Method Development and Validation of Acetazolamide by using UV Spectroscopy

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Abstract:
This UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of Acetazolamide in tablet formulation. It involves absorbance measurement at 288nm (λmax of Acetazolamide) in Ethanol. For UV Spectrophotometric method, linearity was obtained in concentration range of 1-50µg/ml for acetazolamide and with regression 0.9998. Recovery was in the range of 98-101%; the standard deviation and % RSD were found to be < 2 %; shows the high precision of the method, in accordance with ICH guidelines. The method has been successively applied to active pharmaceutical ingredient and was validated according to ICH guidelines.

Keywords: Acetazolamide, UV-spectrophotometric technique.

I. INTRODUCTION

The main objective of validation is to form a basis for written procedures which are designed to assure the drug products have the identity, quality, and purity. Quality, safety and efficacy are most important to designed and develop and build the products. In each step of manufacturing we should concern to maximize the probability that the final product meets all quality and design specification. Analytical monitoring is important to products of pharmaceuticals for to ensure its safety efficacy throughout all phases of its shelf life. Analytical validation is the corner stone of product development. According to ICH typical analytical performance characteristics that should be considered in the validation of the type of the methods are[1]

• Linearity
• Accuracy
• Precision
• Repeatability
• Ruggedness
II. LITERATURE REVIEW

- Dr. K.K. Senthilkumar et al., Determination of Acetazolamide in a fixed dosage form was carried out by UV Spectrophotometric method. The absorbance values were observed for different dilutions of drug at 263.00 nm and which are used for the dilution in Ethanol. This method obeys Beer’s Lambert’s Law in the concentration range of 1-5 μg/mL. The results have been validated statistically and the recovery studies confirmed the accuracy of this proposed method.

- Dudhe P.B et al., A simple, reproducible and economical two spectrophotometric methods have been developed for determination of acetazolamide in tablet dosage form. All solutions were prepared by using methanol as a solvent. Method A is area under curve (AUC), in which area was integrated in the range of 253.00 nm – 273.00 nm and in method B i.e. first order derivative spectroscopy, absorbance values were measured at λmin = 248.83 nm, λmax = 278.94 nm and λzero cross = 263.89 nm. For both methods linearity was established in the range of 5 μg/ml - 30 μg/ml (Method A: R² = 0.9991 and Method B: R² = 0.9991). Validation studies were performed by ICH Q2(R1) guideline for method A and B. Accuracy, precision, assay, limit of detection (LOD) and limit of quantitation (LOQ) studies were done for both methods and results was found within acceptable limit.

- Philip T.R. Hwang et al., A simple, rapid and specific HPLC method has been developed to determine acetazolamide concentrations in human plasma. The assay procedure requires only 250 μl of sample with direct injection of the organic supernatant after protein precipitation with acetonitrile. Chlorothiazide was used as an internal standard. A reversed-phase C18 μBondapak column was employed for the chromatographic separation. The eluent was monitored at 265 nm using a UV variable wavelength detector. The retention times for acetazolamide (ACZ) and chlorothiazide (CTZ) were 6 and 8 min respectively. A linear relationship (r = 0.9995) was obtained over the 1-20 μg/ml concentration range. The limit of sensitivity for ACZ was 0.5 μg/ml, with greater than 85% recovery of ACZ and internal standard. The method was applied to human plasma samples obtained after administration of a 250 mg acetazolamide tablet.

- Dennis J. Chapronet al., A high-performance liquid chromatographic method for the determination of acetazolamide in whole blood, plasma, and urine was developed. Samples of biological fluids containing various concentrations of acetazolamide were spiked with the internal standard, sulfadiazine. Samples were then mixed with a 50% ammonium sulfate solution. Whole blood samples were heated for 25 s in boiling water. All samples were extracted with ethyl acetate; a phosphate buffer (pH 8.0) was used to wash the extracts. Acetazolamide was back-extracted into a glycine buffer (pH 10.0), which was then washed with ether. Separation of acetazolamide and internal standard from other biological constituents was achieved on a 10-μm C18 reverse-phase column using an acetonitrile-methanol-acetate buffer (pH 4.0). The eluant was monitored at 254 nm. All calibration curves were linear, and the results from reproducibility studies were excellent. Application of the method to human pharmacokinetic studies was demonstrated.

- Anjaneyulu Narapusettil et al., The objective of this research was to develop a novel liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination of acetazolamide in human plasma. Methods: An analytical method based on LC-MS/MS (API-4000) has been developed and validated for the quantitative determination of acetazolamide in human plasma using acetazolamide d3 as Internal Standard (IS). After Solid phase extraction (SPE), analyte and the IS were chromatographed on a C18 columns using a isocratic mobile phase composed of 0.1% formic acid buffer and acetonitrile (30:70, v/v) pumped at a flow rate of 0.80 mL/min. Results: Precision and accuracy of the method was determined using five analytical batches in the concentration range of 50.3–12046 μg/mL. All the validation experiments were carried out as per the US FDA guidelines and results met the acceptance criteria. Conclusion: The proposed LC–MS/MS assay method is simple, rapid and sensitive for the determination of acetazolamide in human plasma. A chromatographic run time of 2.0 min, allow us to analyze more than 300 samples in a day.

- M. I. Walash et al., A simple and sensitive spectrophotometric method was developed for the determination of acetazolamide (ACM) in pure form and pharmaceutical preparations. The proposed method is based on the complex formation of acetazolamide with Palladium (II) chloride in acetate buffer pH5.4 and measuring the absorbance at 308 nm. The absorbance- concentration plot was rectilinear over the concentration range of 5-70 μg/ml with a minimum detection limit (LOD) of 0.98 μg/ml, limit of quantification (LOQ) of 2.96 μg/ml and a molar absorptivity ζ=2.7 × 103 L/mol.cm. The factors affecting the absorbance of the formed complex were carefully studied and optimized. The composition of the complex as well as its stability constant was also investigated. The proposed method was applied for the determination of acetazolamide in its tablets and the results obtained were favorably compared with those obtained using the official method. A proposal of the reaction pathway was postulated.

1. Plan of work
Method development and validation of acetazolamide will be done by UV Spectrophotometer method.

Methodology
- Literature survey
- Procurement of drug sample and other chemicals
- Selection of wavelength (λ max)

Method development by UV–Spectrophotometer
1. Selection of Preliminary by UV- Spectrophotometric Conditions
   A) Selection Of Solvent
   B) Selection Of Wavelength
2. Validation Of Proposed Method
3. Linearity And Range
4. Precision
   A) Intra Day Precision
   B) Inter Day Precision
5. Accuracy
6. Ruggedness
7. Sensitivity

III. AIM& OBJECTIVE

- To develop and validate a different dilutions for the estimation of Acetazolamide by using UV–Spectrophotometer method.
The developed method is validated according to ICH guidelines for various parameters specified in ICH guidelines.

<table>
<thead>
<tr>
<th>S.No</th>
<th>NAME</th>
<th>MODEL</th>
<th>MANUFACTURER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UV-Spectrophotometer</td>
<td>2060</td>
<td>Analytical Tech</td>
</tr>
<tr>
<td>2</td>
<td>Ultra Sonicator</td>
<td></td>
<td>Remi</td>
</tr>
<tr>
<td>3</td>
<td>Weighing Balance</td>
<td></td>
<td>Wensar Corporation</td>
</tr>
<tr>
<td>4</td>
<td>Distilled Water</td>
<td>HPLC</td>
<td>Merck Milli QRO- Purification System</td>
</tr>
</tbody>
</table>

**EXPERIMENTAL WORK:**

**4. METHODOLOGY AND DEVELOPMENT**

**METHODOLOGY**

**Preparation of standard stock solution**

Accurately weighed 10 mg of Acetazolamide was transferred to a 100 ml volumetric flask, dissolved in 20 ml distilled water by shaking manually for 10 min. The volume was made up to final strength, i.e. 100 μg/ml.

**Selection of wavelength for analysis of acetazolamide**

Appropriate volume 0.5 ml of standard stock solution of acetazolamide was transferred into a 10 ml volumetric flask, diluted to a mark with distilled water to give concentration of 5 μg/ml, 10μg/ml, 15μg/ml. The resulting solution was scanned in the UV range (200–400 nm). In spectrum acetazolamide showed absorbance maximum at 288 nm.

**Validation Parameter**

The method was validated in terms of linearity, accuracy, precision, and ruggedness.

<table>
<thead>
<tr>
<th>Stocks</th>
<th>Wavelength of stocks</th>
<th>Absorbance(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5μg/ml</td>
<td>288</td>
<td>0.356</td>
</tr>
<tr>
<td>10μg/ml</td>
<td>288</td>
<td>0.594</td>
</tr>
<tr>
<td>15μg/ml</td>
<td>288</td>
<td>0.912</td>
</tr>
</tbody>
</table>

**DRUG PROFILE:**

- Name: Acetazolamide
- BrandName: Diamox Diacarb
- Category: Carbonic Anhydrase Inhibitors
- Dose: 250mg every 6 hours
• Structure:

\[
\begin{align*}
\text{H}_3\text{C} & \text{O} \\
\text{NH} & \text{S} \\
\text{N} & \text{N} \\
\text{S} & \text{O} \\
\text{NH}_2 & 
\end{align*}
\]

• IUPAC Name: N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide
• Molecular formula: \( \text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2 \)
• Molecular weight: 222.237 g/mol

**MECHANISAM OF ACTION:**

**MECHANISM OF ACETAZOLAMIDE:**
- Carbonic Anhydrase catalyses: \( \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \)
- \( \text{H}^+ \) ions produced by the breakdown of \( \text{H}_2\text{CO}_3 \) will exchange the \( \text{Na}^+ \) ions through \( \text{H}^+\text{Na}^+ \) antiporter.
- \( \text{Na}^+ \) will combine with \( \text{HCO}_3^- \) to form the \( \text{NaHCO}_3 \) in the lumen of proximal convoluted tubules (PCT).
- Inhibition of \( \text{HCO}_3^- \) reabsorption.
- Reduces \( \text{Na}^+\text{H}^+ \) exchange.
- \( \text{NaHCO}_3 \) is excreted along with water.
- Hence increased in diuresis.

**RESULTS AND DISCUSSION**

**LINEARITY:**
- Different aliquots of Acetazolamide in the range 0.5–3 ml were transferred into series of 10 ml volumetric flasks, and the volume was made up to the mark with distilled water to get concentrations 5, 10, 15, 20, 25, and 30 \( \mu \text{g/ml} \), respectively.
- The solutions were scanned on a spectrophotometer in the UV range 200–400 nm.
- The spectrum was recorded at 288 nm. The calibration plot was constructed as concentration vs. absorbance.

**RESULTS OF LINEARITY:**

<table>
<thead>
<tr>
<th>Concentration(( \mu \text{g/ml} ))</th>
<th>Absorbance(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0.142</td>
</tr>
<tr>
<td>10</td>
<td>0.273</td>
</tr>
<tr>
<td>15</td>
<td>0.404</td>
</tr>
<tr>
<td>20</td>
<td>0.532</td>
</tr>
<tr>
<td>25</td>
<td>0.659</td>
</tr>
<tr>
<td>30</td>
<td>0.791</td>
</tr>
</tbody>
</table>

[http://ijesc.org/]
ACCURACY:
- To the pre analyzed sample solutions, a known amount of standard stock solution was added at different dilutions i.e. 50%, 100%, and 150%.

RESULTS OF ACCURACY:

<table>
<thead>
<tr>
<th>% Concentration (at specification Level)</th>
<th>N=3</th>
<th>Absorbance</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td></td>
<td>0.4213</td>
<td>2.5</td>
<td>2.498</td>
<td>99.92</td>
<td>99.65</td>
</tr>
<tr>
<td>100%</td>
<td></td>
<td>0.6213</td>
<td>5.0</td>
<td>4.990</td>
<td>99.08</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td></td>
<td>0.9199</td>
<td>10</td>
<td>9.995</td>
<td>99.95</td>
<td></td>
</tr>
</tbody>
</table>

ACCURACY OF 50% ACETAZOLAMIDE:

ACCURACY OF 100% ACETAZOLAMIDE:
ACCURACY OF 150% ACETAZOLAMIDE:

PRECISION:
- Precision of the method was studied as intraday and interday variations.
- Intraday precision was determined by analyzing the 10, 15 and 20 μg/ml of acetazolamide solutions for three times in the same day.

INTERDAY PRECISION:
- Interday precision was determined by analyzing the 10, 15 and 20 μg/ml of acetazolamide solutions daily for 3 days over the period of week.

RESULTS OF PRECISION:

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Intra-day precision</th>
<th>Inter-day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absorbance measured</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>10</td>
<td>0.4113</td>
<td>0.140</td>
</tr>
<tr>
<td>15</td>
<td>0.6147</td>
<td>0.094</td>
</tr>
<tr>
<td>20</td>
<td>0.9210</td>
<td>0.122</td>
</tr>
</tbody>
</table>

PRECISION OF 10% GM/L ACETAZOLAMIDE:

PRECISION OF 15% GM/L ACETAZOLAMIDE:
PRECISION OF 20% GM/L ACETAZOLAMIDE:

RESULTS OF REPEatability:

REPEATABILITY

Repeatability was determined by analyzing 20 μg/ml concentration of acetazolamide solution for six times and the % amount found was 99.69 % RSD < 2.

Results of Repeatability:

<table>
<thead>
<tr>
<th>Concentration (μg/mL)</th>
<th>Absorbance measured (Mean ± SD)</th>
<th>Amount Found (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.8310±0.0324</td>
<td>99.69</td>
<td>0.02</td>
</tr>
</tbody>
</table>

RUGGEDNESS:

• The peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs.

The result showed that the % RSD was less than 2%

RESULTS OF RUGGEDNESS:

<table>
<thead>
<tr>
<th>Analyst</th>
<th>Concentration (μg/mL)</th>
<th>Absorbance measured (Mean ± SD)</th>
<th>Amount Found (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20</td>
<td>0.8116±0.0015</td>
<td>98.98</td>
<td>0.02</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>0.8214±0.0010</td>
<td>99.12</td>
<td>0.01</td>
</tr>
</tbody>
</table>
6. CONCLUSION

This UV-Spectrophotometric technique is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of Acetazolamide in tablet formulation in different dilutions. The validation procedure confirms that this is an appropriate method for their quantification in the formulation. It is also used in routine quality control of the formulations containing this entire compound.

PARAMETERS OF UV SPECTROPHOTOMETER:

<table>
<thead>
<tr>
<th>Validation parameter</th>
<th>Acetazolamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>Water &amp; Ethanol</td>
</tr>
<tr>
<td>Detection wave length</td>
<td>288nm</td>
</tr>
<tr>
<td>Beer's limit</td>
<td>1-30µg/ml</td>
</tr>
<tr>
<td>Linearity</td>
<td>10-50µg/ml</td>
</tr>
<tr>
<td>R²</td>
<td>0.9998</td>
</tr>
<tr>
<td>LOD</td>
<td>2.99µg/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.51µg/ml</td>
</tr>
<tr>
<td>Precision</td>
<td>% RSD&lt;2</td>
</tr>
<tr>
<td>Recovery</td>
<td>98-101</td>
</tr>
</tbody>
</table>

IV. REFERENCES


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