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METHOD DEVELOPMENT AND VALIDATION OF BUMETANIDE BY UV SPECTROPHOTOMETRIC METHOD IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Analytical method development and validation play important role in the discovery, development and manufacture of pharmaceuticals. A simple, rapid and reproducible UV- spectrophotometric method for the quantitative determination of bumetanide in tablet formulation was developed and validated in the present work. Parameters such as linearity, precision and accuracy were studied according to ICH guidelines. The method A was carried out with borate buffer of pH 9 and method B was carried out with phosphate buffer of pH 7. The wavelength 252nm was selected for the estimation of drug using distilled water as a solvent. The drug obeyed Beer-Lambert's law over the concentration range 5-75µg/ml. The accuracy of the method was assessed by recovery studies and was found between 99.03 – 100.3% for method A and 99.5 – 100.5% for method B. The method was successfully applied for routine analysis of bumetanide drug in formulations.

KEYWORDS: Bumetanide, UV Visible spectrophotometer, Zero-order and First-order derivative and ICH guidelines.

INTRODUCTION

Quantitative Analysis is an analysis in which the amount or concentration of an analyte may be determined (estimated) and expressed as a numerical value in appropriate units. Several techniques like ultraviolet/visible spectrophotometry, fluorimetry, titrimetry, electroanalytical techniques, chromatographic methods (thin-layer chromatography, gas chromatography and high-performance liquid chromatography), capillary electrophoresis and vibrational spectroscopies are the main techniques that have been used for the quantitative analysis of pharmaceutical compounds.[1] An important group of methods which find an important place in pharmacopoeias are spectrophotometric methods based on UV absorption.[2] Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. The advantages of these methods are low time and labor consumption. The precision of these methods is also excellent. The use of UV–Vis spectrophotometry especially applied in the analysis of pharmaceutical dosage form has increased rapidly over the last few years. Bumetanide is a loop diuretic used to treat heart failure. Bumetanide [3-(Aminosulfonyl)-5-(butylamino)-4-phenoxy-benzoic] acid is a potent high-ceiling or loop diuretic that has an efficiency 40 to 60 times greater than furosemide.[3] The chemical formula and molecular weight of bumetanide are $C_{17}H_{20}N_2O_5S$ and 364.416, respectively. The world anti-doping agency (WADA) and national football league (NFL) consider the supplement a banned ingredient for athletes. Its alleged use is to disguise steroids by increasing urine output. This compound belongs to the sulfonamide family, although its structure differs considerably from furosemide and others of its class. Bumetanide has been included in the majority of reports related to the screening of diuretics, thus liquid–liquid extraction has been the most widely used method as a clean procedure for the urine matrix. Solid–liquid extraction has been scarcely used for the extraction of this diuretic from human urine.[4] There are no spectroscopic methods for the determination of bumetanide. Hence, the present work was planned to validate the UV spectroscopic method for bumetanide in tablet formulations as per ICH guidelines 9-12.

MATERIALS AND METHODS

Chemicals and reagents

Bumetanide was obtained as a gift sample from Spectrum lab, Hyderabad. All the chemicals used were

of analytical grade. The tablet formulations were procured from a local pharmacy.

Instrumentation
UV 1800 double beam UV Visible Spectrophotometer with a pair of 10mm path length matched quartz cells were used for the study. The UV solutions 2.42 software was used.

Preparation of standard drug solution
Accurately weighed 10mg of bumetanide and dissolved in 50ml methanol in a 100ml volumetric flask. The solution was sonicated for 10mins. The final volume was adjusted to100 ml with methanol (standard stock solution of 100µg/ml). The prepared standard solution was scanned in the range of 200-400 nm for determination of the wavelength of maximum absorption.

Preparation of borate buffer (pH 9)
Boric acid (6.2 grams) was dissolved in 500ml of distilled water. The pH was adjusted to 9 with 1M sodium hydroxide and diluted with water to 1000mL.

Preparation of phosphate buffer (pH 7)
Disodium hydrogen phosphate (0.5grams) was mixed with potassium dihydrogen phosphate (0.301grams) in a 1000mL volumetric flask. The final volume was made up with distilled water.

Validation procedure
The method was validated according to ICH guidelines, in terms of linearity, accuracy, precision, and LOD & LOQ.[5] Method validation helps to validate the analytical method for a diversity of concentrations so that the change in formulation or concentration does not need additional validation. Methods are evaluated to determine its effectiveness for future use.[6]

Linearity
The linearity was determined by plotting concentration against corresponding absorbance.[7] A standard stock solution (100µg/ mL) was further diluted with buffer to obtain 10µg/mL - 60µg/mL solutions. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Accuracy
The accuracy of the proposed method was assessed by recovery studies which were carried out at three different levels i.e. 50%, 100% and 150%.[8] A known amount of standard drug solution was added to the pre-analyzed sample solution at three different levels, absorbance was recorded. The % recovery was then calculated.

Precision
Intra-day precision
Standard stock solutions (1.5 ml, 3 ml, and 6 ml) were taken in a 10 ml volumetric flasks and final volume was made up to the mark with buffer. The absorbances of these solutions were individually measured thrice within a day and recorded.[9]

Inter-day precision
Standard stock solutions (1.5 ml, 3 ml, and 6 ml) were taken in 10 ml volumetric flasks and volume were made up to the mark with buffer. The absorbances of these solutions were individually measured thrice in three days and recorded.

Limit of detection
LOD was calculated based on the standard deviation of response and the slope of the corresponding curve using following equation: LOD = 3.3 σ/ S.

Limit of quantification
LOQ was calculated based on the standard deviation of response and the slope of the corresponding curve using following equation: LOD = 10 σ/ S.

RESULTS
The standard solution of bumetanide in methanol (10 µg/ml) was subjected to a scan individually at series of wavelengths of 200 nm to 400 nm at zero order derivative mode. The first order derivative spectra were taken at a smoothening factor of the instrument using Shimadzu 1800 spectronic UV Visible spectrophotometer. The absorption maximum of bumetanide was found to be at 252nm. An overlain spectrum was depicted in fig 5-8 and summary of validation parameters was represented in table 1.

Table 1: Summary of validation parameters.

Parameters					
Methods	**Order**	**Correlation coefficient**	**%Recovery ± SD**	**Sandell's sensitivity**	**Molar absorptivity**
Method A	Zero-order	0.9992	101.9±0.26	0.0268	13687.88
	First-order	0.9993	100.53±0.90		
Method B	Zero-order	0.9992	100.4±1.56	0.0289	12284.23
	First-order	0.9991	98.4±1.1		

Linearity
Beer Lambert's law was obeyed in the concentration range of 10-60µg/ml. Calibration curves were shown in fig 1-4.

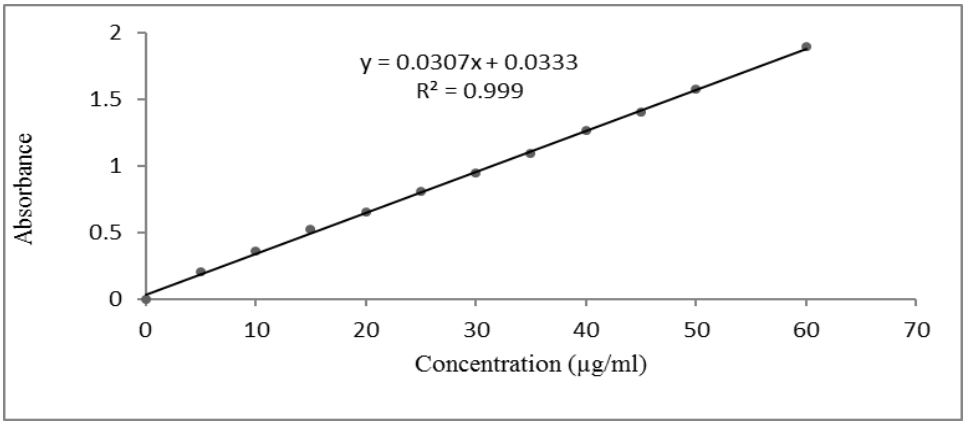

Fig. 1: Calibration curve of method A (zero order).

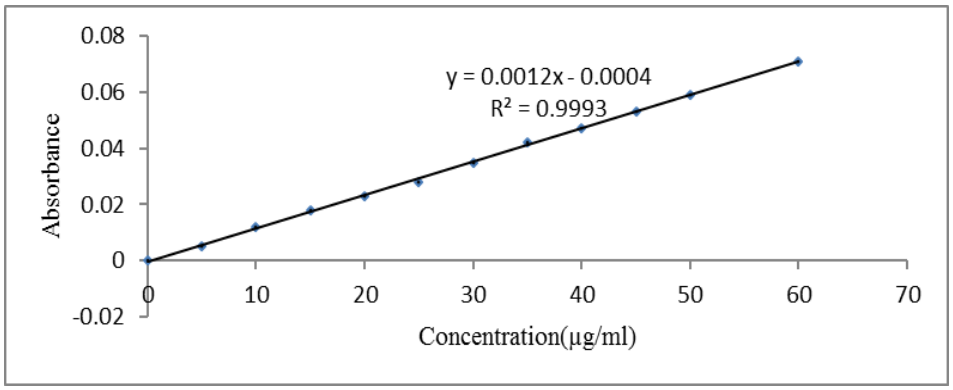

Fig. 2: Calibration curve of method A (first-order derivative).

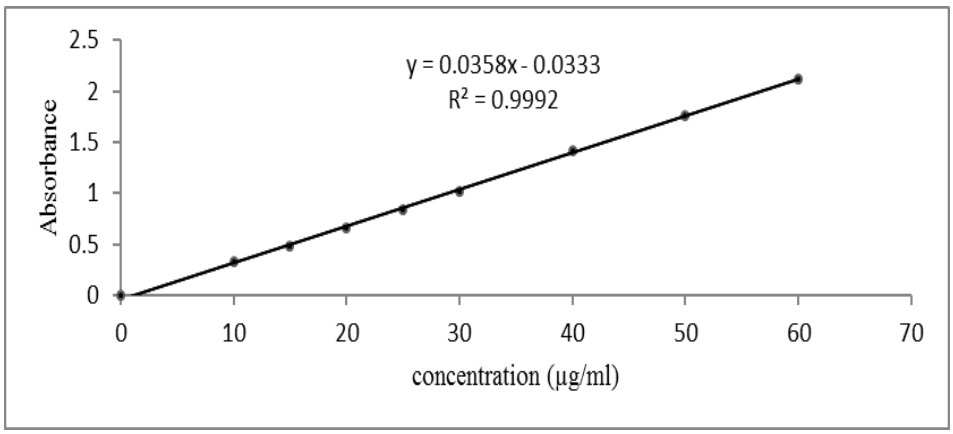

Fig. 3: Calibration curve of method B (zero order).

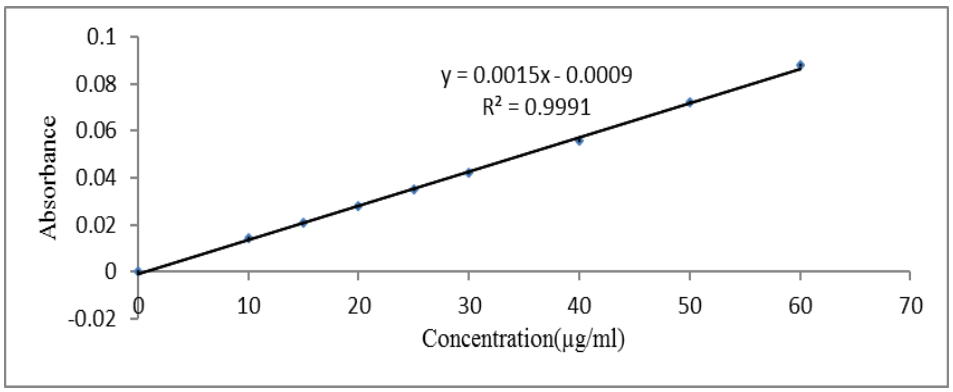

Fig. 4: Calibration curve of method B (first-order derivative).

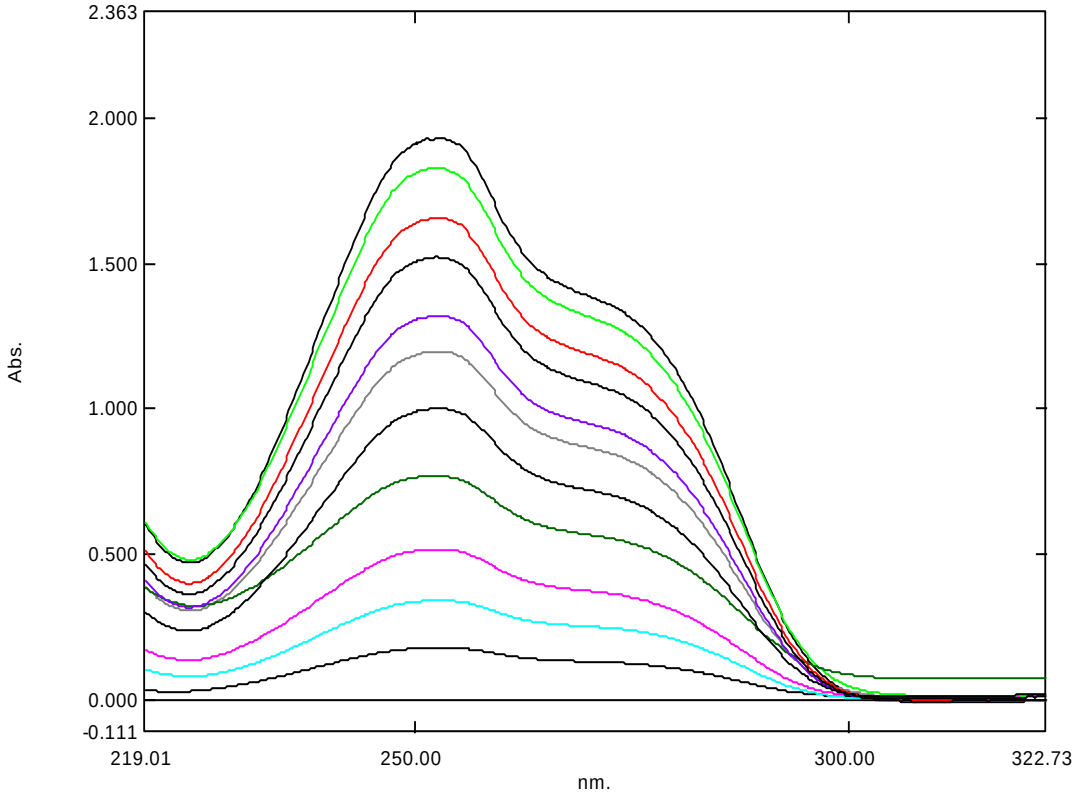

Fig. 5: Overlain spectrum of method A (zero order).

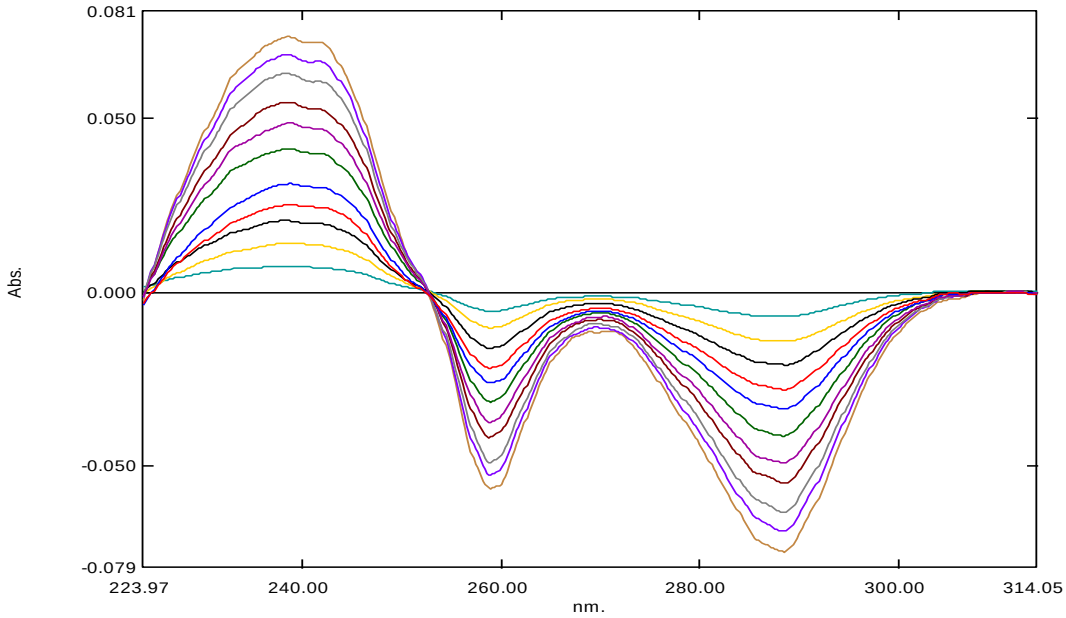

Fig. 6: Overlain spectrum of method A (first-order).

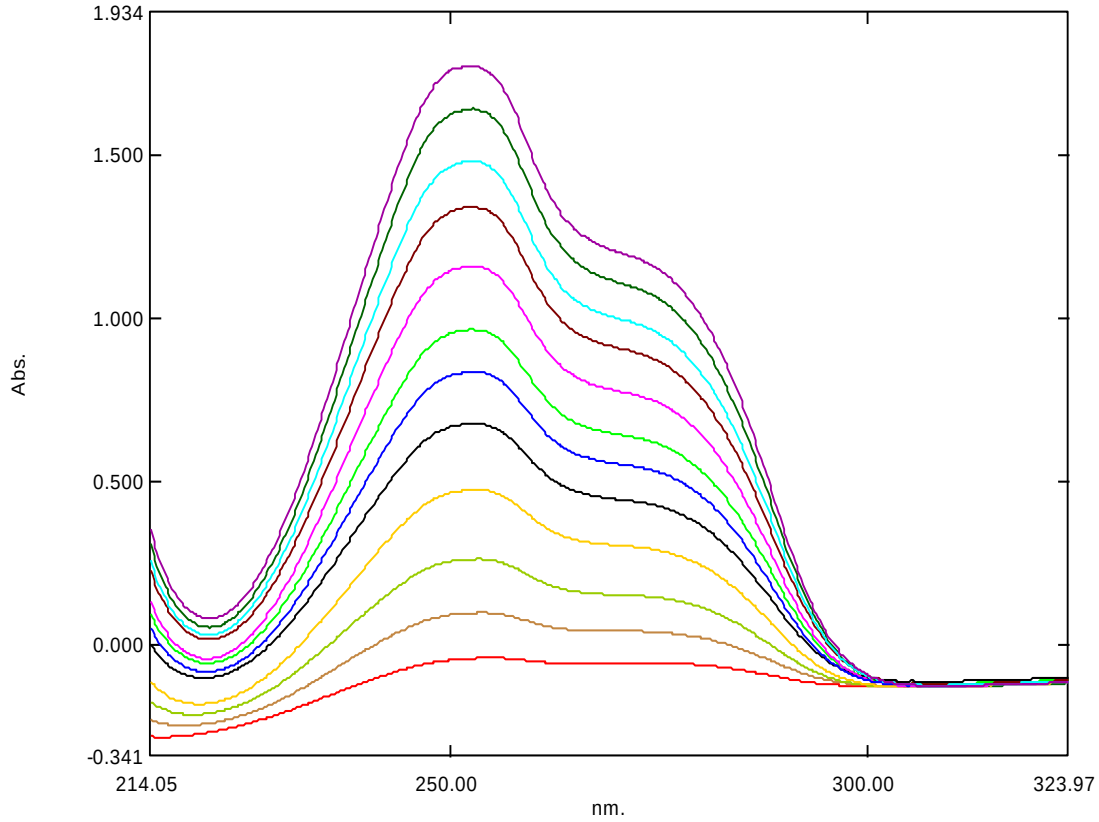

Fig. 7: Overlain spectrum of method B (zero order).

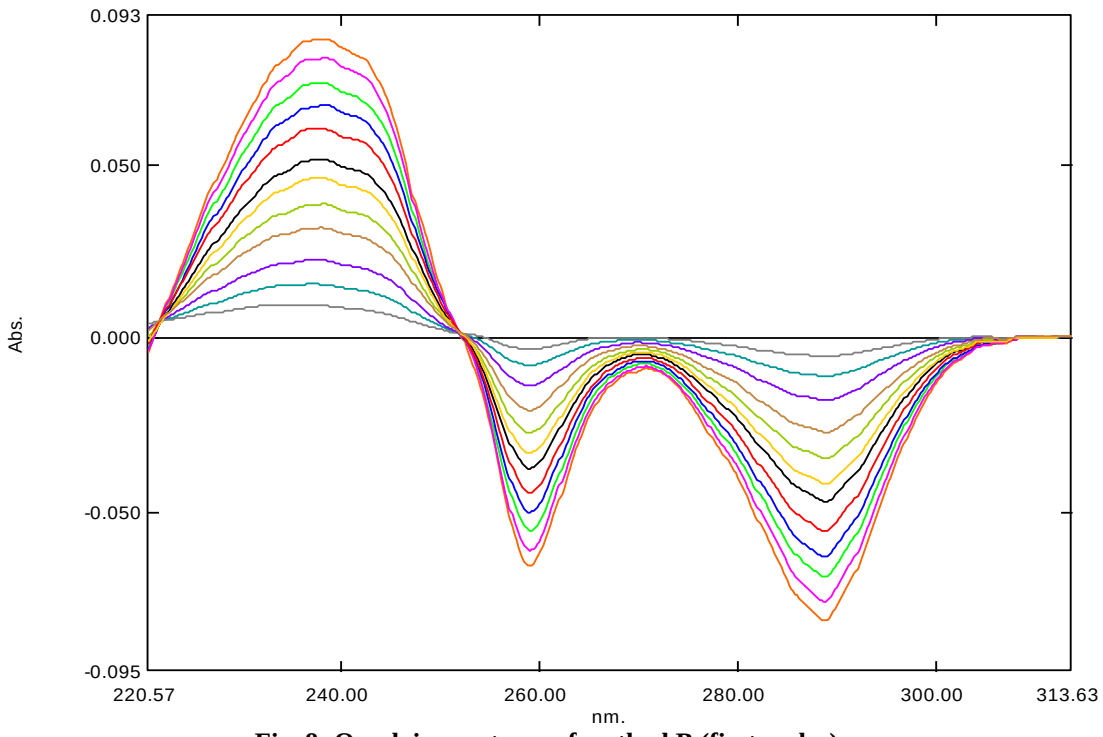

Fig. 8: Overlain spectrum of method B (first-order).

Accuracy

The mean % recovery of concentrations ranging (spike level) 50%, 100%, 150% was found to be 99.03 to 101.37 for method A and 99.35 to 100.52 for method B, respectively. The results were shown in table 2&3.

Table 2: Accuracy data of method A.

Method A	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	*Mean ± SD
Zero-order	10	5	15.1	101.37 ± 0.65
	10	10	10.06	99.03 ± 0.19
	10	15	25.06	100.3± 1.06
First order	10	5	15.04	100.52 ± 1.52
	10	10	19.85	99.35 ± 0.97
	10	15	24.2	98.97 ± 0.36

**Number of experiments – 3.*

Table 3: Accuracy data of method B.

Method B	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	*Mean ± SD
Zero-order	10	5	14.9	99.5 ± 0.88
	10	10	20.01	100.4 ± 0.56
	10	15	24.25	100.5 ± 0.30
First-order	10	5	15	98.4±1.09
	10	10	19.6	98.5±0.49
	10	15	24.1	98.1±0.56

**Number of experiments – 3.*

Precision

The %RSD for the inter-day and intra-day precision were reported to be 1.01 & 0.55 for method A and 1.8 & 1.2 for method B, respectively. The results of precision were shown in table 4-7.

Table 4: Intra-day precision data of method A.

Method A	Concentration (µg/ml)	Amount found (µg/ml)	*Mean ± SD (µg/ml, n=3)	%RSD
Zero-order	15	15.1	101.5± 0.55	0.55
	30	30.36	101.2±0.34	0.33
	60	59.3	98.99±0.29	0.29
First order	15	14.99	99.97±0.55	0.55
	30	29.73	99.11±0.75	0.76
	60	60.26	100.4±1.01	1.01

**Number of experiments – 3.*

Table 5: Inter-day precision data of method A.

Method A	Concentration (µg/ml)	Amount found (µg/ml)	*Mean ± SD (µg/ml, n=3)	%RSD
Zero-order	15	15.13	100.4± 1.01	1.01
	30	30.36	99.11±0.75	0.76
	60	59.3	100.95±0.12	0.13
First order	15	14.9	99.14±1.45	1.46
	30	29.6	99.44±1.68	1.69
	60	59.83	99.97±1.40	1.39

**Number of experiments – 3.*

Table 6: Intraday precision data of method B.

Method A	Concentration (µg/ml)	Amount found (µg/ml)	*Mean ± SD (µg/ml, n=3)	%RSD
Zero-order	15	14.75	99.93± 1.20	1.20
	30	29.02	98.69±1.01	1.01
	60	59.97	99.25±1.48	1.48
First order	15	14.4	101.11±1.02	1.02
	30	29.3	101±1.51	1.51
	60	59.2	100.9±1.54	1.54

**Number of experiments – 3.*

Table 7: Inter-day precision data of method B.

Method B	Concentration (µg/ml)	Amount found (µg/ml)	*Mean ± SD (µg/ml, n=3)	%RSD
Zero-order	15	14.94	98.76± 1.79	1.80
	30	29.95	98.97±1.20	1.21
	60	60.02	98.93±1.91	1.92
First order	15	14.9	99.33±1.62	1.62
	30	30.4	100.44±1.84	1.84
	60	60.9	100.83±1.67	1.66

**Number of experiments – 3.*

LOD & LOQ

The limit of detection and the limit of quantification were determined to be 0.0894 and 0.963 µg/mL for method A and 0.0893 & 0.994 µg/mL for method B.

DISCUSSION

Bumetanide is an UV-absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectroscopic method. The spectral analysis showed the λ max of bumetanide to be 252nm. The linearity of the method was tested in order to demonstrate the proportional relationship of response versus analyte concentration over the working range.[10] It was found to be linear and hence suitable for the estimation of the drug. The slope, intercept, correlation coefficient and optical characteristics were summarized**.** Regression analysis of Beer's law plot revealed a good correlation. The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.[11] The mean % recovery was in the acceptance limit of 98.0 to 102.0%. The RSD was not more than 2.0%. The proposed method was validated as per the ICH guidelines. The precision was measured in terms of inter-day and intra-day, which was determined by a sufficient number of aliquots of a homogeneous sample. The % RSD was found within the range of ± 2.0. This showed that the precision of the method was satisfactory. The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels.[12] The limit of quantification (LOQ) or quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy.[13] The LOD and LOQ of bumetanide were determined by using the standard deviation of response and slope approach as defined by ICH guidelines.[14]

CONCLUSION

The UV-spectrophotometric method for the estimation of bumetanide in bulk and tablet formulation was found to be accurate, precise and robust. This spectrophotometric method for the routine quantitative determination of samples definitely reduces unnecessary tedious sample preparations and the cost of materials and labor. The method was found to be linear over a convenient range, economical and utilized a solvent which can be easily prepared. The mean % recovery was validated as per the ICH guidelines. The %RSD for the inter-day and intra-day precision were reported. The LOD and LOQ of bumetanide were determined by using the standard deviation of response and slope approach as defined by ICH guidelines. Therefore I conclude that the above factors make this method suitable for the estimation of bumetanide in bulk drug and in pharmaceutical dosage forms.

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