile phase was added, if necessary sonicate to dissolve. The volume was adjusted up to the mark with the mobile phase. This is the primary stock solution of bamifylline hydrochloride with a concentration of 1000 µg/ml. The secondary stock solution is prepared by adding 1 ml of primary stock solution in 10 ml volumetric flask and made up the volume with a mobile phase having the concentration range 100

µg/ml. five working standard solutions were prepared for calibration graph by adding defined volumes of the secondary stock solution and diluting with the mobile phase. The concentrations of bamifylline hydrochloride are 2, 4, 6, 8 and 10 µg/ml respectively.

Sample preparation for tablets

Accurately weighed twenty bamifylline hydrochloride tablets and average weight was calculated. Accurately weighed a portion of tablet, powder equivalent to 100 mg of bamifylline hydrochloride and transfer into a 100 ml volumetric flask to this 50 ml mobile phase was added and sonicated for 15 min. Mobile phase was adjusted up to the mark. The solution was filtered using 0.45 µm nylon filter. From the above solution pipette out 1.0 ml into a 100 ml volumetric flask and dilute with mobile phase up to the mark and mix well. Further diluted to get desired concentration. The amount present in the tablet was calculated from plotted calibration graph or utilizing regression equation.

After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 20 µl fixed sample loop of the injection port.

Analytical method validation

Once the chromatographic and the experimental conditions were established, the method was validated by the determination of the following parameters such as specificity, system suitability, linearity, precision, accuracy, robustness, limit of detection (LOD), limit of quantitation (LOQ) as per ICH Q2 (R1) guidelines.

System suitability parameters

The chromatographic systems used for analysis must pass system suitability before going to start the experiment. At first HPLC system is stabilized for forty minutes. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms to evaluate the system suitability parameters such as tailing factor (NMT 1.5), theoretical plate count (NLT 3000) and retention time. The % RSD for the peak area of six replicate injections of Sofosbuvir standard NMT 2.0. The parameters such as tailing factor, % RSD and theoretical plates were studied.

Linearity

A standard stock solution of the bamifylline hydrochloride (1 mg/ml) was prepared with the mobile phase. To study the linearity range of drugs, serial dilutions were made from standard stock solution in the range of 2-10 µg/ml.

Specificity

Specificity of an analytical method is its ability to measure accurately and specifically the analyte of interest without interference from placebo and degradation products. The specificity of the method was established by injecting blank, placebo and standard solution in triplicate and recording the chromatograms.

Precision

The precision of the method was determined by repeatability (intraday) and intermediate precision (interday). Repeatability was

determined by performing six repeated analysis of the same working solution of bamifylline hydrochloride on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on different days and also by another analyst performing the analysis in the same laboratory (between-analysts).

Accuracy

The accuracy of a method is defined as the closeness of a measured value to the true value. The recovery studies were carried out at 50 %, 100 %, and 150 % of the target level in the tablet in triplicate each in the presence of placebo.

Robustness

The robustness was determined by analyzing the same sample under a variety of conditions. The factors considered to be: variations in the flow rate, the organic ratio of mobile phase and pH. There were no significant changes in the chromatographic pattern when the above modifications were made in the experimental conditions, showing thus that the method is robust. The % RSD of bamifylline hydrochloride should be not more than 2.0 %.

LOD and LOQ

Limit of detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated based on using following formulas, $LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma/S$, where σ is the deviation response. S is the slope of the calibration curve.

RESULTS AND DISCUSSION

Method development and optimization

The current study was aimed at developing a sensitive, rapid and accurate reversed-phase HPLC method for the analysis of bamifylline hydrochloride in bulk drug and in pharmaceutical dosage form. In order to get decorous retention time, sharp and well-resolved peak, the parameters such as different flow rates, detection wavelength, a choice of mobile phases containing acetonitrile, methanol, and HPLC grade water were studied. Good quality symmetrical sharp peak, minimum tailing factor in short run time was obtained with C₁₈ column and mobile phase composed of methanol: acetonitrile in the ratio of 90:10 v/v, at a flow rate of 1 ml/minute with maximum lambda max at 263 nm. All the system suitability parameters were computed at the optimised chromatographic conditions. The retention time of 2.913, plate number of 12215 and a tailing factor of 1.112 were obtained for bamifylline hydrochloride. The obtained values of the entire system suitability parameters are within the limits of the agreeable range, which shows that the proposed method is fit for detection of bamifylline hydrochloride in the tablet form. The optimum chromatographic conditions and system suitability parameters are tabulated in table 1.

Table 1: Optimized chromatographic conditions and system suitability parameters

Parameter	Chromatographic conditions
Instrument	Agilent Technologies 1260 series with the G1311C quaternary pump.
Column	Eclipse XDB C ₁₈ column (4.6 mm i.d. X 250 mm, 5 µm particle size)
Detector	1260 series DAD VL photo diode array detector G1315D
Mobile phase	Methanol: acetonitrile (90: 10 % v/v)
Flow rate	1 ml/minute
Detection wavelength	UV at 263 nm
Runtime	10 min
Temperature	Room temperature (25 °C)
Volume of injection loop	20 µl
Retention time *	2.913 min
Theoretical plates [th. pl]*	12215
Tailing factor*	1.112

*= number of six determinations.

Linearity

The calibration curve was constructed between concentrations versus peak area by the prepared in the concentration range of 2-10 µg/ml of stock solution. The linearity range was found to be 2-10 µg/ml and the results are tabulated in table 2. The calibration graph of bamifylline hydrochloride is presented in fig. 2. The regression equation was found to be $Y = 60359x + 2678.1$. The correlation coefficient of bamifylline hydrochloride r^2 was noted as 0.9996 which states that the method was good linear to the concentration versus peak area responses. The results show that a phenomenal relationship between peak area and concentration of the drug in the calibration curve. The standard chromatograms of bamifylline hydrochloride are presented in fig. 3 to fig. 7.

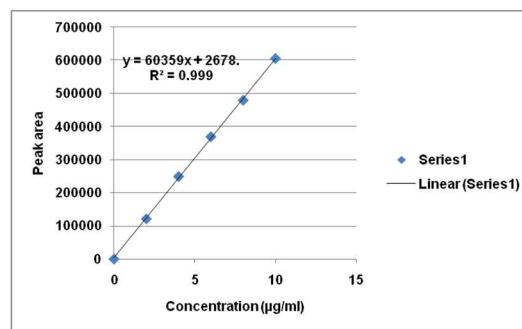


Fig. 2: Calibration graph of bamifylline hydrochloride

Table 2: Calibration data of bamifylline hydrochloride

S. No.	Concentration (µg/ml)	Peak area, (mAu)
1	2	121573
2	4	249585
3	6	369733
4	8	480018
5	10	605006

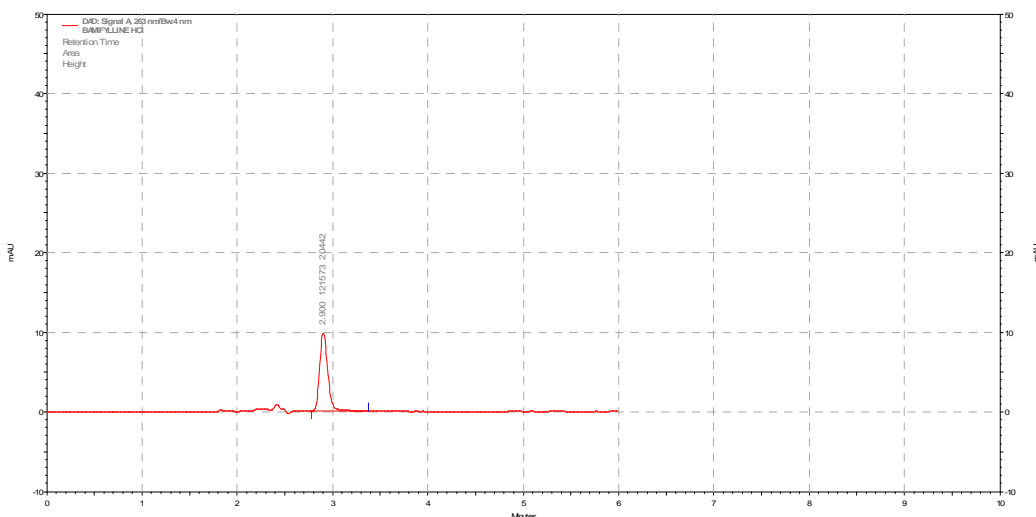


Fig. 3: Standard chromatogram bamifylline hydrochloride (2 µg/ml)

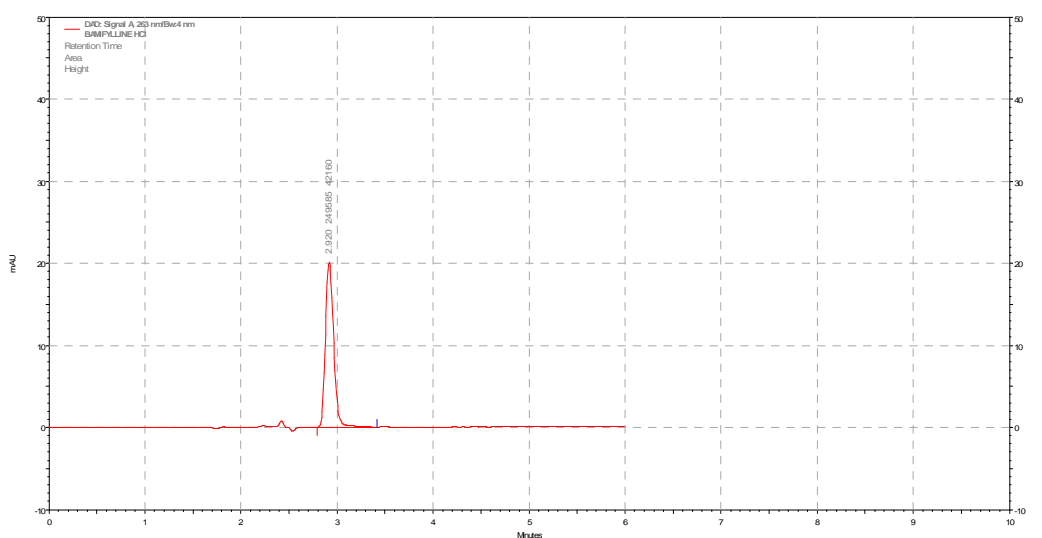


Fig. 4: Standard chromatogram bamifylline hydrochloride (4 µg/ml)

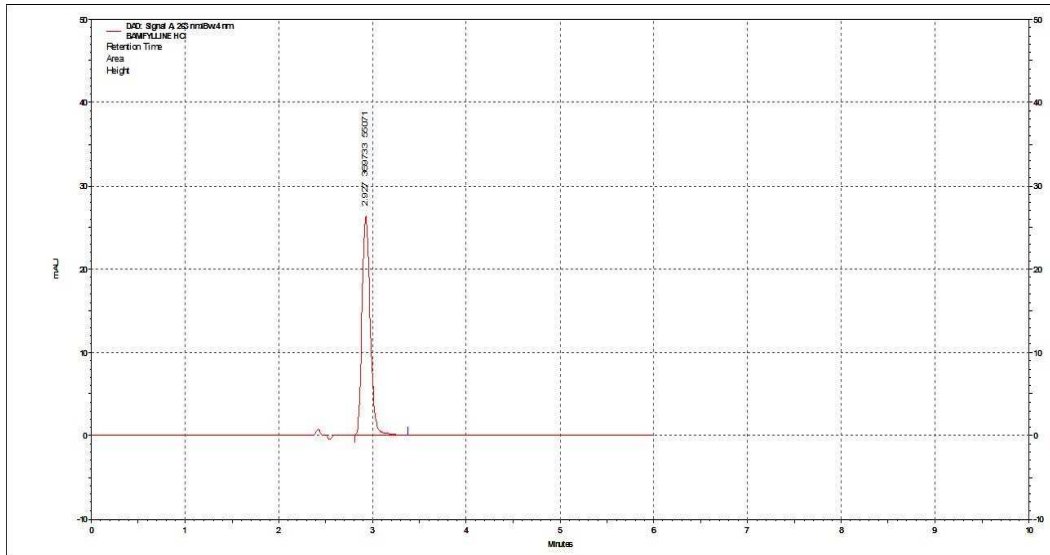


Fig. 5: Standard chromatogram bamifylline hydrochloride (6 µg/ml)

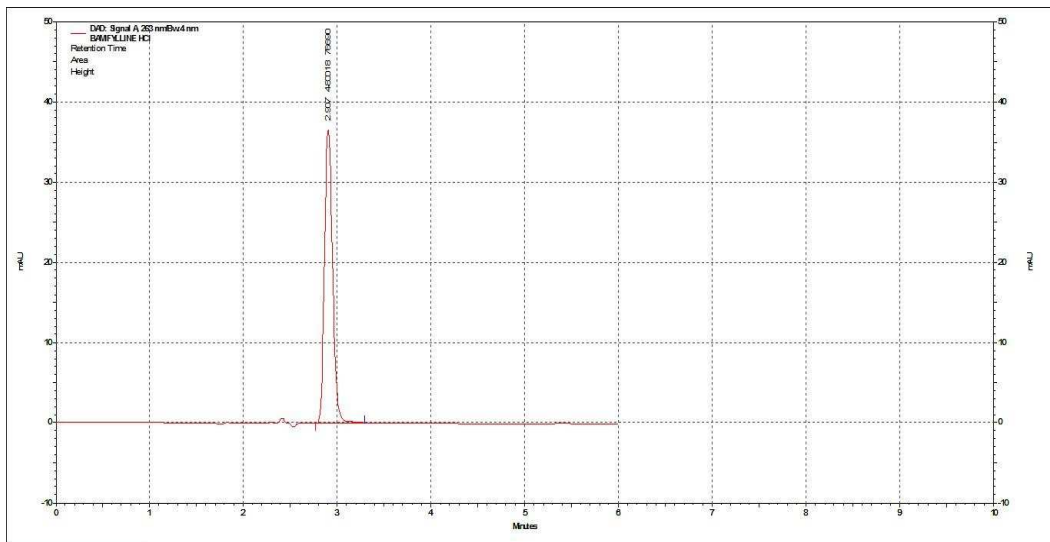


Fig. 6: Standard chromatogram bamifylline hydrochloride (8 µg/ml)

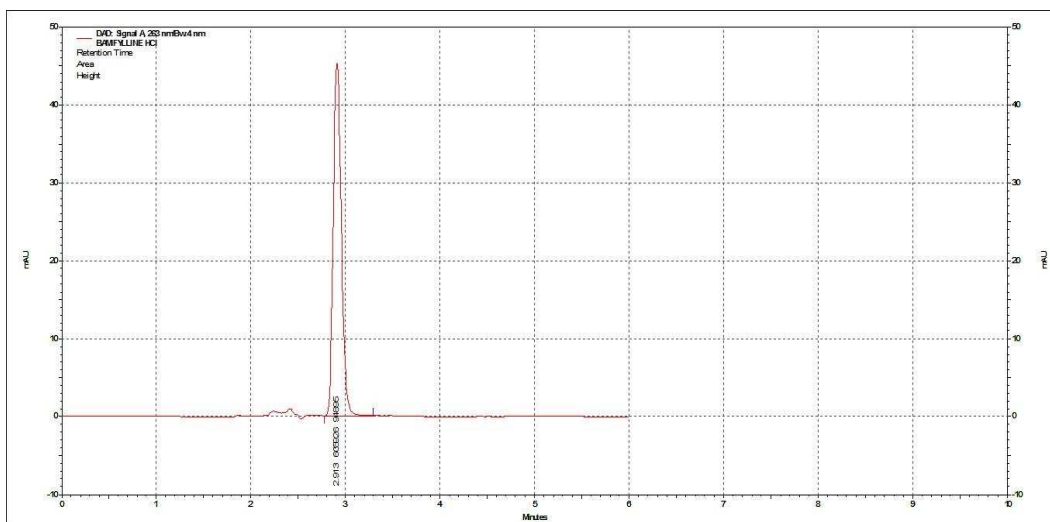


Fig. 7: Standard chromatogram bamifylline hydrochloride (10 µg/ml)

Specificity

Commonly used tablet excipients did not interfere with this method. It shows that the method is specific. Furthermore, the well-shaped peaks also indicate the specificity of the method. The specificity results are tabulated in table 3.

Precision

System precision is shown in table 4. It was noted that the percentage RSD values of precision for Intra-day and inter-day (table 5) precision was 0.0008 and 0.0007 respectively. Intra-day

and inter-day % RSD values lower than 2% clearly assuring that this method was found to be fairly precise and reproducible.

Accuracy

Regarding accuracy, a known amount of the standard drug was added to the fixed amount of preanalyzed sample solution. % recovery was calculated by comparing the area before and after addition of the standard drug. The standard addition method was performed at 50 %, 100 % and 150 % levels. The high value of recoveries obtained for bamifylline hydrochloride indicates that the proposed method was found to be accurate. The recovery results are presented in table 6.

Table 3: Specificity study

Name of the solution	Retention time (t _R) min.
Mobile phase	No peaks
Placebo	No peaks
BMF 10 µg/ml	2.913 min.

Table 4: Results of system precision

Sample	Concentration (µg/ml)	Injection no.	Peak area (mAU)	mean±SD*	% RSD#
Bamifylline	10	1	605006	6053.7±472.3	0.07
		2	605009		
		3	605200		
		4	605098		
		5	605980		
		6	605985		

*Each value is represented as a mean±SD of 5 observations (n=5), SD: Standard Deviation, RSD: Relative Standard Deviation, #Acceptance criteria<2.0.

Table 5: Intra-day and Inter-day precision data of bamifylline

Concentration (µg/ml)	Intra-day precision	Inter-day precision		
		Day 1	Day 2	Day 3
6	369733	369732	369731	369732
	369731	369733	369734	369738
	369737	369734	369727	369726
	369735	369730	369729	369729
	369738	369735	369730	369741
	369730	369738	369736	369735
mean±SD*	3.224±3697	2.732±3697	3.311±3697	5.612±3697
% RSD#	0.000872	0.000739	0.000896	0.001518

*Each value is represented as a mean±SD of 5 observations (n=5), SD: Standard Deviation, RSD: Relative Standard Deviation, #Acceptance criteria<2.0.

Table 6: Accuracy study

The amount of the sample added (µg/ml)	The amount of standard drug solution added (µg/ml)	Percentage of standard added	Mean percent recovery	%RSD*
6	3	50 %	99.7	0.11
6	6	100 %	99.8	0.09
6	9	150 %	99.6	0.13

*Average of triplicate injections

Table 7: Robustness results of bamifylline hydrochloride

Parameter	Optimized	Used	Retention time (min)	Plate count\$	Peak asymmetry #	Remark
Flow rate (±0.2 ml/min)	1.0 ml/min	0.8 ml/min	2.917	12,200	1.190	*Robust
		1.0 ml/min	2.913	12,215	1.25	*Robust
		1.2 ml/min	2.911	12,300	1.21	*Robust
Detection wavelength (±5 nm)	263 nm	258 nm	2.912	12,245	1.113	Robust
		263 nm	2.913	12,215	1.112	Robust
		268 nm	2.913	12,225	1.113	Robust
Mobile phase composition (Methanol: Acetonitrile) (±0.5%)	90:10 v/v	95:05 v/v	2.911	12,649	1.202	*Robust
		90:10 v/v	2.913	12,215	1.25	*Robust
		85:15 v/v	2.919	12,440	1.102	*Robust

Acceptance criteria (Limits): #Peak Asymmetry<1.5, \$Plate count>2000, * Significant change in Retention time.

Robustness

The robustness of the developed method was evaluated by small deliberate changes in method parameters such as flow rate (±0.2

ml/min), detection wavelength (±5 nm) and mobile phase composition (±0.5 %). The % RSD values of robustness which is less than 2 % reveals that the proposed method is robust. The results of robustness study results are shown in table 7. Even though the small changes in the

conditions did not significantly effect on the peak asymmetry, plate count and retention time of bamifylline hydrochloride.

LOD and LOQ

The developed method found to be high sensitivity with LOD of 0.4825 µg/ml and LOQ of 1.4621 µg/ml. The LOD and LOQ values are presented in table 8. The results of LOD and LOQ supported the sensitivity of the developed method.

Analysis of tablet formulation

The developed, validated method was successfully applied for the determination of bamifylline hydrochloride in their tablet dosage form. The assay result (table 9) shows that the amount of the drug was in excellent agreement with the labelled value of the formulation. The representative sample chromatogram of bamifylline hydrochloride is shown in fig. 8. Eventually, summary of validation parameters is shown in table 10.

Table 8: LOD and LOQ results of bamifylline hydrochloride

Limit of Detection (LOD)	0.4825 µg/ml
Limit of Quantitation (LOQ)	1.5922 µg/ml

Table 9: Results of analysis of bamifylline hydrochloride

S.No	Formulation	Labelled amount mg/tablet	Amount found mg/tablet	Mean % assay±SD	% RSD*
1	Bamifix tablets	600	595.01	99.4±1	0.100

*Average of six determinations; SD: standard deviation; RSD: relative standard deviation.

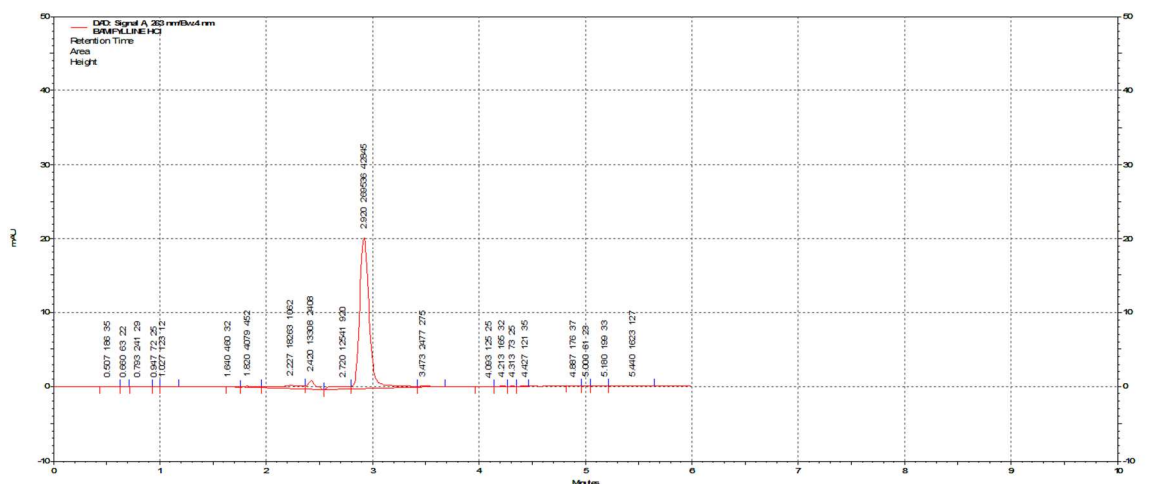


Fig. 8: Bamifylline hydrochloride sample chromatogram

Table 10: Summary of validation parameters

Validation parameters	Results
Detection wavelength (λ_{max})	263 nm
Beer's law limits (µg/ml)	2-10 µg/ml
Regression equation	$Y = 60359x + 2678.1$
Correlation coefficient (r^2)	0.9996
Flow rate	1 ml/minute
Retention time (R_t)	2.913 min
Intra-day Precision (% RSD)	0.0008
Inter-day Precision (% RSD)	0.0007
Accuracy (% recovery)	99.6-99.8 % w/w
Limit of Detection (µg/ml)	0.4825 µg/ml
Limit of Quantitation (µg/ml)	1.4621 µg/ml
Assay (% w/w)	99.4 % w/w

CONCLUSION

In conclusion, a simple, accurate, sensitive, rapid and precise RP-HPLC method was developed and validated for the estimation of bamifylline hydrochloride in pharmaceutical dosage form. Statistical analysis for the above said results obviously demonstrates that the method is fit for the estimation of bamifylline hydrochloride in tablet forms without any interference. This method can help research studies, quality control and routine analysis with lesser resources available. The results of the assay of pharmaceutical

formulation of the developed method are highly reliable and reproducible and is in good agreement with the label claim of the drug. Hence the method can be used for the regular analysis of bamifylline hydrochloride in tablet dosage form without any interference of excipients.

ACKNOWLEDGEMENT

The authors are thankful to Hetero Labs for providing the sample of bamifylline hydrochloride. We are highly grateful to Dr. L. Rathaiah,

honourable chairman, Vignan group of institutions, Vadlamudi, Guntur for providing the necessary laboratory facilities to carry out the present investigation.

CONFLICT OF INTERESTS

Declared none

REFERENCES

- Nishimura Y, Maeda H, Hashimoto A, Tanaka K, Yokoyama M. Relationship between bronchial hyperreactivity and symptoms of cardiac asthma in patients with non-valvular left ventricular failure. *Jpn Circ J* 1996;60:933-9.
- Alciato P, Cantone PA, Fico D, Gagliardini R, Petrella V. Bamifylline in the therapy of asthmatic syndromes. *Minerva Med* 1990;81:93-7.
- Tomai F, Crea F, Gasparidone A, Versaci F, De Paulis R, Polisca P, Chiariello L, *et al.* Effects of adenosine receptor blockade by bamiphylline on ischaemic preconditioning during coronary angioplasty. *Eur Heart J* 1996;17:846-53.
- Ilma Nugrahani, Evalianty agiana Pertiwi, Okky Dwichandra putra. Theophylline-na-saccharine single crystal isolation for its structure determination. *Int J Appl Pharm Sci* 2015;7:15-24.
- Gaganpreet Kaur, Prabhudas K, Amit Singh. Development of mucoadhesive carbohydrate heteropolymer microbeads for sustained release of theophylline. *Int J Appl Pharm Sci* 2015;7:295-9.
- Carlucci G, Colanzi A, Mazzeo P. Determination of bamifylline hydrochloride impurities in bulk material and pharmaceutical forms using liquid chromatography with ultraviolet detection. *J Pharm Biomed Anal* 1990;8:1067-9.
- Nicot G, Lachatre G, Gonnet C, Rocca JL. High performance liquid chromatographic method for determination of bamifylline and its three major metabolites in human plasma. *J Chromatogr* 1983;277:239-49.
- Papadoyannis, Samanidou V, Zotou A. Simultaneous determination of bamifylline and its major metabolite AC-119 by RP-HPLC. *J Liq Chromatogr Relat Technol* 2006;16:3847-61.
- Belliardo F, Lucarelli C. High performance liquid chromatographic method for determination of bamifylline and its major metabolite in human plasma. *J Liq Chromatogr* 1990;535:305-9.
- B Patel, Mrunali R Patel, Tapan S, Goswami. Stability indicating RP-HPLC method for estimation of bamifylline hydrochloride in tablet formulation: development and validation. *Thai J Pharm Sci* 2014;38:168-75.
- Gerlo E, Maes V. High performance liquid chromatographic method for assay of bamifylline in human plasma of neonates. *Am Assoc Clin Chem* 1988;34:1657.
- Rashmin B, Patel, Mrunali R, Patel, Tapan S, Goswami. A new HPTLC method for estimation of bamifylline: Development and validation consideration. *Int J Adv Pharm Anal* 2012;2:83-7.
- Patel R, Patel M, Dubey N, Dubey N, Patel B. HPTLC method development and validation: strategy to minimize methodological failures. *J Food Drug Anal* 2012;20:561-71.
- Ravisankar P, Naga Navya Ch, Pravallika D, Navya Sri D. A review on step-by-step analytical method validation. *IOSR J Pharm* 2015;5:7-19.
- ICH Q2 (R1), Validation of analytical procedures, Text and methodology. International Conference on Harmonization, Geneva; 2005. p. 1-17.

How to cite this article

- Panchumarthy Ravisankar, Shaheem Sulthana, Inturi. Mary Thanuja, A Dihitha Chowdary, J Vyshnavi. Development and validation of a RP-HPLC method for the determination of bamifylline hydrochloride in tablet dosage form. *Int J Appl Pharm* 2017;9(4):76-82.