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VALIDATED SPECTOPHOTOMETRIC METHODS FOR THE ASSAY OF CINITAPRIDE HYDROGEN TARTRATE IN PHARMACEUTICALS

Three simple, selective and rapid spectrophotometric methods have been established for the determination of cinitapride hydrogen tartrate (CHT) in pharmaceutical tablets. The proposed methods are based on the diazotization of CHT with sodium nitrite and hydrochloric acid, followed by coupling with resorcinol, 1-benzoylacetone and 8-hydroxyquinoline in alkaline medium for methods A, B and C, respectively. The formed azo dyes are measured at 442, 465 and 552 nm for methods A, B and C, respectively. The parameters that affect the reaction were carefully optimized. Under optimum conditions, Beer’s law is obeyed over the ranges 2.0-32.0, 1.0-24.0 and 1.0-20.0 µg mL\(^{-1}\) for methods A, B and C, respectively. The calculated molar absorptivity values were 1.2853 \(\times\) 10\(^4\), 1.9624 \(\times\) 10\(^4\) and 3.92 \(\times\) 10\(^4\) L mol\(^{-1}\) cm\(^{-1}\) for methods A, B and C, respectively. The results of the proposed procedures were validated statistically according to ICH guidelines. The proposed methods were successfully applied to the determination of CHT in Cintapro tablets without interference from common excipients encountered.

Keywords: cinitapride hydrogen tartrate, spectrophotometry, diazo coupling reaction, tablets.

Cinitapride [1,2] is chemically designated as 4-amino-N-[1-(3-cyclohexen-1-ylmethyl)-4-piperidinyl]-2-ethoxy-5-itrobenzamide hydrogen L-(+)-tartrate (Figure 1). It acts as an agonist of the 5-HT\(_1\) and 5-HT\(_4\) receptors and as an antagonist of the 5-HT\(_2\) receptors. CHT is indicated for the treatment of gastrointestinal disorders associated with motility disturbances such as gastroesophageal reflux disease, non-ulcer dyspepsia and delayed gastric emptying. The therapeutic importance of CHT requires the development of a sensitive and rapid method for industrial quality control and clinical monitoring. The critical literature survey revealed that very few methods have been reported for estimation of CHT in biological fluids and pharmaceutical formulations, which include LC-MS/MS [3] HPLC [4] polarographic [5] and spectrophotometric methods [6, 7]. Some of these methods suffer from interference from the tablet matrix, whereas others are time consuming or require expensive equipment and are consequently not suitable for routine analysis. To best of our knowledge, there are no reports in the literature about resorcinol, 1-benzoylacetone and 8-hydroxyquinoline as diazo coupling reagents for determination of CHT. In view of this, we have developed simple and sensitive spectrophotometric methods for the assay of CHT in pharmaceutical formulations. The methods are based on the aromatic amino group present in CHT, which was diazotized with nitrous acid at temperature 0-5 °C, and the diazonium salt thus formed was coupled with resorcinol (method A), 1-benzoylacetone (method B) and 8-hydroxyquinoline (method C) in alkaline medium. The colored chromogens formed were measured at 442, 465 and 552 nm for method A, B and C, respectively. The scientific novelty of the present work is that the reagents used in the proposed methods are easily available and the reactions involved with these reagents are simple, rapid and sensitive in their range of determination. The results obtained by the pro-
posed methods were compared favorably with those of the reference method.

![Figure 1. Structure of cinitapride hydrogen tartrate.](image)

**EXPERIMENTAL**

**Apparatus**

All absorption spectra were recorded using double beam UV-Visible spectrophotometer (Shimadzu 1601, Japan) equipped with 1 cm matched quartz cells by using a personal computer loaded with the UV-PC 3.9 software package. An electronic micro balance (Sartorius MC 5, Germany) was used for weighing the solid materials.

**Materials and reagents**

All solvents and reagents used were of analytical grade. Double-distilled water was used throughout the investigation. 0.2% (w/v) sodium nitrite (S.D. Fine Chem., Mumbai, India), 2% (w/v) sulfamic acid (BDH, Mumbai, India) and 20% (w/v) sodium hydroxide (S.D. Fine Chem., Mumbai, India) were freshly prepared in double distilled water. 1 M hydrochloric acid (S.D. Fine Chem., Mumbai, India) was prepared with double distilled water. 0.5% (v/v) resorcinol (S.D. Fine Chem., Mumbai, India) was freshly prepared in double distilled water. 1-benzoylacetone (Sisco research chemicals, Mumbai, India) and 0.5% (w/v) 8-hydroxyquinoline (E-Merck, Mumbai, India) were prepared in methanol (Sisco research chemicals Ltd, Mumbai, India). A standard CHT gift sample was obtained from Symed Labs, Hyderabad, India. Cintaproe tablets were purchased from the local market. Standard stock solution of 200 µg mL\(^{-1}\) CHT was prepared by dissolving accurately weighted 10 mg of pure drug in double distilled water and diluted to 50 mL with the same solvent. The solution was further diluted with double distilled water to get 100 µg mL\(^{-1}\).

**Assay procedure**

**Method A**

Aliquots of the standard CHT (100 µg mL\(^{-1}\)) solution ranging from 0.2-3.2 mL were transferred into a series of 10 mL volumetric flasks. To each flask, 1.0 mL of ice-cold 0.2% sodium nitrite and 1.0 mL of 1 M hydrochloric acid were added. The solution in each flask was well shaken and allowed to stand for 5 min to allow complete diazotization at 0-5 °C. After that, 0.5 mL of 2.0% sulphamic acid was added to each flask. Then volumes of 1.0 mL of 0.5% resorcinol (RCL) and 1.0 mL of 20% sodium hydroxide solutions were added. The contents were diluted to the mark with distilled water and mixed well. The absorbance of the orange colored azo dye was measured at 442 nm against the reagent blank.

**Method B**

Different aliquots of stock reference CHT solution (100 µg mL\(^{-1}\)) ranging from 0.1-2.4 mL were transferred into a series of 10 mL volumetric flasks. To each flask, 1.0 mL of ice-cold 0.2% sodium nitrite and 1.0 mL of 1 M hydrochloric acid were added. The solution in each flask was well shaken and allowed to stand for 5 min to allow complete diazotization at 0-5 °C. To each flask, 0.5 mL of 2.0% sulphamic acid was added. Then volumes of 1.0 mL of 0.5% 1-benzoylacetone (BAC) and 1.0 mL of 20% sodium hydroxide solutions were added. The contents were diluted to the mark with methanol and mixed well. The absorbance of yellow colored azo dye was measured at 465 nm against the reagent blank.

**Method C**

Varying aliquots of stock reference CHT solution (100 µg mL\(^{-1}\)) in the range from 0.1-2.0 mL were transferred into a series of 10 mL volumetric flasks. To each flask, 1.0 mL of ice-cold 0.2% sodium nitrite and 1.0 mL of 1 M hydrochloric acid were added. The solution in each flask was well shaken and allowed to stand for 5 min to allow complete diazotization at 0-5 °C. After that, 0.5 mL of 2.0% sulphamic acid was added to each flask. Then volumes of 1.0 mL of 0.5% 8-hydroxyquinoline (8-HQ) and 1.0 mL of 20% sodium hydroxide solutions were added. The contents were diluted to the mark with methanol and mixed well. The absorbance of the wine red colored azo dye was measured at 552 nm against the reagent blank. In all three methods, the calibration graph was constructed by plotting the absorbance versus concentration of the CHT in µg mL\(^{-1}\).
**Assay procedure for formulations**

Twenty tablets containing CHT were grounded into a fine powder. An amount of the powder equivalent to 10 mg of CHT was weighed into a 50 mL volumetric flask. Then, 35 mL of double distilled water was added and shaken thoroughly for about 30 min to extract the complete drug. The volume was diluted to the mark with double distilled water and filtered using Whatmann No. 41 filter paper. The filtrate was diluted to get 100 µg mL⁻¹ with double distilled water. A suitable aliquot was then subjected to analysis.

**RESULTS AND DISCUSSION**

Diazotization employing coupling reactions is the most generally used method of assay for drugs containing a free aromatic amino group or yielding an aromatic amino group upon hydrolysis or reduction [8, 9]. The coupling procedures vary somewhat, depending on the reactivity of the compounds involved. Some compounds will couple in acidic solution; others will couple in a very alkaline solution [10]. The more alkaline the solution, the faster the coupling reaction of diazonium compounds. The proposed methods are the diazo coupling reaction of the CHT with RCL, BAC and 8-HQ in alkaline medium. Two steps are involved in the reaction that produces the colored dye. In the first step, the studied CHT is treated with nitrite solution in acidic medium at 0-5 °C, which undergoes diazotization to give the diazonium chloride ion. In the second step, the diazonium cation reacts with the coupling reagents such as RCL, BAC and 8-HQ by electrophilic substitution at ortho-position to hydroxyl groups on the benzene ring in RCL, active methylene group in BAC and para-position to the hydroxyl group on the benzene ring in 8-HQ in the presence of sodium hydroxide medium for methods A, B and C, respectively. The resulting colored azo dyes show maximum absorption at 442, 465 and 552 nm for methods A, B and C, respectively (Figure 2). The possible reaction pathways for the proposed methods are depicted in Scheme 1.

In order to optimize the proposed spectrophotometric methods, the effect of experimental variables was studied. Studies were performed by altering each variable in turn while keeping the others constant.

**Effect of acid on diazotization**

The diazotization reaction occurred in acidic conditions. Hydrochloric acid as reaction medium was found to give more satisfactory results than sulphuric acid. Since the CHT does not contain many electron-withdrawing groups, a stronger acid like sulphuric acid was not very effective in the present work for diazotization. Good results have been obtained with hydrochloric acid. Then, the concentration of hydrochloric acid solution was optimized for maximum diazotization. It was found that the absorbance reached its maximum when the amount of hydrochloric acid was 0.8 mL. Evidently, the absorbance kept a constant value when the amount of hydrochloric was higher than 0.8 mL (Figure 3). This indicates that the amount of the formed soluble azo dyes reached its maximum. Thus, 1 mL of 1 M hydrochloric acid was used in all methods.
Scheme 1. The suggested reaction pathway of proposed methods.
Effect of sodium nitrite concentration

For the maximum formation of azo dye, the influence of sodium nitrite concentration was investigated (Figure 4). The results demonstrate that the increase in absorbance tends to have a constant value for the sodium nitrite solution concentration equal to or higher than 0.6 mL, so 1.0 mL volume was selected in order to ensure an excess of reagent in the flask to guarantee the reaction completion. The interference of excess of nitrous acid was removed by adding 0.5 mL of 2% sulfamic acid before the coupling reaction.

The effect of reaction time on the diazotization process was studied. The minimum time required for complete diazotization process was found to be less than 5 min, and the absorbance remained constant after 5 min (Figure 5). Thus, in all further experiments, the reaction time for the diazotization process was fixed at 5 min.

Effect of coupling reagents concentration

The influence of concentration of RCL, BAC and 8-HQ was investigated in the proposed methods by measuring the absorbance at specified wavelengths in the standard procedure for solutions containing a fixed concentration of CHT and varying amounts of coupling reagents. These results indicate that the maximum absorbance was obtained with 1 mL of reagent in all methods for quantitative determination of the investigated drug. The higher concentration of reagents does not affect the sensitivity of methods in all methods (Figure 6). This clearly indicates that all CHT had reacted with coupling reagents and the amount of formed azo products reached its maximum.

Figure 3. Effect of hydrochloric acid on the reaction product of 20 µg mL⁻¹ CHT in method A and 10 µg mL⁻¹ CHT in methods B and C.

Figure 4. Influence of sodium nitrite on the reaction product of 20 µg mL⁻¹ CHT in method A and 10 µg mL⁻¹ CHT in methods B and C.
Effect of sodium hydroxide

The stability and formation of azo dye depends upon the nature of reaction medium. A study was conducted to determine the most effective alkali and the optimum alkali concentration to be used. Sodium hydroxide was found to be more suitable for coupling reaction compared to sodium carbonate or aqueous ammonia because the formed dye was stable and more intense in sodium hydroxide medium. The results of the study revealed that 1 mL volume of 20% (w/v) sodium hydroxide was optimal for all methods to produce maximum absorbance.

Effect of solvent

The choice of diluent for the reaction mixture was also studied. Water, methanol, ethanol, acetone, and isopropanol were tested as diluting solvents. Water was the best diluent for method A. But in the case of methods B and C, the highest absorbance and reproducible values were obtained when methanol was used as a diluting solvent.

Effects of reaction time

The colored dyes developed rapidly after the addition of reagents and attained maximum intensity immediately at room temperature (27±1 °C). A stability study of the chromogens was carried out by measuring the absorbance values at time intervals of 10 min. It was found that the formed azo dyes were stable for two hours in methods A and B but in the case of method C stability of chromogen was one more hour.
Validation of the proposed methods

Calibration curves, linearity and sensitivity

Under optimum experimental conditions, a linear relation was obtained between absorbance and concentration of CHT in the range 2.0-32.0 µg mL\(^{-1}\) in method A, 1.0-24.0 µg mL\(^{-1}\) in method B and 1.0-20.0 µg mL\(^{-1}\) in method C. The regression analysis of the plot using the method of least squares was made to evaluate the intercept (a), slope (b), regression coefficient (r\(^2\)) and standard deviations of slope and intercept. In all cases, Beer’s law plots were linear with good correlation coefficients, as shown in Table 1. The moderately high sensitivity of the methods was indicated by the fairly high value of molar absorptivity and low values of Sandell’s sensitivity. The sensitivity of the developed methods A, B and C was checked in terms of limits of detection and quantitation values. The detection limit or LOD is the lowest amount of analyte in a sample that can be detected not quantitated under the experimental conditions. The LOD and LOQ [11] were determined using the formula: 

\[
\text{LOD or LOQ} = kSD_a/b, \quad \text{where } k = 3.3 \text{ for LOD and } 10 \text{ for LOQ, } S_Da \text{ is the standard deviation of the intercept, and } b \text{ is the slope of regression line.}
\]

Precision of proposed methods

Intra-day precision (repeatability) of the proposed method was evaluated by carrying out the determination of six replicates of standard CHT at three different concentration levels within Beer’s limit on the same day. Inter-day precision (intermediate precision) also was evaluated by assaying the pure drug solution in six replicates at the same concentration levels on five consecutive days. Table 2 summarizes the intra-day and inter-day precision of the proposed methods. The results reveal that precision of proposed were fairly high as indicated by the low values of %RSD and %RE.

Accuracy and recovery

The accuracy and validity of the proposed methods for the determination of the drug in commercial dosage form were studied by recovery experiments via standard addition technique. In this study, pre-analyzed tablet powder was spiked with pure CHT at two different levels and the total amount of drug was determined by the proposed methods. Each determination was repeated three times. The recoveries of the pure drug added to the tablet powder are shown in Table 3. The results reveal that the proposed methods are not liable to interference by tablet fillers, excipients and additives usually formulated with pharmaceutical preparations. The recovery values indicate that the accuracy of method is not affected by co-formulated substances.

Application of the methods to the analysis of tablets

The applicability of the developed methods was evaluated by analyzing commercial samples containing CHT. The results of the assay are given in Table 4 and compared with the reference method [6] for the parallel study of same-batch tablets and the results were statistically evaluated by applying Student’s t-test and F-test. As can be seen from Table 4, calculated t-value and F-value at the 95% confidence level did not exceed the tabulated values of 2.228 and 5.05, respectively. It is evident from the results that the proposed methods are applicable to the analysis of CHT in its tablets forms with comparable analytical performance.

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Table 1. Analytical and regression parameters of proposed methods: Y is absorbance and C is the concentration in µg mL\(^{-1}\).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{\text{max}}) / nm</td>
<td>442</td>
<td>465</td>
<td>552</td>
</tr>
<tr>
<td>Color</td>
<td>Orange</td>
<td>Yellow</td>
<td>Wine red</td>
</tr>
<tr>
<td>Beer’s law limit, µg mL(^{-1})</td>
<td>2.0-32.0</td>
<td>1.0-24.0</td>
<td>1.0-20.0</td>
</tr>
<tr>
<td>Molar absorptivity, L mol(^{-1}) cm(^{-1})</td>
<td>1.2853×10(^4)</td>
<td>1.9624×10(^4)</td>
<td>3.92×10(^4)</td>
</tr>
<tr>
<td>Sandell’s sensitivity, µg cm(^{-2})</td>
<td>0.043</td>
<td>0.028</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Regression equation \((Y = a + bC)\):

| Slope (b)                                      | 0.0233        | 0.0355        | 0.0709        |
| Intercept (a)                                  | 0.0276        | 0.0163        | 0.0096        |
| Correlation coefficient (r\(^2\))              | 0.999         | 0.9994        | 0.9997        |
| Standard deviation of slope (S\(_{b}\))        | 2.848×10\(^{-4}\) | 3.56×10\(^{-4}\) | 5.7×10\(^{-4}\) |
| Standard deviation of intercept (S\(_{a}\))    | 5.427×10\(^{-3}\) | 4.8×10\(^{-3}\) | 6.38×10\(^{-3}\) |
| Detection limit, LOD, µg mL\(^{-1}\)           | 0.76          | 0.45          | 0.3           |
| Quantification limit, LOQ, µg mL\(^{-1}\)      | 2.33          | 1.36          | 0.9           |

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CONCLUSIONS

This paper presents that RCL, BAC and HQ are new diazo coupling reagents for the spectrophotometric determination of CHT. The proposed methods are selective as the free aromatic amino group preferentially interacts with nitrous acid to form the diazo-tized product which is later coupled with RCL, BAC or 8-HQ to form very stable dye stuff. The proposed method concerned with 8-HQ was superior as compared with that of the other coupling reagents and the performance order of the proposed methods is 8-HQ > BAC > RCL according to higher molar absorptivity and lower detection limits. The proposed methods provide adequate sensitivity, selectivity and free from interferences. From a practical point of view, the developed methods need minimum sample treatment, which allowed us to achieve a high analytical productivity. These advantages make the proposed methods very suitable for routine analysis of CHT in quality control laboratories.

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REFERENCES

VALIDIRANE SPEKTROFOTOMETRIJSKE METODE ZA ANALIZU CINITAPRID HIDROGEN TARTARATA U FARMACEUTSKIM PREPARATIMA

Razvijene su tri jednostavne, selektivne i brze spektrofotometrijske metode za određivanje cinitaprid hidrogen tartarata (CHT) u farmaceutskim tabletama. Predložene metode se zasnivaju na diazotovanju CHT sa natrijum nitritom i hlorovodničkom kiselinom i kuplovanju sa resorcinolom (metoda A), 1-benzoilacetonom (metoda B) i 8-hidrokoksiihlinolinom (metoda C) u alkalnoj sredini. Nastale azo boje su merene na 442, 465 i 552 nm za metode A, B i C, respektivno. Optimizovani su parametric koji utiču na reakciju. Pod optimalnim uslovima Beer-ov zakon važi u opsezima 2,0-32,0, 1,0-24,0 i 1,0–20,0 µg/ml za metode A, B i C, redom. Rezultati razvijenih procedura su statistički validirani u skladu sa smernicama ICH. Ove metode su uspešno primenjene za određivanje CHT u tabletama Cintapro bez interferencije sa uobičajenim puniocima.

Ključne reči: cinitaprid hidrogen tartarata, spektrofotometrija, diazo kuplovanje, tablete.