ABSTRACT

Tuberculosis cases have significantly increased within the past decade, especially among AIDS patients, hence the importance of isoniazid, a first line anti-tubercular agent. This has prompted many investigators to develop methods for the rapid determination of isoniazid in pure form as well as in pharmaceutical formulations.

A method is described for the determination of isoniazid, in pure form and in pharmaceutical formulations. The method is based on the coupling of isoniazid and vanillin in an ethanolic hydrochloric acid medium and the spectrophotometric determination at the absorption maximum (405 nm). A yellow coloured hydrazone was formed. Beer’s law was obeyed in the concentration range of 1-12 μg/ml at 405 nm.

The proposed method was applied in the analysis of commercially purchased brands of isoniazid tablets and showed good accuracy and precision. Excipients used in the pharmaceutical formulation showed no interference in the analysis.

The method offers the advantages of rapidity, simplicity and sensitivity and low cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

Keywords: Isoniazid, vanillin, spectrophotometric assay method, hydrazone, tuberculosis
MATERIALS AND METHODS

Jenway 6505 Ultraviolet/Visible Spectrophotometer with matched cuvettes, Ethanol (BDH, Analar), Hydrochloric acid (BDH, Analar), Vanillin powder (BDH, Analar), Isoniazid Chemical Reference, Distilled water, Commercial formulations were purchased from local sources.

Preparation of isoniazid stock solution

Isoniazid stock solution was prepared by accurately weighing 100mg of pure sample and dissolving in distilled water in a 100ml volumetric flask and made up to volume. This was shielded from light before use.

Determination of absorption maximum

A 5ml aliquot of Isoniazid solution was transferred into a 25ml volumetric flask. 4ml of 3% Vanillin solution was added. It was made up to volume using 0.5M ethanolic hydrochloric acid. This was then allowed to stand for about 10minutes and the absorption maximum determined after scanning using the UV/Visible spectrophotometer. It was obtained as 405nm.

Determination of effective reagent concentration

Preliminary experiments were done in order to ascertain the effect of concentration and the volume of vanillin at the wavelength of maximum absorption, 405nm.

To a series of isoniazid solution, varying concentrations (1-5%) were added and the analytical procedure followed. After 10 minutes, the absorbance of each solution was read at 405nm. It was observed that the analytical signal increased with an increase in reagent concentration up to 5%. The concentration of vanillin therefore utilized was 3%.

Similarly, by fixing the vanillin concentration as 3% in a series of isoniazid solution, different volumes of vanillin in the range of 1 - 6 ml were added. The analytical procedure was then followed. After 10 minutes, the absorbance was read. It was observed that 4ml of 3% vanillin solution was optimal for the formation of colour with maximum intensity. Therefore, 4ml of 3% vanillin solution was utilized for all measurements.

Validation of the analytical method

This was done by assessing the accuracy, precision, reproducibility, sensitivity and selectivity of the method.

Analytical method

A 5 ml aliquot of Isoniazid solution was transferred into a 25ml volumetric flask. 4ml of 3% Vanillin solution was then added and made up to volume with 0.5M ethanolic hydrochloric acid. It was left to stand for 10 minutes and the absorbance taken at 405nm against a reagent blank.

Analysis of pharmaceutical formulations

Twenty tablets were weighed. They were then powdered and an amount equivalent to 100 mg was dissolved in distilled water. This was then filtered. The filtrate was made up to mark in a 100 ml volumetric flask. 5ml of the aliquot was then treated as described above.

Preparation/validation of calibration curve

Several volumes of isoniazid corresponding to 0.1-0.5mg were obtained upon dilution of the stock solution. This was then transferred to 25ml volumetric flasks. 4ml of vanillin solution was added followed by addition of 0.5M ethanolic hydrochloric acid and then thorough mixing. It was left to stand for full colour development for 10minutes before taking absorbance reading of the solutions. This experiment was repeated on 3 separate days and the mean values taken. From this, a calibration curve was plotted and regression analysis carried out.

The calibration curve was validated by following the same procedure but changing the serial concentrations. The average absorbance readings were correlated directly with the concentrations using the calibration curves.

RESULTS AND DISCUSSION

Isoniazid readily reacts with vanillin in an ethanolic hydrochloric acid solution yielding an intense yellow coloured hydrazone as shown in the equation having an absorption maximum at 405nm [Figure 1]. The reagent blank does not absorb around this wavelength. The hydrazone formed was stable in the temperature range 20 - 40°C. The colour of the product formed was stable for about 2 hours at room temperature (30°C) followed by a steady decrease in absorbance values.
Beer’s law was obeyed in the range 1 - 12μg/ml at the λmax. A regression analysis of the Beer's law plot showed a good correlation (r = 0.96) and a regression equation of y = 4.278x [Figure 2].

The proposed method was applied in the assay of some pharmaceutical formulations. The results obtained compare well with claimed label values [Tables (1) & (2)]

### Table 1: Evaluation of accuracy and precision of the proposed method

<table>
<thead>
<tr>
<th>Conc. prepared mg/ml</th>
<th>Conc. recovered* mg/ml</th>
<th>% recovery</th>
<th>% error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.987 ± 0.07</td>
<td>98.7</td>
<td>1.3</td>
</tr>
<tr>
<td>0.2</td>
<td>0.212 ± 0.24</td>
<td>106</td>
<td>6</td>
</tr>
<tr>
<td>0.3</td>
<td>0.298 ± 0.15</td>
<td>99.3</td>
<td>0.7</td>
</tr>
<tr>
<td>0.4</td>
<td>0.407 ± 0.32</td>
<td>101.75</td>
<td>1.75</td>
</tr>
<tr>
<td>0.5</td>
<td>0.487 ± 0.03</td>
<td>97.4</td>
<td>2.6</td>
</tr>
</tbody>
</table>

*Mean value of 3 determinations at each concentration ± SD

### Table 2: Analysis of Isoniazid in pharmaceutical formulations using the proposed method

<table>
<thead>
<tr>
<th>Tablet sample code</th>
<th>Label claim (mg)</th>
<th>Actual strength found (mg)</th>
<th>% of label claim found by the proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>300</td>
<td>302.52</td>
<td>100.84</td>
</tr>
<tr>
<td>B</td>
<td>300</td>
<td>300.21</td>
<td>100.07</td>
</tr>
<tr>
<td>C</td>
<td>300</td>
<td>300.21</td>
<td>100.07</td>
</tr>
<tr>
<td>D</td>
<td>300</td>
<td>297.63</td>
<td>99.21</td>
</tr>
</tbody>
</table>

*Mean value of 3 determinations at each concentration ± SD

The assay method in the United States Pharmacopoeia requires the use of HPLC system. It specifies that the tablets should contain not less than 90% and not more than 110% of the labeled amount of isoniazid. Isoniazid in pure form as well as in pharmaceutical formulations has been determined using the proposed method. Based on the application of the proposed method, four brands of Isoniazid tablets analyzed showed between 99.21 - 100.84% of the label claim values. This is in conformity with the requirements in the United States Pharmacopoeia. The accuracy and precision obtained can be favourably compared with that obtained by the official method. The method also showed a relative freedom from interference by the usual tablet Excipients. The proposed method allows for the determination of isoniazid in pure form as well as in pharmaceutical formulations.

This proposed method can be applied for the routine quality control studies of isoniazid as it offers the advantages of rapidity, simplicity and sensitivity and low cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.
REFERENCES


