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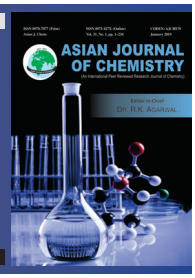

RP-HPLC Estimation of Bumetanide and its Impurities in Oral Solid Dosage Form

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A novel and simultaneous stability indicating RP-HPLC method has been developed for quantitative analysis of bumetanide in fixed dose pharmaceutical formulations. Bumetanide and its degradation products are well separated by the Discovery C_{18}, 250 × 4.6 mm, 5 µm column as a stationary phase and (50:50 v/v) of 0.1 % *o*-phthalaldehyde and acetonitrile as a mobile phase. All the compounds are monitored using photodiode array detector at 254 nm with an isocratic method and the flow rate of 1.0 mL/min was maintained. Validation of method was performed as per International Council for Harmonization (ICH) guidelines and the parameters namely; precision, accuracy, specificity, stability, robustness, linearity, limit of quantitation (LOQ) and limit of detection (LOD) were evaluated. The linearity of the proposed method was found to be 0.315-1.875 µg/mL for bumetanide and its impurities. The developed method is more economical and suitable for laboratory use because of solvent consumption is very less. Hence, the developed method can be used for the determination of bumetanide and its impurities in drug product stability studies and pharmaceutical formulations.

Keywords: RP-HPLC, Bumetanide, Degradation products.

INTRODUCTION

Bumetanide is chemically designated as 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoic acid and used as loop diuretics (sulfamyl category) in the treatment of heart failure, renal insufficiency and hepatic cirrhosis cases [1-3]. It is commonly used in the patients with high doses of furosemide (diuretic) that is non-effective and more active than the furosemide about 40-60 times on a weight basis [4]. Bumetanide also acts as an inhibitor of chloride (Cl^-) co-transporter NKCC1 (cation-chloride co-transporter) to keep the intracellular Cl^- levels in the neurons at a definite level [5,6].

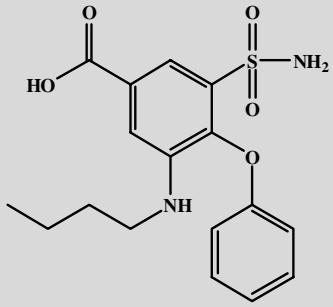
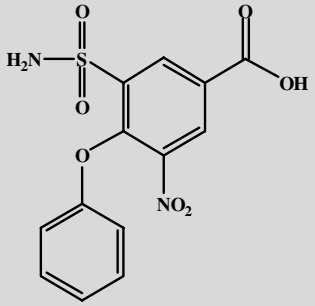
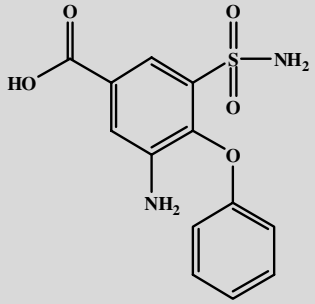
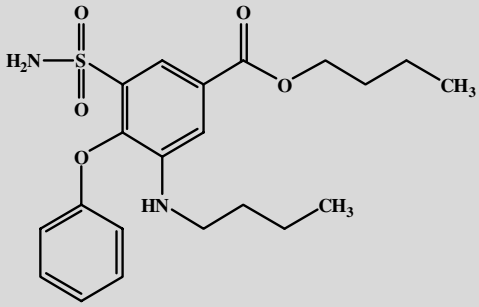
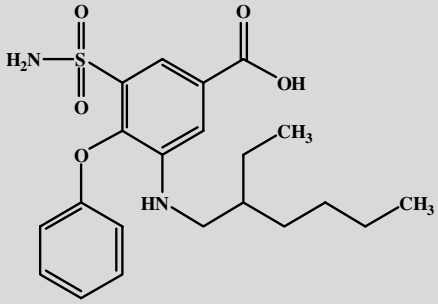
Various techniques have been reported for the analysis of bumetanide, either alone or with other drugs combination, such as fluorimetric method [7], coulometry [8] and HPLC methods [9,10]. Bumetanide levels in biological fluids have been measured by radioimmuno assay [11], potentiometry [12], gas chromatography [13], GC-mass spectrometry [14], liquid chromatography tandem mass spectrometry (LC-MS/MS) [15] and HPLC with photometric detection [16]. Although, all reported methods requires huge solvent and difficult for the laboratory scale validations.

Hence, attempted to develop and validate a simple, precise, and sensitive, stability indicating reverse-phase HPLC method for the determination of bumetanide in drug product along with its impurities in very simple mobile phase with isocratic method. The method is comprehensively validated as per the guidelines of International Conference on Harmonization (ICH) [17-19]. The molecular structure of bumetanide and the molecular formula and structure of its known impurities are presented in Table-1.

EXPERIMENTAL

Standard bumetanide (purity, ≥ 99.9%) was supplied by Spectrum Laboratories (Hyderabad, India). Acetonitrile, NaH_2PO_4 monohydrate and orthophosphoric acid are HPLC-grade/analytical grade chemicals and were purchased from Merck KGaA (Darmstadt, Germany). Centrifuge tubes and 0.45 µm membrane filters were obtained from Millipore Pvt. Ltd., Bangalore, India. Millipore water from MilliQ water purification system was used. The instrument, Waters photodiode array (PDA) detector was used and the electronic analytical balance (Thermo Fisher Scientific, Hyderabad, India) was used for weighing purposes.

TABLE-1
IUPAC NAMES AND MOLECULAR STRUCTURES OF BUMETANIDE AND ITS IMPURITIES

Impurity Name	Structure	m.f.
Bumetanide (**BUME**)		$C_{17}H_{20}N_2O_5S$
BUME-A: 3-Nitro-4-phenoxy-5-sulphamoylbenzoic acid		$C_{13}H_{10}N_2O_7S$
BUME-B: 3-Amino-4-phenoxy-5-sulphamoylbenzoic acid		$C_{13}H_{12}N_2O_5S$
BUME-C: Butyl-3-(butylamino)-4-phenoxy-5-sulphamoylbenzoate		$C_{21}H_{28}N_2O_5S$
BUME-D: 3-(2-ethylhexylamino)-4-phenoxy-5-sulphamoylbenzoic acid		$C_{21}H_{28}N_2O_5S$

Chromatographic conditions: Waters HPLC instrument with PDA detector was used for HPLC method development and validation of the samples. The technique was developed using a Discovery C18, 5 µ particle size and 250 × 4.6 mm column as a stationary phase. *o*-Phthalaldehyde (0.1 %) as buffer and acetonitrile (50:50, v/v) was used as the mobile phase. To prepare all the solutions, the mixture of acetonitrile and Milli Q water in 50:50 v/v was used as diluent. The mobile phase was degassed and thoroughly mixed before use. The flow rate of mobile phase was maintained at 1.0 mL/min and the eluted

compound bumetanide was monitored at 254 nm. The injection volumes (50 µL) were set for both standards and samples. The temperature of the column was preserved at 50 ºC.

Preparation of standard stock solution: Accurately weighed 0.5 mg of bumetanide working standard was transferred into a 10 mL volumetric flask, sonicated by adding the diluent until the complete dissolving of the drug and makeup to the mark using diluent.

Preparation of working standard solution: The standard stock solution of 0.25 mL was pipetted into a 10 mL volumetric flask and diluted the volume with diluent and mixed well.

Preparation of sample solution: Bumetanide tablet powder equivalent to 12.5 mg was taken into a 50 mL volumetric flask, added 35 mL of diluent, sonicated for 30 min and makeup to the volume with diluent. Centrifuged and filtered the sample and filtrate were used to inject into the HPLC system.

Specificity: Bumetanide and its impurities were injected into the optimized system to demonstrate the specificity of the developed method in the formulation of bumetanide [19]. The effectual separation of known impurities and degradants of bumetanide peak by forced degradation studies of bumetanide tablets were conducted at different stress conditions like acid (2N HCl), base (2N NaOH), peroxide (20 % H_2O_2), thermal (105 ºC in hot air oven) and photolytic stress (photostability chamber at 200 watt-hour/m^2).

Linearity: The linearity of bumetanide and its impurities were verified in the concentration ranges between 0.3125 µg to 1.825 µg. Each solution was prepared and injected in triplicate. Least-square regression analysis method was used for treating calibration graphs, which were obtained by plotting peak area *versus* concentration data.

Precision and accuracy: Six sample preparations were performed on the inter-day and intra-day and precision were represented by the calculated peak area of relative standard deviation (% RSD). The accuracy of developed method was determined by spiking API on placebo various concentrations ranging from 50 % to 150 % of targeted test concentration. Preparations were performed at higher and lower concentrations for wrapping the lasting levels in the triplicate from and keep the data in the range parameter.

RESULTS AND DISCUSSION

Method development: For the effective separation of bumetanide with its four known impurities, several mobile phase compositions and different stationary phases were investigated in the preliminary studies. All analytes have the different retention behaviours and hence it is a challenging development to separate all analytes in shorter method without interfering Placebo components and degradation impurities. Method development started with evaluating retention of bumetanide and its impurities in reversed-stationary phases (C8 and C18 columns), Phosphate buffer with organic modifiers and keeping impurities retention as a target. Further, chromatographic experiments were optimized by choosing orthophosphoric acid buffer with acetonitrile which is helped to retain bumetanide, stationary phases Hypersil BDS C18 and kromosil 250 mm column, with 55:45 mobile phase composition. Finally, the best separation method was found with 0.1 % *o*-phthalaldehyde (OPA) buffer, stationary phase Discovery C18, 250 mm × 4.5 mm and 5 µm column with the isocratic method. Symmetrical and high intensity chromatogram peaks of bumetanide and its impurities were recorded with the above chromatographic conditions and system suitability data are summarized in Table-2.

TABLE-2
SYSTEM SUITABILITY

Component	Retention time	Tailing factor	Theoretical plates
BUME	5.47	1.2	24425
BUME-A	3.62	1.1	10500
BUME-B	4.25	1.1	13258
BUME-C	6.26	1.1	77788
BUME-D	8.11	1.1	82319

The developed method was validated as per the International Council for Harmonization (ICH) guidelines [16-19]. The validation parameters namely, precision, accuracy, specificity, robustness, linearity, limit of quantitation (LOQ) and limit of detection (LOD) are evaluated.

Validation: The linear curve plotted in concentration ranges of 0.3125-1.875 µg/mL for bumetanide and its impurities and the results are presented in Table-3. The linear regression equation of bumetanide was found to be Y = 10551x + 155.2. 3815 X + 36.3 with the correlation coefficient greater than 0.999. The linear regression equation for BMUE-A was found to be Y = 108373x+678.47450, BUME-B was found to be Y = 103295x + 1561.8, BUME-C was found to be Y=118913x + 175.9 and BUME-D was found to be Y= 129820x + 669.9 with the correlation coefficient greater than 0.999 for all impurities. The linearity plots belong to bumetanide and its impurities are presented in Fig. 1. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by producing serial dilutions. Accuracy was performed on three levels of 50, 100 and 150 %.

TABLE-3
LINEARITY OF BUME AND ITS IMPURITIES

Stock solution (mL)	Volume of flask (mL)	Conc. of BUME (mg/L)	Conc. of BUME A, B, C & D (mg/L)	Linearity Level (%)
0.25	10	0.3125	0.3125	25
0.50	10	0.625	0.6250	50
0.75	10	0.9375	0.9375	75
1.00	10	1.25	1.2500	100
1.25	10	1.5625	1.5625	125
1.50	10	1.875	1.8750	150

Acid degradation studies: Acid degradation study was conducted by addition of 2 N HCl to a bumetanide solution prepared by adding 20 mg of bumetanide to diluent and kept the solution on shaking water bath for 30 min at 60 ºC. The solution was cooled to room temperature, neutralized with 2 N NaOH and made up with diluent. The obtained chromatogram and stress plots are presented in Fig. 2.

Base degradation studies: Base degradation study was conducted by addition of 2 N NaOH to a bumetanide solution prepared by adding 20 mg of bumetanide to diluent and kept

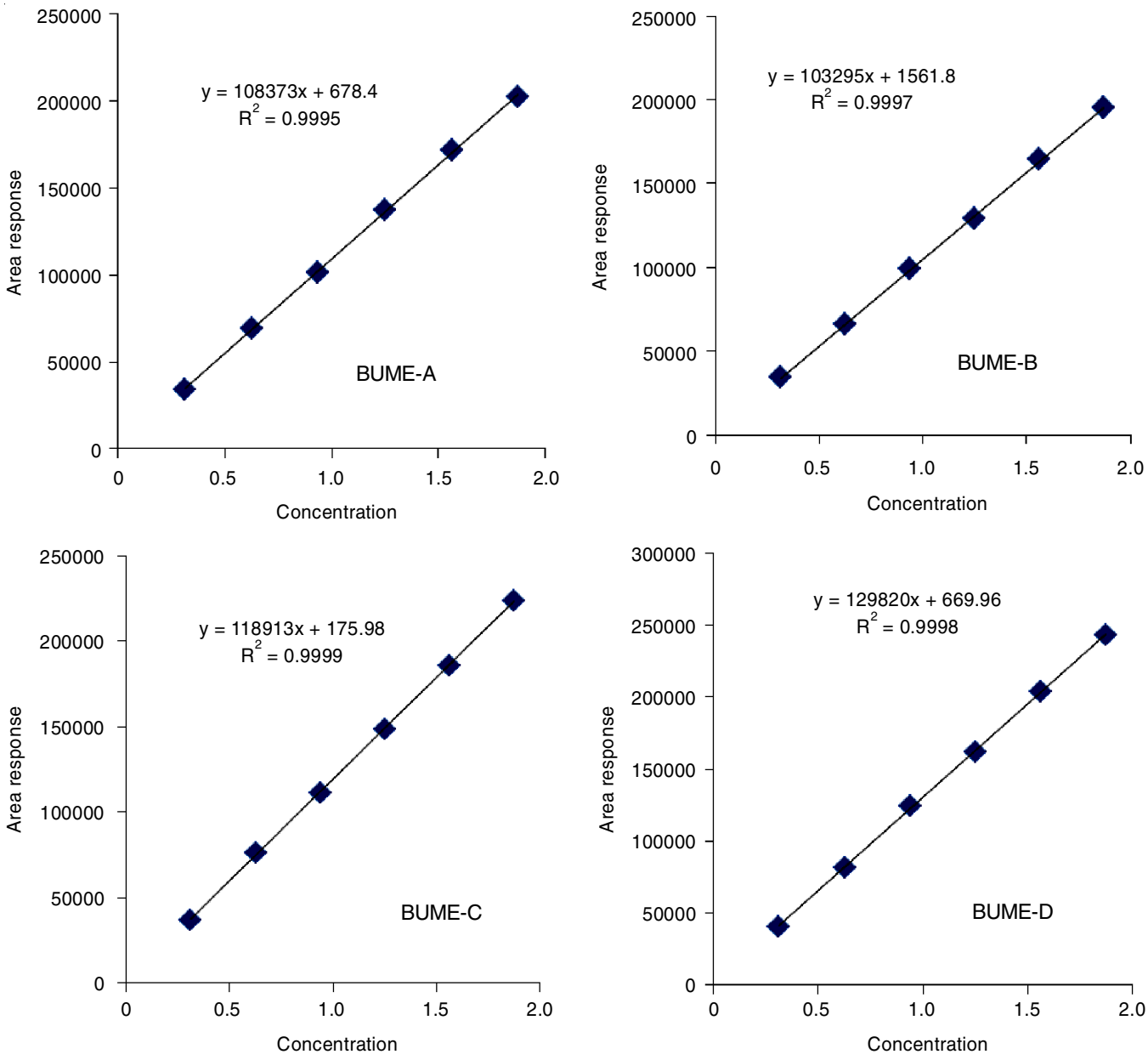


Fig. 1. Linearity plots of bumetanide impurities

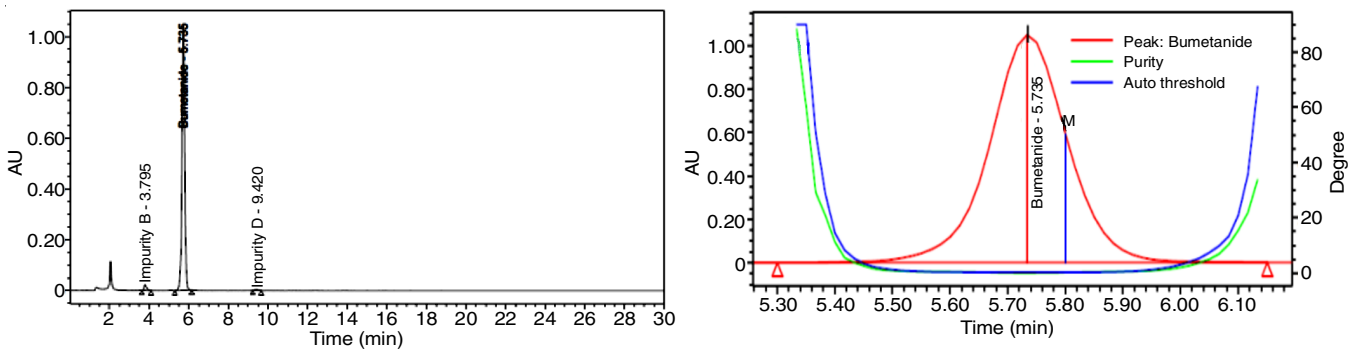


Fig. 2. Chromatograms and purity plot of bumetanide under acid stress

the solution on shaking water bath for 30 min at 60 ºC. The solution was cooled to room temperature, neutralized with 2 N HCl and made up with diluent. The obtained chromatogram and stress plots are presented in Fig. 3.

Oxidative degradation studies: Oxidative degradation study was conducted by addition of 20 % H_2O_2 to a bumetanide solution prepared by adding 20 mg of bumetanide to diluent, kept the solution in room conditions for 30 min and made up

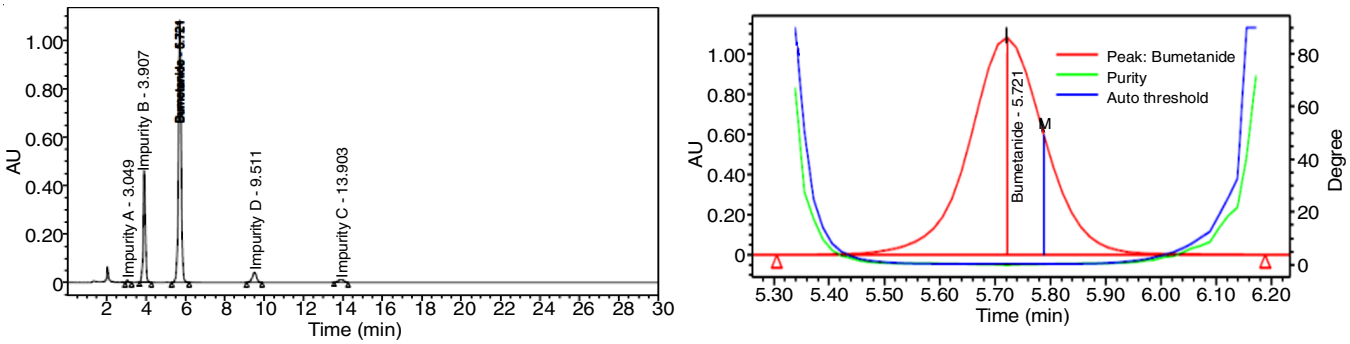


Fig. 3. Chromatograms and purity plot of bumetanide under base stress

with diluent. The obtained chromatogram and stress plots are presented in Fig. 4.

Thermal degradation studies: Thermal degradation study of bumetanide was performed at 105 ºC for 6 h. After completion of the stress, control sample was used for sample preparation. The obtained chromatogram and stress plots are presented in Fig. 5.

Photolytic degradation studies: Photolytic degradation study of bumetanide was performed in the photolytic chamber (1.2 million lux per hour and 200 watt-hour/m^2) for 16 h. After completion of the stress on the sample, the same samples were used for the sample preparation. The obtained chromatogram and stress plots are presented in Fig. 6.

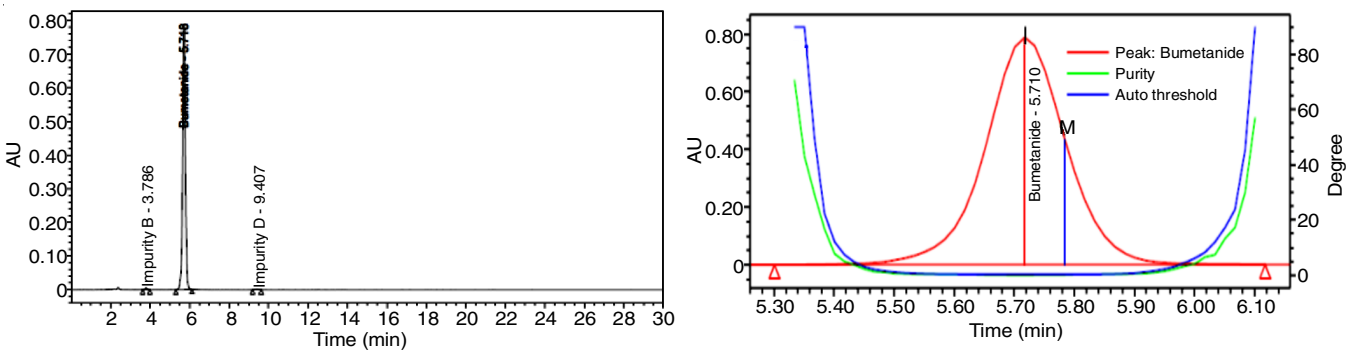


Fig. 4. Chromatograms and purity plot of bumetanide under peroxide stress

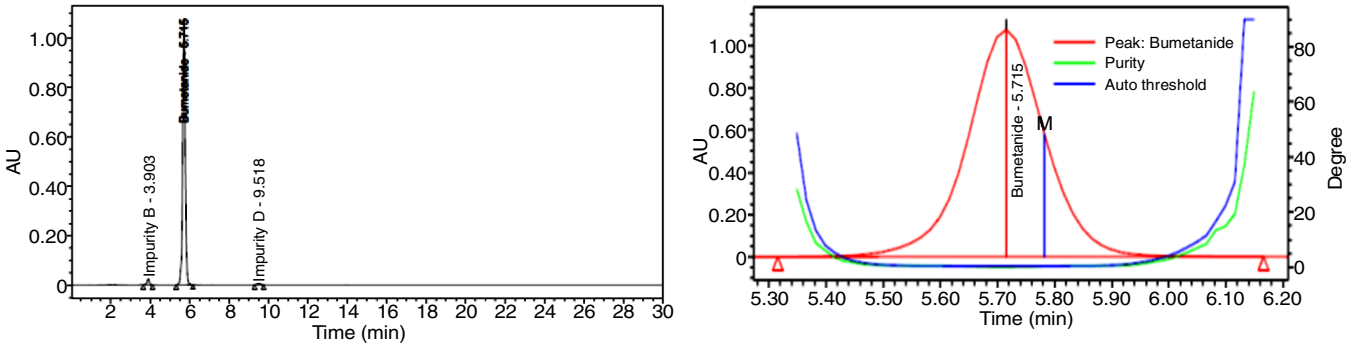


Fig. 5. Chromatograms and purity plot of bumetanide under thermal stress

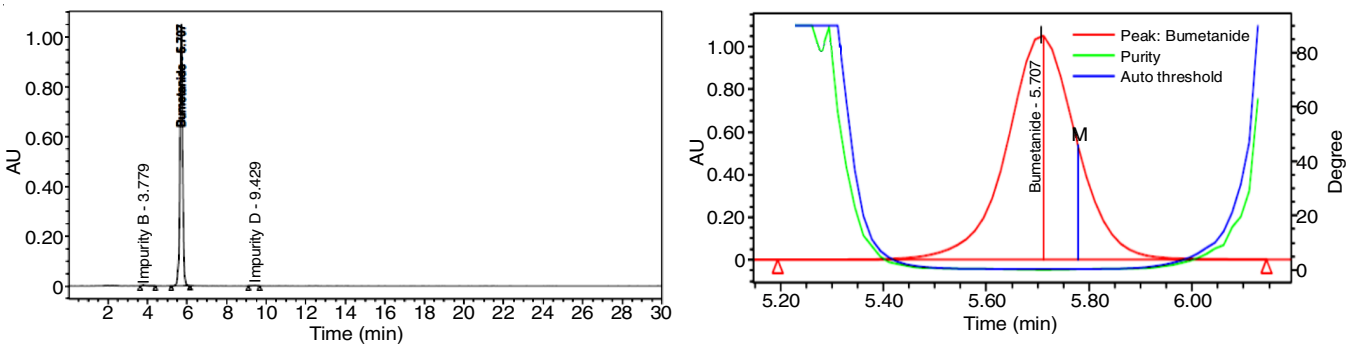


Fig. 6. Chromatograms and purity plot of bumetanide under photolytic stress

All the stress conditions applied were enough to degradation. Under acidic and basic stress conditions, bumetanide was degraded up to 2.3 and 20 %, respectively. Under oxidative and thermal stress studies, bumetanide was degraded up to 1.0 and 2.5 %, respectively. Bumetanide was stable under photolytic stress conditions. Hence, from these stress studies, bumetanide was not stable in acidic, basic and thermal conditions. The degradation products of bumetanide were effectively separated by the developed method. Hence, the method is considered to be extremely selective and specific for future use.

Accuracy: The accuracy of developed method was determined by spiking API on placebo various concentrations ranging from 50 to 150 % of targeted test concentration. The recovery values of impurities BUME-A, BUME-B, BUME-C and BUME-D were in the range of 95.45-98.74, 97.07-99.45, 92.54-96.86 and 96.72-98.98, respectively and the results are depicted in Table-4.

TABLE-4
ACCURACY

Component	50 %	100 %	150 %
BUME-A	97.13	95.45	98.74
BUME-B	98.77	99.45	97.07
BUME-C	92.54	96.86	96.06
BUME-D	97.50	96.72	98.98

Precision: Relative standard deviations (% RSD) of all impurities were calculated to characterize the precision. Six sample solutions of targeted concentrations were injected into the system on the same day for determination of intra-day precision, and the same concentrations of six sample solutions were injected into the system for three consecutive days for determination of inter-day precision. The obtained percentages of RSD were 0.85, 0.88, 1.03 and 0.70 for the impurities BUME-A, BUME-B, BUME-C and BUME-D, respectively and the results are presented in Table-5.

TABLE-5
PRECISION

S. No	BUME-A	BUME-B	BUME-C	BUME-D
Prep'n-1	0.476	0.474	0.567	0.453
Prep'n-2	0.483	0.480	0.554	0.452
Prep'n-3	0.486	0.468	0.555	0.454
Prep'n-4	0.484	0.478	0.566	0.460
Prep'n-5	0.480	0.473	0.562	0.450
Prep'n-6	0.487	0.476	0.556	0.453
RSD (%)	0.85	0.88	1.03	0.70

Precision of spiked chromatogram: Six samples were prepared by spiking the all impurities on the test solution to get 0.2 % of the impurities in the sample and evaluated the precision of the test method. The finalized spiked chromatogram is presented in Fig. 7.

Stability: The stability of bumetanide and its impurities (BUME-A, BUME-B, BUME-C and BUME-D) in the spiked sample was examined at room temperature for 96 h. All the spiked samples were kept in the air-tight volumetric flask on bench-top for observing the stability of the samples and found that all prepared samples are stable up to 96 h.

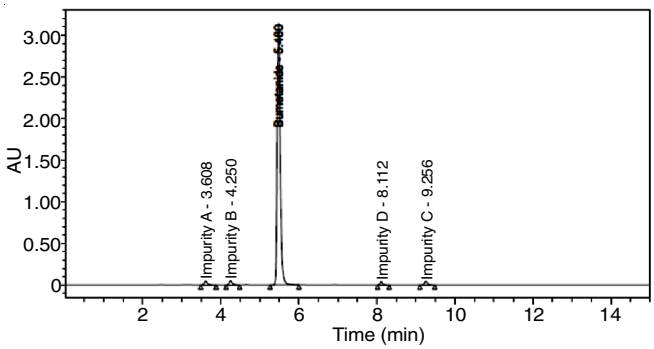


Fig. 7. Finalized spiked chromatogram

Limit of detection (LOD) and limit of quantitation (LOQ): In order to examine the LOD and LOQ, dissimilar concentrations of solutions were prepared by spiking known amounts of impurities and spiked bumetanide in the diluent. Detection limits and quantitation limits were determined by the signal-to-noise (S/N) approach and calculated by using average S/N ratio from all the analyses at each concentration level. The concentration which gives S/N 3 can be readily detected was reported as the LOD. The LOD was found to be 0.026 µg for bumetanide, 0.024 µg for BUME-A, 0.0255 µg for BUME-B, 0.027 µg for BUME-C and 0.027 µg for BUME-D. LOQ was found to be 0.074 µg for BUME, 0.072 µg for BUME-A, 0.0745 µg for BUME-B, 0.737 µg for BUME-C and 0.727 µg for BUME-D (signal to noise ratio of 10:1).

Robustness: Robustness was performed with 10 % organic variation in mobile phase composition and 0.2 mL/min flow rate. By changing the retention time and chromatographic conditions slightly, the number of theoretical plates and tailing factors were studied and robustness of the developed method was assessed. From the results, minor variations in chromatographic conditions had an insignificant effect on the chromatographic parameters. Hence, from the recovery studies, the developed method was very accurate and appropriate for intended use.

Conclusion

The novel and simultaneous stability indicating RP-HPLC method was developed for quantitative analysis of bumetanide and its impurities in the oral solid dosage form. The present developed method was validated by testing its specificity, accuracy, precision, linearity, stability, limits of detection and limits of quantitation. The shorter time run enables rapid determination of bumetanide drug and its impurities. The method was found to be specific and stability indicating and this method show an excellent performance in terms of speed and sensitivity. Hence, this method can be applicable for assessing the impurities in finished dosage forms. The developed method is more economical and suitable for laboratory use as solvent consumption is very less. Further, the method can be used for determination of bumetanide and its impurities in drug product stability studies and pharmaceutical formulations. Moreover, conventional reported HPLC methods may be replaced by the proposed HPLC method because of its superiority.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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