Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 3 Issue 8 August 2019

Research Article

New Stability Indicating RP-UFLC Method for the Determination of Bumetanide – A Potent Diuretic

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Received: June 19, 2019; **Published:** July 18, 2019

DOI: 10.31080/ASPS.2019.03.0343

Abstract

Bumetanide is a potent loop diuretic. A new ultrafast liquid chromatographic method has been developed for the assay and stability studies of Bumetanide in tablets and validated. Shimadzu Model CBM-20A/20 Alite UFLC system (Shimadzu Co., Kyoto, Japan) equipped with SPD M20A prominence photodiode array detector with C18 column (250 mm × 4.60 mm i.d. 5µm particle size) was used with a mobile phase composition acetic acid: acetonitrile: water (0.1: 80: 20 v/v) and flow rate of 1.2 mL/min (UV detection at 220nm). Bumetanide has shown linearity 0.1–100 µg/mL with linear regression equation y = 70867x+3608 (r2 = 0.9996). The LOQ was found to be 0.0964 µg/mL and the LOD was found to be 0.03091 µg/mL. Bumetanide was subjected to forced degradation and the method was validated as per ICH guidelines.

Keywords: Bumetanide; RP-UFLC; Stability Indicating; Validation; ICH Guidelines

Introduction

Bumetanide, is a medication used to treat swelling and high blood pressure which include swelling as a result of heart failure, liver failure, or kidney problems. Bumetanide is a loop diuretic and works by decreasing the reabsorption of sodium by the kidneys [1]. Bumetanide (Figure 1) can lessen symptoms such as shortness of breath and swelling in your arms, legs, and abdomen. Bumetanide tablets are indicated for the treatment of edema associated with congestive heart failure, hepatic and renal disease, including the nephrotic syndrome. It is taken by mouth, or by injection into a vein or muscle and effects generally begin within an hour and lasts for about six hours. People with sulfa allergy are allergic to Bumetanide. Bumetanide was patented in 1968 and came into medical use in 1972 and it is available as a generic medication.

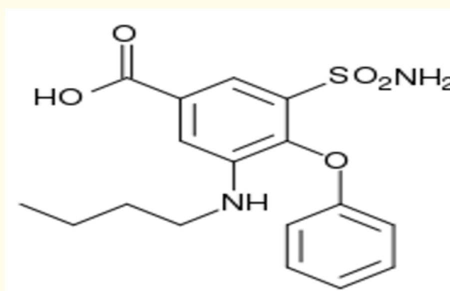


Figure 1: Chemical structure of Bumetanide

Bumetanide was determined in biological fluids like plasma, urine etc by HPLC [2,3] and with amperometric detection [4] and LC-MS [5] where as a very few methods were developed for its as-

say in pharmaceuticals using HPLC [6,7], automated flow injection with fluorimetric detection [8], UPLC [9], HPTLC [10] and spectrophotometry [11]. In the present study the authors have developed a robust and economic stability indicating method for the quantification of Bumetanide in tablets. The method was validated as per ICH guidelines [12-13].

Materials and Methods

Instrumentation

Chromatographic separation was achieved by Shimadzu Model CBM-20A/20 Alite UFLC system (Shimadzu Co., Kyoto, Japan) equipped with SPD M20A prominence photodiode array detector on C18 column (250 mm × 4.60 mm i.d. 5µm particle size) maintained at room temperature.

Preparation of bumetanide drug solution

Accurately weighed 25 mg of Bumetanide was taken in a 25mL volumetric flask and volume is made up to the mark with HPLC grade acetonitrile (1000 µg/mL) and dilutions were made with mobile phase and filtered through 0.45 µm membrane filter prior to injection.

Method validation

Linearity

A series of (0.1–100 µg/mL) Bumetanide solutions were prepared from the stock solution with mobile phase and 20 µL of each of these solutions were injected in to the UFLC system. The mean peak area of Bumetanide were calculated from the chromatograms and a calibration curve was drawn by taking the concentration of the Bumetanide solutions on the x-axis and the corresponding mean peak area values on the y-axis.

Precision, accuracy and robustness

Intraday and inter-day precision were studied using three different concentrations of Bumetanide on the same day and on three consecutive days respectively and the % RSD was calculated. The accuracy of the assay method was evaluated in triplicate at three concentration levels (50, 100 and 150%), and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Bumetanide in the drug product and the percentage recovery was calculated. The robustness of the method was assessed by exposing the drug solution to different analytical conditions purposely changing from the original optimized conditions. The effects so obtained were summarized to calculate the % RSD and has to be less than 2.0% specifying that the proposed method was robust.

Forced degradation studies

Forced degradation studies were performed to determine the ability of the drug to withstand its properties in the applied stress conditions. Bumetanide was exposed to different stress conditions such as acidic hydrolysis, basic hydrolysis and thermal treatment.

Acidic degradation was performed by treating the drug solution with 1mL of 0.1N HCl, heated at 80 ºC for about 30 minutes on a water bath. The stressed sample is then cooled neutralized with 1mL 0.1N sodium hydroxide solution and the solution was made up to volume to the required concentration with the mobile phase. 20 µl of the solution was injected in to the UFLC system.

Alkaline degradation, was performed by treating the drug solution with 1mL 0.1 N NaOH heated at 80ºC for 30 minutes on a water bath. The solution is then cooled and neutralized with 1mL 0.1N hydrochloric acid and diluted with mobile phase. 20 µl of the solution was injected in to the UFLC system.

Thermal degradation was performed by heating the drug solution at 80ºC for 30 minutes on a water bath. The solution is then cooled and diluted with mobile phase.20 µl of the solution was injected in to the UFLC system.

Assay of bumetanide tablets

Twenty tablets were procured, crushed and powdered. Powder equivalent to 25 mg Bumetanide was extracted using the mobile phase in a 25 ml volumetric flask. The solution was sonicated for half an hour and filtered through 0.45 mm membrane filter and 20 µL of this solution was injected in to the UFLC system and the peak area was noted at its retention time from the resultant chromatogram.

Results and Discussion

A new reverse phase ultrafast acting stability indicating method was developed for the quantification of Bumetanide in tablets. The previously reported liquid chromatographic methods were compared with the present method in Table 1.

A C18 column (250 mm × 4.60 mm i.d. 5µm particle size) was selected with mobile phase composition acetic acid: acetonitrile: water (0.1: 80: 20 v/v) and flow rate of 1.2 mL/min (UV detection at 220nm) for the determination of Bumetanide by which a sharp

peak was observed at 2.588 min (Run time 10 min). The optimized chromatographic conditions were shown in Table 2.

Method/Mobile phase (v/v)/Reagent	λ (nm)	Comment	Reference
HPLC	Exicitation (338) Emission (433)	Fluorimetric detection (In plasma and urine) (Acetophenone as Internal standard)	2
HPLC Methanol: water: glacial acetic acid (66:34:1)	Exicitation (228) Emission (418)	Fluorimetric detection (In plasma and urine)	3
HPLC Acetonitrile: KH_2PO_4 buffer (pH 4.0) (50:50)	-	Ampheometric detection (in Urine)	4
LC–ESI-MS/MS Methanol: 5mM Aq. ammonium trifluoroacetate	-	Human plasma (Tamsulosin as internal standard)	5
HPLC Methanol: Water (70: 30)	335	Low linearity 1.0-10 μg/ml	6
HPLC Phosphate buffer (pH=7.8): Acetonitrile (70:30)	216	Low linearity 0.6-1.6 μg/ml	7
HPLC Alkaline medium	Exicitation (314) Emission (370)	Fluorimetric detection	8
UPLC Water: Acetonitrile (30: 70)	254	PDA	9
HPTLC Toluene: Ethyl acetate: Formic acid (7: 3.5: 0.5)	335	100-800 ng/spot	10
Spectrophotometry Borate buffer (pH 9.0) Phosphate buffer (pH 7.0)	252	Low linearity 5.0-75 μg/ml	11
UFLC Acetonitrile: Water: Glacial acetic acid (80: 20: 0.1)	220	High linearity 0.1-100 μg/ml	Present method

Table 1: Review of previously published methods with the present method.

Parameter	Optimized chromatographic conditions
Mobile Phase	Acetonitrile: Water: Glacial acetic acid (80: 20: 0.1)
Stationary Phase	C18 Phenomenex column (250 mm × 4.60 mm i.d. 5μm particle size)
Flow Rate	1.2 mL/min
Detection wavelength	220 nm
Column temp.	(25°±2°C)
Injection Volume	20 μL
Detector	SPD M20A prominence photodiode array detector
Elution	Isocratic mode
Total Run Time	10 mins
Retention time	2.588 mins

Table 2: Optimized chromatographic conditions.

Linearity

Bumetanide has shown linearity over the concentration range 0.1–100 μg/mL (Table 3) (% RSD 0.0017-0.9467) with linear regression equation $y = 70867x+3608$ ($r^2 = 0.9996$) (Figure 2). The LOQ was found to be 0.0964 μg/mL and the LOD was found to be 0.03091 μg/mL. The system suitability parameters for the Bumetanide has shown that the tailing factor was less than 2 (or <1.5-2.0) and the theoretical plates were more than 2000.

Precision, accuracy and robustness

Intraday and inter-day precision were studied at three different concentration levels of Bumetanide on the same day and on three consecutive days respectively and the % RSD was found to be 0.0087-0.1002 (Intraday) (Table 4) and 0.0069-0.0342 (Inter day) (Table 5) respectively (<2.0) demonstrating that the method is precise. The accuracy of the method was proved by the standard addi-

tion method and the recovery values were 0.24-0.52 (<2.0) with a recovery of 99.53-99.68% (Table 6). The robustness of the assay method was established by introducing small changes in the chromatographic conditions which include detection wavelength (218 and 222 nm), percentage of acetonitrile in the mobile phase (78 and 82%) and flow rate (± 0.1 ml/min). Robustness of the method was studied using 10 µg/mL of Bumetanide (Table 7) and the % RSD was found to be 0.109-0.946 (<2.0).

Conc. (µg/mL)	*Mean peak area	% RSD
0.1	7224	0.3105
0.5	3612	0.9467
1	72246	0.0644
5	361230	0.1064
10	705788	0.0419
20	1453843	0.0088
50	3546132	0.1003
80	5779680	0.0017
100	6999440	0.0038

Table 3: Linearity of Bumetanide.

*Mean of three replicates.

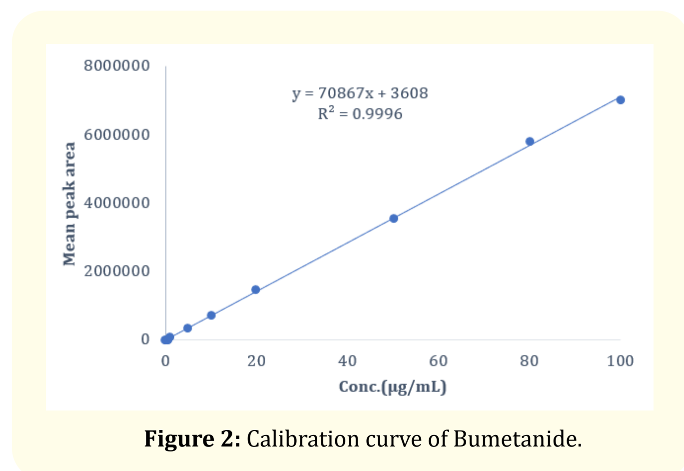


Figure 2: Calibration curve of Bumetanide.

Conc. (µg/mL)	*Mean peak area	Statistical Analysis
		*Mean ± SD (% RSD)
10	705788	705569 ± 295.0497(0.0418)
10	705687	
10	705234	
20	1453843	1453812 ± 127.911(0.0087)
20	1453671	
20	1453921	
50	3546132	3546519± 355.590(0.1002)
50	3546595	
50	3546831	

Table 4: Intraday precision study of Bumetanide.

*Mean of three replicates

Conc. (µg/mL)	*Mean peak area			*Mean ± SD (% RSD)
	Day 1	Day 2	Day 3	
10	705701	705758	707598	70572± 48.746 (0.0069)
20	1453985	1453521	1458970	1453825 ± 1090.369 (0.075)
50	3546254	3546625	3548521	3547133± 1215.987 (0.0342)

Table 5: Inter day precision study of Bumetanide.

*Mean of three replicates

Spiked Conc. (µg/mL)	Formulation (µg/mL)	Total Conc. (µg/mL)	*Conc. obtained (µg/mL) ± SD (%RSD)	% Recovery
10 (50%)	20	30	29.86 **± 0.0717 (0.24)**	99.53
	20	30		
	20	30		
20 (100%)	20	40	39.87 **± 0.1236 (0.31)**	99.68
	20	40		
	20	40		
30 (150%)	20	50	49.83 **± 0.2591 (0.52)**	99.67
	20	50		
	20	50		

Table 6: Accuracy study of Bumetanide.

*Mean of three replicates

Parameter	Condition	*Mean peak area	*Mean peak area ± SD (RSD)
Flow rate (± 0.1ml/min)	1.1	714236	707015 ± 6692.39 (0.946)
	1.2	705788	
	1.3	701021	
Detection wavelength (±2 nm)	218	706924	706960 ± 1190.916 (0.168)
	220	705788	
	222	708169	
Mobile phase composition Acetic acid: Acetonitrile: Water	0.1: 72:28	706239	706442 ± 774.0278 (0.109)
	0.1: 80:20	705788	
	0.1: 78:22	707296	

Table 7: Robustness study of Bumetanide

*Mean of three replicates

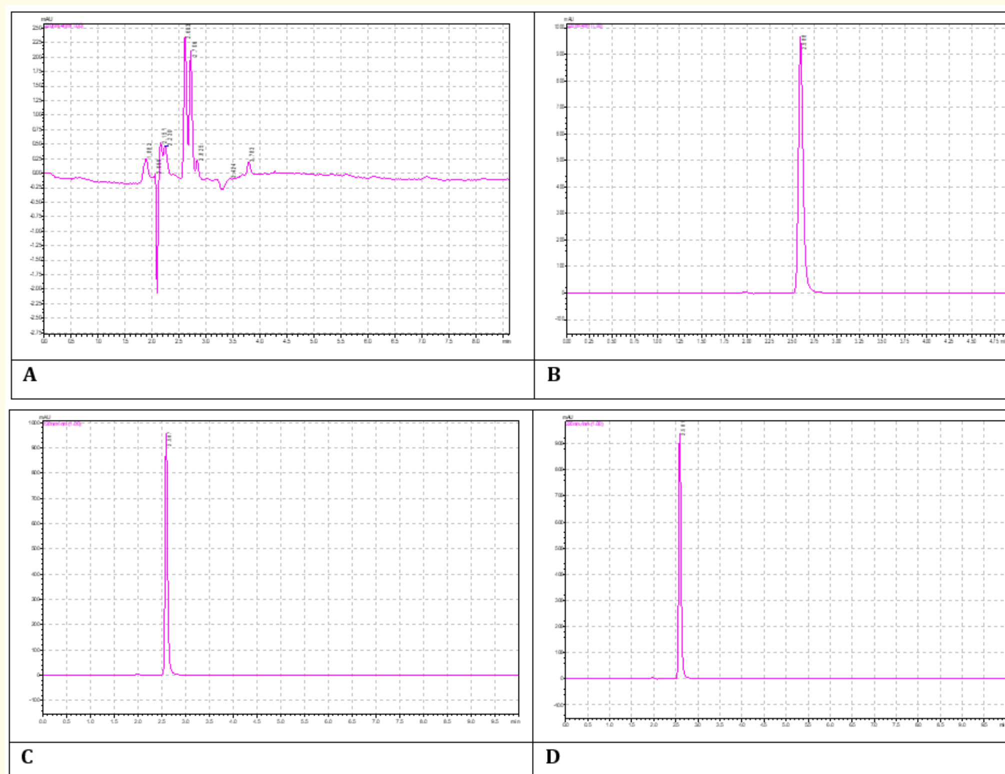
Forced degradation studies

Bumetanide was eluted at 2.588 min. Bumetanide has undergone acidic hydrolysis (6.46%), thermal degradation (1.71%) and alkaline hydrolysis (0.74%) which is less than 10% indicating that the Bumetanide is more resistant towards all forced degradation conditions applied and only during alkaline hydrolysis an extra peak was observed at 2.289 min along with the drug peak at 2.603 min. The system suitability parameters were well in the acceptance criteria (Table 8). The individual chromatograms observed during the forced degradation studies were shown in Figure 3.

Stress condition	Rt (min)	% Recovery*	% Drug degradation	Theoretical Plates (>2000)	Tailing factor (<1.5)
Standard drug	2.594	100	------	8876.430	1.430
Acidic degradation 0.1N HCl/ 80°C/ 30 min	2.607	93.54	6.46	7761.454	1.444
Alkaline degradation 0.1N NaOH/ 80°C/ 30 min	2.603 2.289	99.26	0.74	7456.420	1.427
Thermal degradation 80°C/30 min	2.599	98.29	1.71	8763.680	1.412

Table 8: Stress degradation studies of Bumetanide.

*Mean of three replicates



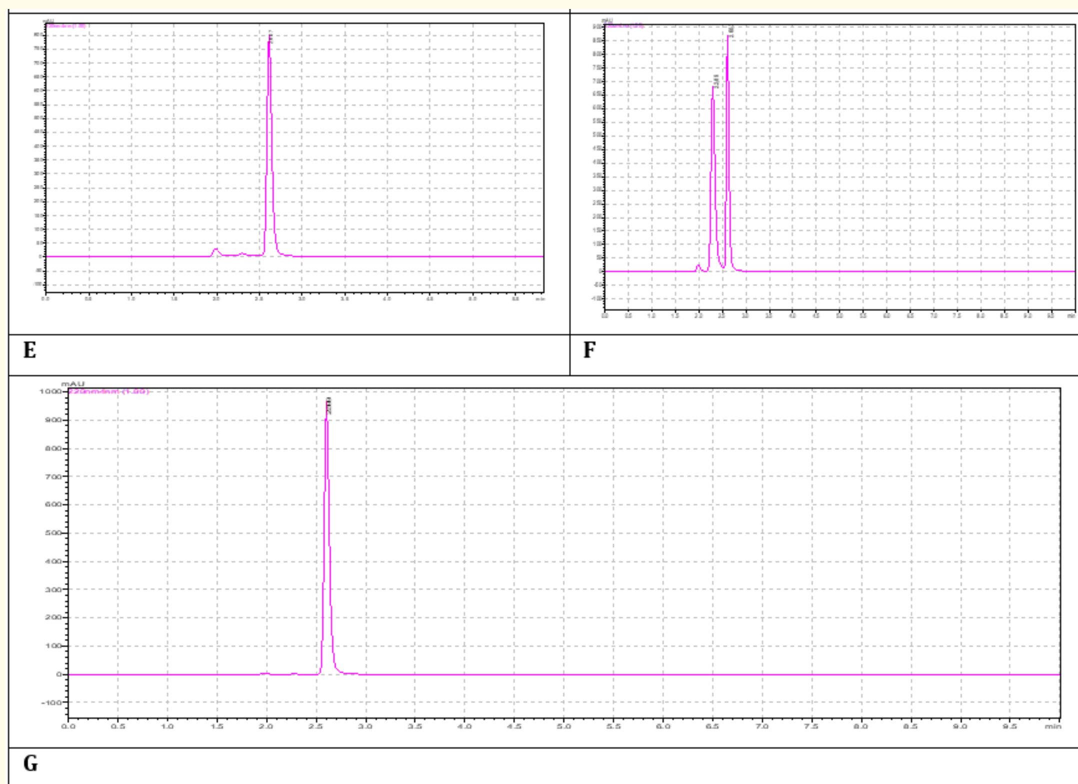


Figure 3: Typical chromatograms of A) Mobile phase B) Bumetanide API (50 µg/mL) C) Bumex (Label claim: 1 mg) (50 µg/mL) D) Burinex (Label claim: 2 mg) (50 µg/mL) E) Acidic degradation F) Alkaline degradation and G) Thermal degradation.

Assay of bumetanide tablets

Assay was performed by using two different brands of Bumetanide tablets consisting of 0.5, 1 and 2 mg API and found that the amount of Bumetanide was found to be 99.60-99.80 (Table 9) and there is no interference of excipients.

S. No.	Brand name	Label claim (mg)	Observed amount (mg)	% Recovery*	Manufacturer (India)
I	Bumex	1	0.998	99.80	Validus pharmaceuticals LLC
II	Burinex	2	1.993	99.65	Leo pharma
III	Bumex	0.5	0.498	99.60	Genentech, Inc

Table 9: Assay of Bumetanide tablets.

*Mean of three replicates

Conclusion

The RP-UFLC techniques were validated as per ICH guidelines and found to be simple, economical and robust for the quantification of Bumetanide tablets.

Acknowledgement

The authors are grateful to M/s GITAM (Deemed to be University), Visakhapatnam for providing the research facilities and Genentech, Inc (India) for supplying gift samples of Bumetanide. The authors have no conflict of interest.

Bibliography

1. Feit PW. "Aminobenzoic acid diuretics. 2. 4-Substituted-3-amino-5-sulfamoyl benzoic acid derivatives". *Journal of Medicinal Chemistry* 14.5 (1971): 432-439.
2. Smith DE. "High-performance liquid chromatographic assay for Bumetanide in plasma and urine". *Journal of Pharmaceutical Sciences* 71.5 (1982): 520-523.
3. Wells TG., *et al*. "Measurement of Bumetanide in plasma and urine by High-performance liquid chromatography and application to Bumetanide disposition". *Journal of Chromatography* 570.1 (1991): 235-242.
4. Legorburu MJ., *et al*. "Quantitative determination of the loop diuretic Bumetanide in urine and pharmaceuticals by high-performance liquid chromatography with amperometric detection". *Journal of Chromatographic Science* 39 (2001): 425-430.
5. Dinesh SP., *et al*. "Application of a rapid and sensitive liquid chromatography-tandem mass spectrometry method for determination of Bumetanide in human plasma for a bioequivalence study". *Journal of Pharmaceutical and Biomedical Analysis* 66 (2012): 365-370.
6. Rao JR., *et al*. "Stability indicating RP- HPLC method for Bumetanide in bulk drug and tablet formulation". *Asian Journal of Research Chemistry* 2.3 (2009): 266-269.
7. HUANG Qin., *et al*. "HPLC determination of the dissolution of Bumetanide tablets". *Chinese Journal of Pharmaceutical Analysis* 27.8 (2007): 1285-1287.
8. Solich P., *et al*. "Automated flow injection fluorimetric determination and dissolution studies of Bumetanide in pharmaceuticals". *Analytica Chimica Acta* 438.1-2 (2001): 131-136.
9. Chaitanya B., *et al*. "Method development, validation and stability studies for determination of Bumetanide in bulk and pharmaceutical dosage form by RP-UPLC". *International journal of pharmacy and pharmaceutical sciences* 10.3 (2018): 35-42.
10. Mohan Kumar., *et al*. "Development and validation of a stability-indicating HPTLC method for analysis of Bumetanide in the bulk drug and tablet dosage form". *Research Journal of Pharmacy and Technology* 3.1 (2010): 239-243.
11. Chaitanya B., *et al*. "Method development and validation of Bumetanide by UV spectrophotometric method in bulk and pharmaceutical dosage form". *European Journal of Biomedical and Pharmaceutical Sciences* 5.3 (2018): 443-450.
12. ICH Validation of analytical procedures: Text and methodology Q2 (R1), International Conference on Harmonization (2005).
13. ICH Stability Testing of New Drug Substances and Products Q1A (R2), International Conference on Harmonization (2003).

Volume 3 Issue 8 August 2019
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