DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF BUMETANIDE IN TABLET FORMULATION

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Received 3-1-2014 Accepted 25-3-2014

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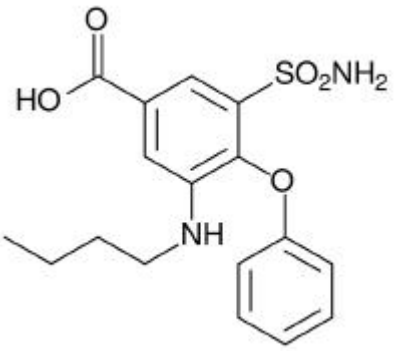
ABSTRACT

A remarkably simple, selective and precise method to determine bumetanide in tablet dosage form was developed and validated using Reverse Phase High Performance Liquid Chromatography (RP-HPLC). The LC separations was achieved on a bondapak C_{18} column in the isocratic mode using Glacial acetic acid: Tetrahydrofuran: Water: Methanol (2:5:45:50 v/v/v/v), as a mobile phase at flow rate 1.0 mL/min with UV detection at 254 nm. The retention time for bumetanide was 11.3 min. The method was validated by determining its sensitivity, precision, accuracy as per ICH guidelines. The % recovery was found to be as is simple, rapid, precise and accurate and hence, can be applied for routine quality control analysis of this drug in tablet dosage form.

KEYWORDS: High performance chromatography, Bumetanide, Validation, Accuracy.

INTRODUCTION

Bumetanide chemically known as 3-butylamino-4-(phenoxy)-5-sulfamoyl benzoic acid (figure-1). Bumetanide, a loop diuretic drug interferes with renal cAMP and/or inhibit the sodium-potassium ATPase pump (Tripathi, 1985).



Bumetanide appears to block the active reabsorption of chloride and possibly sodium in the ascending loop of Henle, altering electrolyte transfer in the proximal tubule. This result is excretion of sodium, chloride, and water, and hence diuresis.

Hitherto, there are few analytical method reported for estimation of bumetanide. The determination of bumetanide in small volumes of plasma by HPLC with tandem mass spectroscopy detection was described by Gradeen *et al*. (1990). Quantification of bumetanide with fluorescence detection in human plasma, serum and urine was advocated by Zivanov Stakic *et al*. (1989). These methods are complicated, costly, time consuming rather than a simple HPLC with UV detection. So, it is unsuitable to use these highly sensitive methods for the routine quantitative assay of bumetanide in tablets where the content of active pharmaceutical ingredients was high in the formulation.

The aim of present work was to develop and validate a simple, fast, reliable isocratic RP-HPLC method with UV detection for the determination of bumetanide in tablet dosage forms. The important features and novelty of the proposed method include simple sample treatment with sonicate of small amount of powder sample at ambient temperature, short analysis time (less than 30 min.), short elution time (less than 6 min), precision (R.S.D less than 2%.) and high recovery (greater than 95%) confirmation of the applicability of the developed method was done according to ICH guidelines (Cooper *et al*., 1989).

MATERIALS AND METHODS:

Drugs and chemicals

Experimental drugs and chemicals working standard of bumetanide, Glacial acetic acid, tetrahydrofuran, Methanol and water (HPLC grade) for HPLC were provided by the Ind Swift, Panchkula, India. The commercial sample was supplied in tablet dosage form 5 mg of bumetanide for oral administration.

Instrument

High performance liquid chromatography, (Waters, USA) consisted of a pump LC-10 A TVP series) equipped Rheodyne model 7161 injector valve with 20 mL loop (Rheodyne Inc. cotati, CA, USA) and on UV-visible detector of type SPD-10 AVP was used as an instrument.

Chromatographic conditions

Chromatographic separation were achieved using Bondapak C_{18} column in the isocratic mode using Glacial acetic acid: Tetrahydrofuran: Water: Methanol (2:5:45:50) (v/v/v/v) as the mobile phase at the flow rate 1.0 mL/min. The mobile phase was prepared freshly and degassed by sonicating for a minute before use (Toshiba, Delhi, India). The elution was monitored at 254 nm and the injection volume was 10ml and at an ambient temperature.

Solutions

(a) Diluent preparation: 2 mL Glacial acetic acid, 5 mL Tetrahydrofuran and 45 mL methanol were mixed and mixture was sonicated for 5 minutes.

(b) Standard preparation: 27 mg of bumetanide was taken in 100 mL volumetric flask and allowed to dissolved in diluent, sonicate for 5 minutes. Then 2 mL of this solution was transferred in 100 mL of volumetric flask and volume was adjusted with mill-Q water (5.5 ppm).

(c) Sample preparation: A 5 mg tablet of bumetanide was dissolved in 900 mL of water contained in a beaker. The contents of the beaker were allowed to mix with the help of paddles rotating at 50 rpm. Six such beakers were prepared and after 30 minutes samples from each beaker were withdraw with the help of pipettes.

Method validation

The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines (ICH Guidelines, 1996, Green, 1998). The method was validated for system suitability, Resolution, Specificity, Method precision, Accuracy, Linearity, Robustness and stability in analytical solution.

Analytical performance characteristics

System suitability studies

System suitability test was performed to confirm the reproducibility of the equipment to be used for the intended validation. The test was performed by prepared standard solution of 5.5 ppm and

injected six times. System suitability parameters like assymetry, theoretical plates and retention period were calculated.

Resolution

Sample solution (5.5 ppm) of bumetanide and Impurity-B was injected to check the resolution between them.

Specificity

The specificity was determining by analyzing peak. The possible interferences were analyzed by the peak purity, which was calculated using software empower. A standard solution was injected and purity analysis was done then a dissolution sample of bumetanide at sample point (30 min) was injected monitored.

Method precision

Method precision or intra-assay precision data were obtained by repeated analysis. In one laboratory on one day, aliquots of homogeneous sample, each of which independently prepared according to standard procedure Six injections of standard solution of 5.5 ppm were injected to check the system suitability. Then six times sample was prepared and each of these sample was injected in duplicate. All of these values gives rise to assay value obtained from method precision.

Accuracy

Accuracy of the method was determined by replicate analysis of three sets of samples at high, middle and low quality control concentrations and comparing the difference between the nominal value and that actually found. Accuracy was expressed as % nominal concentration.

Procedure

i. Accuracy/Recovery was determined by spiking stock solution of Bumetanide in three different concentrations i.e. 25%, 100% and 125% of target concentration of Bumetanide (5.5 ppm) as follows:

A) Stock Solution Preparation: 49.55 mg of Bumetanide was transferred to a 200 mL volumetric flask, 10mL. of Methanol was added to it and the volume was made up to 200 mL with the same.

B) For 25% solution: 5 mL. of stock solution was added to dissolution vessel containing 895 mL of dissolution media and placebo.

C) For 100% solution: 20 mL of stock solution was added to dissolution vessel containing 880 mL of dissolution media and placebo.

D) For 125% solution: 25 mL of stock solution was added to dissolution vessel containing 875 mL of dissolution media and placebo.

ii. Each dissolution sample was injected.

iii. Percentage recovery of Bumetanide was calculated.

Linearity

Calibration curves were constructed by plotting peak areas vs. concentrations of Bumetanide and the regression equations were calculated. The calibration curves were plotted over the concentration range 1.35 to 6.75 mg mL^{-1} for Bumetanide. Each concentration was injected. A calibration curve was plotted between concentration and area for Bumetanide. Correlation coefficient, v-intercept, slope of regression line and residual sum of squares were calculated from the graph.

Robustness

In order to demonstrate that the analytical method is capable to yield reproducible results, a small but deliberate variation in method parameters during normal usage such as change in agitation

rate, change in temperature of dissolution media was made.

Stability in analytical solution

In order to determined the stability of product in solution form, stability study of solution was performed by measuring the areas of dissolution sample of Bumetanide tablets-5mg at zero h and after 1, 2, 4, 6 and 8 hrs at room temperature. The variation in areas of Bumetanide was observed and % cumulative relative standatd deviation (RSD) was calculated and recovered.

RESULTS AND DISCUSSION

Screening and optimization

Selection of the detection wavelength

The UV spectra of bumetanide in Glacial acetic acid: Tetrahydrafuran: Water: methanol (2:5:45:50/ v/v/v/v) in the region between 190-400 nm was obtained. Maximum absorbance of bumetanide shows at 254 nm, hence this was selected as on optimum detection wavelength for quantification of bumetanide.

Selection of column

Proper selection of stationary phase depends upon the nature of the sample, molecular weight and solubilities. As bumetanide is non-polar C_8 and C_{18} reverse phase column was selected. Non polar compounds are very attractive with reverse phase columns, so the elution of compound from the column was influenced by polar mobile phase.

Selection of mobile phase

A mixture of Glacial acetic acid: Tetrahydrofuran: Methanol: Water (2:5:45:50 v/v/v/v) was selected as mobile phase and the effect of composition of mobile phase for bumetanide was thoroughly investigated. Different mobile composition (5:10:45:45 v/v/v/v, 1:10:30:60 v/v/v/v) were optimized respectively to get symmetrical peak with short run time. A short run time and the stability of peak asymmetry were observed in ratio of 2:5:45:50 v/v/v/v of Glacial acetic acid, Tetrahydrofuran. Water methanol and it was found to be optimum mobile phase.

Validation of the proposed method

System suitability

The % RSD of peak areas of bumetanide and its retention time in minutes were with in 2% indicating the suitability of the system (Table 1). These results indicate the applicability of this method to routine with no problems, its suitability being proved.

Table 1. Results from system suitability study

Parameters	Values
Retention Time	11.4 minutes
Tailing factor	1.02
Number of theoretical plates	5597.5
Area (% RSD)	0.13

Resolution

Resolution between bumetanide and impurity -B should not be less than 15.0. As illustrated in table 2 the system parameter lie well within the acceptance criteria therefore, the system and chromatographic parameters are suitable for use.

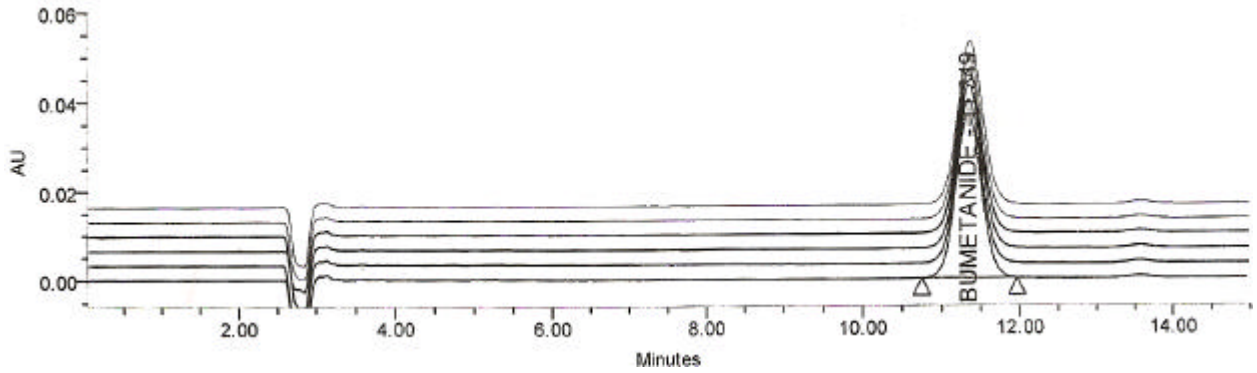


Fig. 2: A typical chromatogram of system suitability for bumetanide

Table 2. Data studied for resolution of bumetanide.

Name	Retention Time (min)	Relative retention	Resolution w.r.t Bumetanide
Impurity-B	3.840	0.34	17.9
Bumetanide	11.349	-	-

Specificity

As may be seen from the results (Table 3) purity angle is less than purity threshold hence, the peak of bumetanide in standard solution and dissolution sample pure and the analytical method of dissolution is specific.

Table 3. Observation for specificity studies

S.No.	Product	Status	Purity Angle	Purity threshold	Peak Purity
1	Bumetanide	Standard solution	0.118	0.345	Passes
2	Bumetanide tablets-5mg	Dissolution sample	0.098	0.304	Passes

Precision

The overall results of analysis shown in table 4 observed during day 1 and day 2 of the precision studies. As in observed from Table 4 the absolute difference in mean percent release of two dissolution tests is 4.08; less than 5% therefore, the method can be declared as precise.

Table 4. Overall results of precision studies

S.NO.	Days	System	Analyst	% Release of bumetanide (mean of 6 beaker)
1	Day-1	TDT-06P	AK	100.74
2	Day-2	TDT-082L	KS	96.66
Absolute difference				**4.08**

Accuracy / Recovery

The accuracy/recovery was confirm by recovery studies by spiking stock solution of bumetanide in the three different concentration i.e., 25%, 100% and 125% of target concentration of bumetanide. As per procedure the results of accuracy is summarized in Table 5.

Table 5 Accuracy/Recovery study for bumetanide

S.No.	No. of beakers	Spiked concentration of label claim of bumetanide	Amount of bumetanide added (mg)	Amount recovered (mg)	Recovery (%)
1	1	25	1.239	1.279	103.23
	2	25	1.239	1.276	102.99
	3	25	1.239	1.276	102.99
2	1	100	4.955	4.734	95.54
	2	100	4.955	4.772	96.31
	3	100	4.955	4.860	98.08
3	1	125	6.194	6.003	96.92
	2	125	6.194	6.010	97.03
	3	125	6.194	6.041	97.53
Percent recovery					**95.54% - 103.23%**

Percent recovery was found to be in range 95.54% to 103.23% which is well with in range of 95-105%. The high percent recovery indicates that no interference will be produced due to excipients used in formulation. Hence, the developed method was found to be accurate.

Linearity

Linearity was tested for the concentration ranging from 1.35-6.75 mg per ml for Bumetanide. The peak area ratio of Bumetanide was plotted against concentration. A linear responses was observed over the examine concentration range. The results are tabulated in table 6. The standard derivation of slope and interrupt value were low. The correlation coefficient (r2) was found to be as 0.9994 indicates that the concentration of bumetanide had good linearity.

Table 6 Overall results of Linearity

	Correlation Coefficient	0.9994
	Slope (m)	148071.99
	Intercept (c)	24444.970
	Residual sum of squares	483430031.5

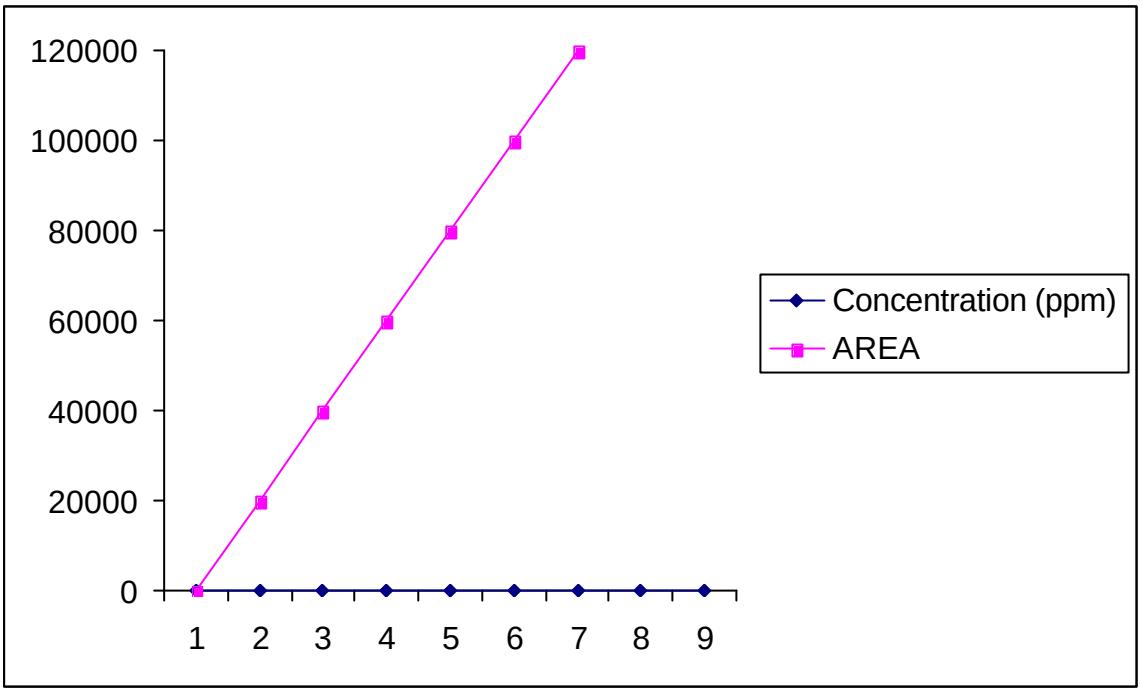


Fig.3: Linear calibration curve of Bumetanide

(i) Robustness (Change in temp. of dissolutions media + 1^{0C})

RSD of mean % release with slight variation in temperature dissolution media should not be more than 5.0%. As may be seen from results (Table 7) RSD of mean % release of three dissolution tests with slight variation in temperature of dissolution media is found to be 1.30% for bumetanide which is well within acceptance criteria. So analytical method is robust.

Table 7 Change in temperature of dissolution media

Temperature of dissolution media	% release of the sampling time (mean)
36.5	98.25
37.5	96.22
38.5	98.55
%RSD	1.30

(ii) Change in agitation rate + 2rpm

RSD of mean % release with slight variation in agitation rate should not be more than 5.0%. As may be seen from results (Table 8), RSD of % release of there dissolution tests with slight change in agitation rate is found to be 0.85% for bumetanide, which is well with is acceptance criteria. So analytical method is robust.

Table 8 :	**Agitation rate**	**% Release at the sampling time**
	48 rpm	99.06
	50 rpm	100.74
	52 rpm	99.72
	% RSD	0.85

Stability of solution

As evident from Table 9, there is slight variation in areas of test situation of bumetanide tablets 5 mg with time. After 8 hours the cumulative % RSD rate is 1.47 for bumetanide, which is well within acceptance criteria that is less than 2.0%. Therefore, it can be establish that the product in solution form is stable for at least 8h.

. **Table 9 Result of stability of solution**

Time of sampling	Area	Cumulative % RSD
Initial	792782	--
1 h	789836	0.26
2 h	785111	0.49
4 h	771846	1.18
6 h	771420	1.28
8 h	764793	1.47

The proposed method is simple, accurate, precise and selective for the estimation of bumetanide in tablet dosage form the mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claim. Hence, it can be easily and conveniently adopted for routine analysis of bumetanide in formulations.

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