IR Quantification of Pyridoxine hydrochloride in Bulk And Oral Dosage Form

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ABSTRACT:
Simple and sensitive infrared spectrophotometric method have been developed for the estimation of pyridoxine hydrochloride in tablet dosage form. Beer’s concentration range was found to lie between 7.5-15mg. The correlation coefficient for the method was found to be 0.9987 and the developed method was analyzed for specificity, linearity of response, precision and accuracy; thus the proposed method could be adopted for routine analysis of bulk drug and its formulation.

Keywords: Infrared spectroscopy (IR), Potassium thiocyanate (KSCN), Potassium bromide disc, Pyridoxine hydrochloride.

INTRODUCTION:
Pyridoxine is one form of vitamin B₆; its hydrochloride salt pyridoxine hydrochloride is used as vitamin B₆ dietary supplement. The biological functions of the naturally occurring forms of vitamin B₆ – pyridoxol, pyridoxal, and pyridoxamine depend on the metabolism of each to a common coenzyme form, pyridoxal phosphate. Vitamin B₆ is widespread in foods of both plant and animal origin, occurring in greatest concentrations in meats, whole-grain products, vegetables and nuts. Vitamin B₆ is the generic descriptor for all 3-hydroxy-2-methylpyridine derivatives exhibiting qualitatively the biological activity of Pyridoxine [3-hydroxy-4, 5- bis (hydroxymethyl)-2-methylpyridine-3-ol]. The metabolically active form of vitamin B₆ is pyridoxal phosphate, which functions as a coenzyme for reactions involving amino acids. Deficiencies of vitamin B₆ are manifested as dermatologic, circulatory, and neurologic changes.

Several analytical methods [1-7] have been reported for pyridoxine hydrochloride with other pharmaceutical agents. Literature review indicates that the proposed methods have not been reported for estimation of pyridoxine hydrochloride in bulk and in formulations.

Figure 1: Pyridoxine hydrochloride

MATERIALS AND METHOD:
All the chemicals used throughout the experiment were of highest purity of IR grade. Potassium bromide (KBr), Potassium thiocyanate (Internal Standard), Pyridoxine hydrochloride (Bulk Material) was obtained as a gift sample from a reputed company. Pyridoxine hydrochloride tablet (Dosage form) was purchased from local market.

Instrumentation:
All spectral measurements were made on ABB-IR instrument (Model no .MB 3000) with KBr press (model no.M15).

Method:
Calibration of the standard:
Potassium thiocyanate was used as an internal standard which was preground, dried, and then reground with dry KBr to make a concentration of about 0.2% by weight of potassium thiocyanate. The final mixture was stored over phosphorus pentoxide. Five different concentration of standard and KBr-KSCN were prepared by mixing...
known weights of the standard substance with a known weight of the KBr-KSCN mixture and then ground by using agate mortar & pestle under IR lamp. A standard calibration curve was constructed using peak area and concentration as presented in Figure.2&3. The values obtained by proposed methods are presented in Table 1. The discs were prepared using KBr press and the infrared spectrum was recorded in absorbance mode; the calibration curve was obtained by plotting the area of the IR absorption at 2060 cm$^{-1}$ (prominent band) against the concentration of the substance.

ASSAY
Weighed 10 tablets of pyridoxinehydrochloride, average weight was determined and ground to fine powder; weighed an aliquot quantity of tablet powder transferred to 100ml volumetric flask. Added 50 ml of acetone and the above mixture was shaken for 30 minutes and filtered the solution through whatmann filter paper. The filtrate was collected and poured in to petridish and evaporated. The residue contains the extracted pyridoxine hydrochloride. Aliquot quantity of pyridoxine hydrochloride residue is weighed and mixed with the KBr/KCNS mixture and then homogenized by using agate mortar & pestle. The final mixture was transferred to KBr press to form a disc and the infrared spectrum was recorded in absorbance mode. The sample absorbance was interpolated on the respective linearity chart of the Pyridoxine hydrochloride and the concentration was determined. The amount of drug present in each tablet was calculated and the assay results are presented in the Table .2.

Recovery studies:
The recovery studies were carried out on spiked samples by adding predetermined amount of standard drugs to the respective sample. The recovery study was performed at two levels (25% & 50%) to confirm the precision and accuracy of the above said method. The percentage recovery was calculated and the recovery results are presented in Table.2.

RESULTS AND DISCUSSION:
Pyridoxine hydrochloride was found to obey beer’s law in the concentration range 7.5mg-15mg. Pyridoxine hydrochloride showed good linearity as indicated by correlation coefficient value (0.9987). The results of the analysis showed that the amount of drug present in the formulation was in good agreement with the label claim of the formulation. The accuracy of the proposed method was determined by recovery study. The recovery studies were carried out on spiked samples at two levels 25%, and 50%. The percentage recovered was found to be around 99.0% and is given in Table 2. The optical characteristics such as Beer’s law limits and Sandell’s sensitivity, the regression characteristics like slope (m), intercept (c), correlation co-efficient (r) were calculated and the results were summarized in the Table-3.

CONCLUSION:
The percentage recovery of the method is around 99 – 100.5 %. The correlation coefficient for the method was found to be 0.9987 and the recovery studies indicates that there is no interference of other ingredients present in the formulation. Thus the method is simple, precise, accurate, less time consuming and could be used for routine analysis.

ACKNOWLEDGEMENT:
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REFERENCES:
Figure 2: Calibration curve for pyridoxine hydrochloride

Figure 3 Calibration graph of pyridoxine hydrochloride standard

Table 1: Estimation of pyridoxine hydrochloride in tablet

<table>
<thead>
<tr>
<th>Pyridoxine hydrochloride</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Label claim (mg)</td>
<td>40</td>
</tr>
<tr>
<td>Amount present in tablet (mg)</td>
<td>39.82</td>
</tr>
<tr>
<td>Amount found (%) ±SD *</td>
<td>99.56 ± 0.057</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.0579</td>
</tr>
</tbody>
</table>

* Each value is the mean of 3 determinations.
Table 2: Recovery study for pyridoxine hydrochloride

<table>
<thead>
<tr>
<th>Method</th>
<th>Label claim</th>
<th>Amount of drug added (mg)*</th>
<th>Amount of drug recovered (mg)*</th>
<th>% Recovery</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBr disc method using internal standard</td>
<td>40mg</td>
<td>2.5</td>
<td>2.49</td>
<td>99.60%</td>
<td>0.04</td>
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<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>5.02</td>
<td>100.40%</td>
<td>0.06</td>
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</tbody>
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Table 3: Optical parameters for the proposal method

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Values (IR Method)</th>
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<tbody>
<tr>
<td>1</td>
<td>Beer’s law</td>
<td>7.5 – 15 mg</td>
</tr>
<tr>
<td>2</td>
<td>Regression equation (y = mx+c)</td>
<td>y = 8.5108x - 41.579</td>
</tr>
<tr>
<td>3</td>
<td>Slope (m)</td>
<td>8.5108</td>
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<tr>
<td>4</td>
<td>Intercept ©</td>
<td>-41.579</td>
</tr>
<tr>
<td>5</td>
<td>Correlation coefficient</td>
<td>0.9987</td>
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