Bioanalytical method development and validation of UV spectrophotometric method for estimation of Bumetanide spiked in human urine

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Abstract

The main purpose of this study was to develop a simple precise, rapid and accurate UV-visible spectrophotometric method for determination of Bumetanide in spiked human urine by extracting the Bumetanide from spiked human urine using ethyl acetate after extraction it was scanned between 200-400nm by using UV detector and its absorbance maxima was found to be 222nm. The calibration curve was linear in the range of 1-17 µg/ml. the recovery and assay studies of bumetanide were within 93-94.85% indicating that the proposed method can be estimation of bumetanide.

Keywords: Bumetanide; UV Spectrophotometric; Validation; Method development

1. Introduction

Bumetanide is chemically designated as 3-(butyl amino)-4-phenoxy-5-sulfamoylbenzoic acid [3] and used as loop diuretics (sulfamyl category) [1][2]. in the treatment of heart failure, renal insufficiency and Shepatic cirrhosis cases [6]. 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 Bumetanide also acts as an inhibitor of chloride (Cl−) co-transporter NKCC1 (cation-chloride cotransporter) to keep the intracellular Cl− levels in the neurons at a definite level [1][2]. Bumetanide is used to reduce extra fluid in the body (edema) caused by conditions such as heart failure, liver disease, and kidney disease. This can lessen symptoms such as shortness of breath and swelling in your arms, legs, and abdomen. Bumetanide is a "water pill" (diuretic) that causes you to make more urine. This helps your body get rid of extra water and salt. Bumetanide is a type of medicine called a diuretic. This helps get rid of extra fluid in your body. Bumetanide is only available on prescription. It comes as tablets and as a liquid that you swallow. Bumetanide also comes mixed with another diuretic calledamiloride to treat oedema.

Diuretics are drugs which cause a net loss of Na+ and water in urine. Diuretic drugs increase the urinary output of electrolytes and water from the kidney by interfering with one or more reabsorptive processes occurring at different segments of the nephron. They are used adjust the volume and composition of body fluids in a variety of clinical situations including hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis. Bumetanide works by decreasing the reabsorption of sodium by the kidneys. The main difference between bumetanide and furosemide is in their bioavailability and potency. About 60% of furosemide is absorbed in the intestine, and there are substantial inter- and intra-individual differences in bioavailability (range 10-90%). About 80% of bumetanide is absorbed, and its absorption does not change when it is taken with food.

1.1. IUPAC name [3].

3-butylamino-4-phenoxy-5-sulfamoylbenzoic acid, is synthesized from 4-chlorobenzoic acid.
1.2. Structure

![Structural formula of Bumetanide](image)

**Figure 1** Structure of Bumetanide

1.3. Introduction to UV spectroscopy

UV spectroscopy is a type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Any molecule has either π, π or σ or combination of these electrons. These bonding (σ and π) and non-bonding electrons absorb the characteristic radiation and undergo transition from ground state to excited state. By the characteristic absorption peaks and the nature of the electron present the molecular structure can be elucidated. UV spectroscopy obeys the Beer-Lambert law, Beer law: This law can be stated as follows: "When a beam of monochromatic radiation is passed through a solution of absorbing substances, the intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substances exponentially".

\[ I = I_0 e^{-k_1 c} \]

Where,

- \( I_0 \) = intensity of light incident upon sample cell
- \( I \) = intensity of light leaving sample cell
- \( c \) = molar concentration of solute
- \( k_1 \) = constant Lambert's law

This law can be stated as follows “When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light”.

\[ I = I_0 e^{-k_2 l} \]

Where,

- \( I_0 \) = intensity of light incident upon sample cell
- \( I \) = intensity of light leaving sample cell
- \( l \) = length of sample cell (cm.)
- \( k_2 \) = constant After combining equation 1 and 2 and deriving we get the following equation 3 of Beer Lambert law as:

\[ A = \log \frac{I_0}{I} = \varepsilon cl \]

Where,

- \( A \) = absorbance
- \( I_0 \) = intensity of light incident upon sample cell
- \( I \) = intensity of light leaving sample cell
- \( C \) = molar concentration of solute
- \( l \) = length of sample cell (cm.)
- \( \varepsilon \) = molar absorptivity
2. Material and methods

2.1. Instrument
A double beam UV-Visible spectrophotometer “ELICO SL 210” and double beam UV-Visible spectrophotometer “SYSTRONIC 2203”.

2.2. Chemicals
Bumetanide was obtained as gift sample from pharmaceutical industry. BUMEX tablets was purchased from local market. Analytical reagent grade Acetonitrile, water, Dihydrogen potassium phosphate, dipotassium hydrogen phosphate was used.

2.3. Extraction of bumetanide from human urine
One microlitres of urine was transferred into centrifuge tube and spiked with fixed aliquots of working standard solution of Bumetanide and vortexed then acidified with 4ml of KH2PO4 P 20ul of 1M NaOH and 8ml of ethyl acetate was added to it and then tubes were mechanically shaken for 20 min and centrifuged at 1800 g for 5min and the organic phase was transferred to a second tube containing 8ml of 0.1M KH2PO4 (7.5) and shaken for 20min and then the mixture was centrifuged and organic layer was separated and evaporated to dryness at temp 40 and the residue was dissolved in 1ml of mobile phase ACN:water (50:50)and 0.5ml of residue was diluted with mobile phase and make up to 50ml with water and absorbance was measured at 222nm [7][8].

3. Method development

3.1. Preparation of standard solution
Standard solution of Bumetanide was prepared by taking 10mg in 10ml volumetric flask containing distilled water and the volume was made up to the mark with acetonitrile and distilled water (6:4) as Diluent (stock solution).Then from this 0.1 ml was pipetted out and transferred into another 10 ml volumetric flask and the volume was made up to the mark with distilled water to give 10µg/ml solution and this was scanned between 200 to 400nm and its absorbance was measured at 222nm.

3.2. Selection of wavelength

![Figure 2 UV spectrum of standard solution (10µg/ml)](image)

Solutions of 10µg/ml of Bumetanide were prepared and the solution was scanned in the spectrum mode from 200nm to 400nm. The maximum absorbance of Bumetanide was observed at 231nm (figure 2).
3.3. Optimization of parameters
All the optimization parameters at room temperature. Bumetanide was found to yield clear colorless solution with ACN:water(6:4), showing maximum absorbance at 222nm. Different concentrations and different volumes were tried for all the solvents. The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance.

3.4. Preparation of calibration curve
Standard stock solution of Bumetanide further diluted to get concentration in the range of 1-18µg/ml. The resultant absorbance of the solution were measured at 222nm against with ACN:water(6:4) as blank.

4. Method validation
The developed method was validated according to ICH guidelines. The proposed method was validated in terms of specificity, linearity, precision, accuracy, robustness, ruggedness, LOD (Limit of detection) and LOQ (Limit of Quantification).

4.1. Specificity
4.1.1. Definition
The ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components (figure 3).

![Figure 3 Blank spectrum](image)

4.2. Linearity
4.2.1. Definition
Ability to obtain test results which are directly proportional to the concentration of analyte in the sample[5].

The proposed spectroscopic method was found to be linear in the range of 1-17µg/ml with correlation coefficient was 0.9991 (figure 4), slope 0.0031 and intercept 0.0529 was shown in table 1.

4.3. Precision
4.3.1. Definition
Degree of agreement between a series of measurement obtained from multiple sampling of same homogenous sample.
The precision of the proposed method was estimated in terms of inter-day and intra-day precision wherein the method was repeated for 6 times respectively. The results shown in table 2 indicating %RSD of less than 2% each level clearly indicate that the proposed method was precise enough for the analysis of drug [5].

\[
\%\text{RSD} = \frac{\text{SD of measurement}}{\text{mean value of measurement}} \times 100
\]

4.4. Accuracy

4.4.1. Definition

Closeness of test results obtained by that procedure to the true values [7].

The accuracy of the method was determined by performing recovery studies by spiking standard solution to that of sample solution at three different levels i.e., 50%, 100%, 150% or 80%, 100%, 120%. Values of %recovery greater than 95-105% indicate that the proposed method was accurate for the analysis of drug and the results were reported in table 3.

\[
\%\text{Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100
\]

4.5. Robustness

4.5.1. Definition

Deliberate changes in the method are made such as wavelength. The ±nm from the fixed wavelength.

The robustness of the proposed method was evaluated by changing wavelength. The %RSD was calculated. The low values of %RSD obtained after small deliberate changes in method indicates that the method was robust and the results were presented in table 4.

4.6. Ruggedness

4.6.1. Definition

The degree of reproducibility of the results obtained by the analysis of the sample under a variety of conditions such as different analyst and different instrument.

The ruggedness of the proposed method was evaluated by varying conditions different analyst and different instrument ("ELICO SL 210" and "SYSTRONIC 2203"). The %RSD was calculated. The low values of %RSD obtained by changing the conditions indicates that the method was rugged and the results were presented in table 5.

4.7. LOD

4.7.1. Definition

It is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated under the stated experimental condition.

\[
\text{LOD} = \frac{3.3 \times \text{SD}}{\text{slope}}
\]

4.8. LOQ

4.8.1. Definition

It is the lowest amount of analyte in the sample which can be quantitatively determined with acceptable precision and accuracy under the stated experimental conditions.

\[
\text{LOQ} = \frac{10 \times \text{SD}}{\text{slope}}
\]
4.9. Assay

Preparation of standard solution: 10mg of Bumetanide drug was accurately weighed and transferred into 10ml of volumetric flask and the volume was made up to the mark with distilled water to get concentration of 1000µg/ml. From this 0.1 ml was pipetted out and transferred into 10ml of volumetric flask and the volume was made up to the mark to get 10µg/ml solution and its absorbance was measured at 222nm.

Preparation of test solution: 10 tablets were weighed and powdered. Powdered tablet equivalent to 10 mg of Bumetanide was weighed accurately and it was taken into 10ml volumetric flask then volume was made up to the mark with distilled water. From the above solution 0.1 ml of solution was pipetted out and taken in 10ml volumetric flask. The volume was made up to 10ml to get 10 µg/ml solution and its absorbance was measured at 222nm.

The % Assay is calculated by using the following formula:

\[
\% \text{ Assay} = \frac{\text{absorbance of the sample}}{\text{absorbance of the standard}} \times \frac{\text{concentration of the standard}}{\text{concentration of the sample}} \times 100
\]

\[
\% \text{ Assay} = 94.85\%
\]

5. Results and discussion

5.1. Linearity

![Figure 4 Linearity study](chart)

Table 1 Results of linearity study

<table>
<thead>
<tr>
<th>S. NO.</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Absorbance maximum(nm)</td>
<td>222</td>
</tr>
<tr>
<td>2</td>
<td>Linearity and range(µg/ml)</td>
<td>1-18µg/ml</td>
</tr>
<tr>
<td>3</td>
<td>Slope</td>
<td>0.1168</td>
</tr>
<tr>
<td>4</td>
<td>Correlation coefficient</td>
<td>0.9991</td>
</tr>
<tr>
<td>5</td>
<td>Y-intercept</td>
<td>0.0529</td>
</tr>
</tbody>
</table>

5.2. Precision

\[
\% \text{RSD} = \frac{\text{SD of measurement}}{\text{mean value of measurement}} \times 100
\]
Table 2 Results of precision studies

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Intraday precision (%RSD)</th>
<th>Inter-day precision (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5µg/ml</td>
<td>0.129%</td>
<td>0.1329%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2528%</td>
</tr>
</tbody>
</table>

5.3. Accuracy

Table 3 Results of accuracy studies (50%, 100%, 150%)

<table>
<thead>
<tr>
<th>Level</th>
<th>Amount of standard added (µg/ml)</th>
<th>Pre-analysed sample (µg/ml)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>1</td>
<td>5</td>
<td>97.14%</td>
</tr>
<tr>
<td>100%</td>
<td>2</td>
<td>5</td>
<td>98.76%</td>
</tr>
<tr>
<td>150%</td>
<td>3</td>
<td>5</td>
<td>99%</td>
</tr>
</tbody>
</table>

5.4. Robustness

Table 4 Results of ruggedness studies

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Concentration</th>
<th>Analyst</th>
<th>%RSD</th>
<th>Instrument</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5µg/ml</td>
<td>Analyst 1</td>
<td>0.7144%</td>
<td>Instrument1 (ELICO)</td>
<td>0.001123</td>
</tr>
<tr>
<td>2</td>
<td>5µg/ml</td>
<td>Analyst 2</td>
<td>0.1744%</td>
<td>Instrument (SYSTRONIC)</td>
<td>0.001123</td>
</tr>
</tbody>
</table>

5.5. LOD (Limit of Detection)

\[
LOD = \frac{3.3 \times SD}{\text{slope}}
\]

The LOD of the proposed method was found to be 0.0234 µg/ml.

5.6. LOQ (Limit of Quantification)

\[
LOQ = \frac{10 \times SD}{\text{slope}}
\]

The LOQ of the proposed method was found to be 0.07054 µg/ml.

5.7. Assay

The % Assay is calculated by using the following formula:

\[
% \text{ Assay} = \frac{\text{absorbance of the sample}}{\text{absorbance of the standard}} \times \frac{\text{concentration of the standard}}{\text{concentration of the sample}} \times 100
\]

\[
% \text{ Assay} = \frac{0.605}{0.6378} \times 5/5 \times 100
\]

\[
% \text{ Assay} = 94.85\%
\]
6. Conclusion

Therefore, a simple rapid method with cost effectively and less economically method was developed and validated by using UV-Visible spectrophotometry.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All authors declared that there are no conflicts of interest.

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