Precipitation and complex formation reactions based titrimetric and spectrophotometric methods for the determination of diphenhydramine hydrochloride

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Two simple, accurate and selective methods for the determination of diphenhydramine in pharmaceutical grade sample and in dosage forms are described. The titrimetric method is based on the precipitation of the chloride content of the drug as AgCl with a known excess of silver nitrate and back titration of the residual silver nitrate with thiocyanate using iron(III) as indicator. The reaction stoichiometry and the range of determination have been evaluated. The spectrophotometric method involves the displacement of thiocyanate of mercuric thiocyanate from the ionized chloride and the subsequent reaction of the liberated thiocyanate with iron(III) to form the familiar red coloured complex, Fe(SCN)$_2^+$ which is measured spectrophotometrically at 460 nm. The Beer’s law is obeyed over the range 5-45 μg mL$^{-1}$ of diphenhydramine HCl, the molar absorptivity and Sandell sensitivity being 3.45 × 10$^4$ L mol$^{-1}$ cm$^{-1}$ and 84.5 ng cm$^{-2}$, respectively at 460 nm. The reaction variables have been optimized. The methods have been found to be very simple, cost-effective and reliable for three dosage forms containing diphenhydramine hydrochloride.

Diphenhydramine hydrochloride (DPH), 2-(diphenylmethoxy) N, N-dimethyl ethylamine hydrochloride, is a conventional antihistamine of the H$_1$ type (receptor antagonist), which can block most of the reactions of histamine in the body. Like many other antihistamines, it has pronounced sedative properties. It also has antiemetic, anticholinergic and local anaesthetic properties.

Diphenhydramine is found in many pharmaceutical preparations, it is usually given orally but it has also been given in severe allergies by deep intramuscular or slow intravenous injection. It is used in cough mixtures, for the control of symptoms of parkinson’s disease, for prevention and treatment of nausea and vomiting. It has also been given with methaqualone as a hypnotic and a 2% water miscible cream has been used for allergic dermatoses and burns.

The therapeutic importance of DPH has prompted the development of many methods for its determination in body fluids and pharmaceutical preparations. The alkalimetric titration method$^2$ suffers from interference from ephedrine. Non-aqueous titrimetric methods$^3$-$^7$ which are the methods of general applicability require scrupulously anhydrous medium. DPH has been determined by precipitating it with potassium iodobismuthate ion, filtering of the precipitate and titrating the unreacted bismuth in the filtrate with EDTA$^8$, the steps being complex and time-consuming. Titrations with sodium tetraphenylborate$^9$ requires carefully controlled pH.

Majority of the spectrophotometric methods suggested for DPH involve extractions of the chromogen into the organic solvent before measuring the absorbance. They are based on reactions such as ion-pair formation$^{10}$-$^{12}$, charge-transfer complex formation$^{13}$-$^{14}$, addition compound formation$^{15}$-$^{16}$ and ternary complex formation$^{17}$-$^{19}$. They involve tedious extraction step and suffer from such disadvantages as low sensitivity$^{17}$-$^{19}$, careful pH control$^{10}$-$^{12}$, insufficient accuracy and precision, and/or longer extraction time. Methods based on reduction$^{20}$ reaction and formation of mixed aggregates with surfactants$^{21}$ have also been reported. Recently a flow injection method based ion-pair formation with bromocresol green has been described by Prapatson et al.$^{22}$.

In the present investigations, precipitation reaction and displacement followed by complex formation reactions involving the ionized chloride of DPH have been utilised for micro titrimetric and sensitive spectrophotometric determination of DPH. The chloride content of DPH is precipitated with a known excess of...
silver nitrate and the unreacted precipitant being back titrated with potassium thiocyanate using iron(III) as indicator. The spectrophotometric procedure involves the reaction of chloride with mercuric thiocyanate to form soluble chloro mercurate(II) complex ion with the liberation of thiocyanate ions which then react with iron(III) to form red coloured complex which is measured at 460 nm. The absorbance is proportional to the thiocyanate ion concentration which in turn is proportional to the chloride and DPH concentration.

**Experimental Procedure**

The absorbance measurements were made on an Elico model SL-171 digital spectrophotometer provided with 1-cm matched glass cells. All chemicals used were of analytical reagent-grade. Double distilled water, second time distilled over alkaline potassium permanganate was used throughout. Silver nitrate (AR grade, Indian Drugs and Pharmaceuticals Ltd., Hyderabad, Assay: 99.9%) solution (−0.04 M) was standardized by using pure sample of NaCl\(^{2-}\) (Merck, Assay: 99%). Potassium thiocyanate (AR grade, S.D. fine chem Ltd., Mumbai, Assay: 99%) (−0.02 M) was standardized by Volhard method\(^{24}\).

Iron(III) (AR grade, S.D. Fine Chem Ltd., Mumbai, Assay: 99%) indicator was prepared by dissolving −10 g of iron(III) alum in 1:1 nitric acid (AR grade, S.D. Fine Chem Ltd., Mumbai, Assay: 69-71%) (100 mL) and boiling the solution to expel the oxides of nitrogen. Iron(III) nitrate (AR grade, BDH sample, Mumbai, Assay: 98.5%) reagent was prepared by dissolving 15.1 g of sample in 45 mL of 72% perchloric acid (AR grade, S.D. Fine Chem Ltd., Mumbai, Assay: 70-72%) and diluting to 100 mL with water. A saturated solution of mercuric thiocyanate (GR grade, Loba Chemie, Assay: 99%) in methanol (AR grade, S.D. fine chem Ltd., Mumbai, Assay: 99.5%) and 1:1 nitric acid were prepared in the usual way. Chloride-free nitrobenzene (AR grade, S.D. fine chem Ltd., Mumbai, Assay: 99%) was used for titrimetric work.

Pharmaceutical grade DPH was gifted by Parke-Davis, India Ltd., and was used as such. A stock standard solution containing 4 mg mL\(^{-1}\) DPH was prepared for titrimetry. This solution was diluted stepwise to provide a working solution of 100 µg mL\(^{-1}\).

**Titrimetric method**

To a 10 mL aliquot of solution containing 6-40 mg of DPH, 2 mL of 1:1 nitric acid were added followed by 5 mL of 0.04 M silver nitrate solution by means of a pipette. The contents were shaken for a minute, 2 mL of nitrobenzene were added and shaken vigorously until the silver chloride was coagulated. Then, iron(III) indicator (0.5 mL) was added, and the excess of silver nitrate titrated with 0.02 M potassium thiocyanate to a permanent red colour end-point. A blank was run in the same way with 10 mL of chloride free water.

**Spectrophotometric method**

Into a series of 10 mL standard flasks were transferred 0.5, 1.0, 1.5 .... 4.5 mL of 100 µg mL\(^{-1}\) of DPH solution by means of a micro burette. A 1 mL volume of iron(III) reagent and 2 mL of mercuric thiocyanate reagent solution were added and diluted to volume with water. The absorbance of the solution was measured at 460 nm against the reagent blank after 5 min. The increase in absorbance was plotted against the drug concentration.

**Formulations**

Twenty tablets were finely powdered. An amount of the powder equivalent to 200 mg of DPH was extracted with three 30 mL portions of water and filtered into a 100 mL standard flask. Washed the filter and diluted to mark with water and subjected to analysis using the general procedures. In respect of benadryl syrup, 10 mL containing 25 mg of DPH was used as such for titrimetric analysis. For spectrophotometric work the syrup was sequentially diluted to obtain 100 µg mL\(^{-1}\) of DPH and subjected to analysis.

**Results and Discussion**

**Optimisation of experimental variables**

The stoichiometric study revealed that the reaction between DPH and AgNO\(_3\) proceeds in the molar ratio of 1:1 which is in conformity with the following reaction scheme:

\[
\text{DP. HCl} \rightleftharpoons \text{DPH}^+ + \text{Cl}^-
\]

\[
\text{Cl}^- + \text{Ag}^+ \rightleftharpoons \text{AgCl} (S)
\]

A 0.5 mL of iron(III) indicator and 2 mL 1:1 nitric acid were found to give satisfactory results. Since AgCl is more soluble than AgSCN leading to low values for chloride content and drug recovery, 2 mL of nitrobenzene were added to eliminate this source of error\(^{25}\).

Fig. 1 shows the absorption spectra of the complex and the reagent blank against the blank and water.
respectively. The absorption is maximum at 460 nm which is in agreement with the earlier observations in perchloric acid medium. Since the method is essentially the measurement of iron(III) thiocyanate complex, variables that influence the sensitivity and stability of the colour were optimised. Of the several acids tested as the reaction medium, perchloric acid was preferred because of high sensitivity and stability of the colour and lower blank absorbance.

Different sources of iron(III) may be used, such as iron(III) nitrate, iron(III) alum and iron perchlorate. Iron(III) nitrate was used since it gave better sensitivity and because of high chloride content in iron perchlorate. A 1 mL volume of the reagent solution was found optimum in a total volume of 10 mL, and 2 mL of mercuric thiocyanate were found to yield optimum absorbance. The colour formation was complete in 5 min and stable for a further period of 2 h. Solvents such as water, ethanol and methanol have been used to prepare mercuric thiocyanate solution and in the present investigation methanolic solution was found to give a higher sensitivity.

**Analytical parameters**

Titrimetry was found to be applicable in the range 6-40 mg, outside which the results were deviant. The relationship between the titration end-point and the drug amount was examined by calculating the correlation coefficient value, r, via linear least squares treatment and was found to be 0.9750 indicating that the reaction between DPH and AgNO₃ occurs stoichiometrically in the ratio of 1:1 in 6-40 mg range.

Beer's law is obeyed in the range 5-45 μg mL⁻¹ of DPH. The apparent molar absorptivity and Sandell sensitivity were 3.45 × 10³ L mol⁻¹ cm⁻¹ and 84.5 ng cm⁻², respectively. The linear plot gave the regression equation.

\[ A = -0.0098 + 0.0121 C \]

Where, A, is the absorbance and C concentration in μg mL⁻¹, and with a correlation coefficient of 0.9620 (n=10). The limit of detection was 0.74 μg mL⁻¹ and the limit of quantification 2.47 μg mL⁻¹.

**Accuracy and precision**

To find out the accuracy and precision of the methods, seven replicate determinations in the same solution containing three different levels of DPH were performed. The percentage recovery, the RSD and range of error at 95% confidence level presented in Table 1 indicate high accuracy and precision of the methods.

<table>
<thead>
<tr>
<th>Drug taken, mg</th>
<th>Drug found*, mg</th>
<th>Error, %</th>
<th>RSD, %</th>
<th>Range of error, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10.19</td>
<td>1.90</td>
<td>1.31</td>
<td>1.30</td>
</tr>
<tr>
<td>15</td>
<td>15.09</td>
<td>0.60</td>
<td>0.88</td>
<td>0.87</td>
</tr>
<tr>
<td>25</td>
<td>25.25</td>
<td>1.00</td>
<td>0.74</td>
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<td>30</td>
<td>29.75</td>
<td>0.83</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*Values obtained for seven determinations.

<table>
<thead>
<tr>
<th>Drug taken, μg</th>
<th>Drug found*, μg</th>
<th>Error, %</th>
<th>RSD, %</th>
<th>Range of error, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>101.67</td>
<td>1.67</td>
<td>1.85</td>
<td>1.84</td>
</tr>
<tr>
<td>250</td>
<td>248.39</td>
<td>0.64</td>
<td>0.78</td>
<td>0.78</td>
</tr>
<tr>
<td>300</td>
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<td>1.30</td>
<td>1.09</td>
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<td>401.65</td>
<td>0.41</td>
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<td>0.87</td>
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</table>
The time required for analysis in either method is less than 15 min. There is no need for preliminary separation of acetone which interferes in the spectrophotometric procedure are not present in either the reagents employed or in the dosage forms used. Hence the methods are free from error due to them.

**Conclusions**

The present methods have been successfully applied for the determination of DPH in capsules and syrup. The results summarized in Table 2 suggest that the methods can be economically used for the accurate analysis of formulations containing DPH. The results also indicate that excipients like starch, talc, lactose, sodium alginate and magnesium stearate do not interfere in the determination.

**Acknowledgements**

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**References**