Indirect spectrophotometric determination of diltiazem hydrochloride in pure form and pharmaceutical formulations

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Abstract: Three simple, accurate, and sensitive spectrophotometric methods (A, B and C) have been described for the indirect assay of diltiazem hydrochloride (DIL.HCl), either in pure form or in pharmaceutical formulations. The first method (A) is based on the oxidation of DIL.HCl by N-bromosuccinimide (NBS) and determination of un consumed NBS by measuring the decrease in absorbance of amaranth dye (AM) at a suitable $\lambda_{\text{max}} = 521$ nm. Other methods (B) and (C) involve the addition of excess ceric ammonium sulfate (CAS) and subsequent determination of the unconsumed oxidant by a decrease in the red color of chromotrope 2R (C2R) at a suitable $\lambda_{\text{max}} = 528$ nm or a decrease in the orange-pink color of rhodamine 6G (Rh6G) at $\lambda_{\text{max}} = 525$ nm, respectively. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 3.0 – 9.0, 3.5 – 7.0 and 3.5 – 6.3 $\mu$g ml$^{-1}$ for methods A, B and C, respectively. The apparent molar absorptivity, Sandell’s sensitivity, detection and quantification limits were calculated. The proposed methods have been applied successfully for the analysis of the drug in its pure form and its dosage form. No interference was observed from a common pharmaceutical adjuvant. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

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Keywords: spectrophotometry; Diltiazem HCl; oxidation reaction; N-bromosuccinimide; ceric ammonium sulfate; pharmaceutical formulations

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1 Introduction

Diltiazem hydrochloride is an important coronary vasodilator drug inhibiting calcium ion entry into smooth muscle cells by blockade of slow calcium channels, used in the treatment of angina pectoris, systemic hypertension, and supraventricular arrhythmias. Diltiazem has been shown to have a moderate effect on platelets, decreasing their (refers to treated of both human and veterinary) aggregator’s response to platelet activating factor [1]. The drug has also, been reported to have effects on plasma rennin activity [2]. The compound is cis-d-3-Acetyloxy-5-(2-dimethylaminoethyl) -2, 3- dihydro-2-(4-methoxyphenyl)-1, 5-benzothiazepin-4 (5H)-one hydrochloride (Scheme 1). Diltiazem is a white, odorless, crystalline powder with a bitter taste, freely soluble in water, chloroform, formic acid, and methyl alcohol, while sparingly soluble in ether.

![Scheme 1 Chemical structure of diltiazem hydrochloride.](image)

The assay procedure listed in British Pharmacopoeia [3] and USP (Id acronym at 1st usage) [4], where in the drug and its formulations are estimated by an HPLC (Id) method. The other methods for its estimation include gas chromatography (GC) [5,6], capillary GC [7], capillary electrophoresis [8], Raman spectroscopy [9], voltammetry and amperometry [10], HPLC [11-23], colorimetry [24,25], and spectrophotometry [26-32]. The present work describes the optimization of conditions for the determination of diltiazem hydrochloride in bulk and tablet formulations through oxidation-reduction reactions.

2 Experimental

2.1 Apparatus

All absorption spectra were made using a UV–VIS spectrophotometer (JASCO 530V, Japan) with a scanning speed of 400 nm/min and a bandwidth of 2.0 nm, equipped with 10 nm matched quartz cells.
2.2 Materials and reagents

All chemicals used were of analytical grade and all solutions were freshly prepared in doubly distilled water.

Pure DIL.HCl was obtained from Glaxowellcome Egypt. Stock DIL.HCl solution (200 µg ml\(^{-1}\)) was prepared by dissolving 20 mg in a 100 ml calibrated flask with water. The working standard solutions were obtained by further dilution of stock solution with water.

An aqueous solution of 5.0×10\(^{-4}\) M amaranth (E. Mark) was prepared by dissolving an accurate weight of dye in least amount of water and completed to the mark in a 100 ml calibrated flask. A 100 µg ml\(^{-1}\) solution of N-bromosuccinimide (Aldrich) was freshly prepared by dissolving an accurate weight in least amount of warm water in 100 ml calibrated flask and then diluted with water to the mark.

An aqueous solution of 1.0×10\(^{-3}\) M chromotrope 2R (Aldrich product) and 2.0×10\(^{-4}\) M rhodamine 6G (BDH) were prepared by dissolving an accurate weight of dye in least amount of water and completed to the mark in a 100 ml calibrated flask. A 3.0×10\(^{-3}\) M solution of ceric ammonium sulfate (CAS) was prepared by dissolving a known weight of CAS in the least amount of 1.0 M sulfuric acid in a 100 ml calibrated flask and then diluted with the same acid to the mark. A solution of 1.0 % KBr, 0.5 M HCl and 1.0 M H\(_2\)SO\(_4\) was prepared.

2.3 General procedure and calibration

2.3.1 Method A

To each 10 ml volumetric flask containing 30-90 µg ml\(^{-1}\) DIL.HCl solution, 1.0 ml of 0.5 M HCl, 1.5 ml of 100 µg ml\(^{-1}\) NBS and 1.0 ml of 1.0 % KBr were transferred. After 5.0 min, 1.3 ml of 5.0×10\(^{-4}\) M AM dye was added mixed throughout and completed to the mark with water. The absorbance was measured at 521 nm against a blank solution prepared in the same manner. Calibration graph was prepared by plotting absorbance of the dye against the drug concentration. The amount of drug in any sample was calculated from its calibration curve.

2.3.2 Method B and C

An aliquot of DIL HCl containing 35 – 70 µg ml\(^{-1}\) was added to an excess volume (1.3 ml) of 3.0×10\(^{-3}\) M CAS containing 1.0 ml of 1.0 M H\(_2\)SO\(_4\). The solution mixture was heated on a boiling water bath for 10 min. The solution was cooled and 1.0 ml of 1.0×10\(^{-3}\) M C2R was added for method B, or 0.8 ml of 2.0×10\(^{-4}\) M Rh6G was added to hot solution for method C. The volume was completed to 10 ml with water and a decrease in color intensities of C2R or Rh6G were measured spectrophotometrically at their corresponding maximum wavelengths of 528 or 525 nm, respectively. The concentration range was determined in each case by plotting the concentration of DIL.HCl against absorbance at the corresponding λ\(_{max}\).
2.4 Procedure for dosage forms

Tablets and capsules: At least ten tablets or capsules of the drug were weighed into a small dish powdered and mixed well. A portion equivalent to 100 mg was weighed and dissolved in 100 ml of water, shaken well and filtered through a sintered glass crucible (G4). A 1.0 ml aliquot of the test solution was diluted to 100 ml in a calibrated flask. An aliquot of the diluted drug solution was then treated as described above in procedure A, B and C.

3 Results and discussion

We have successfully developed sensitive indirect spectrophotometric methods for the assay of DIL.HCl in bulk and pharmaceutical formulations. The methods are based on the oxidation of DIL.HCl containing a sulfur atom which is liable to atmospheric oxidation forming the S-oxide derivative. The absorption spectra of the reaction products in methods A, B, and C show characteristics $\lambda_{\text{max}}$ value (Fig. 1). The factors affecting the color development, reproducibility, sensitivity, and adherence to Beer’s law were investigated.

3.1 Method A

This method involves two steps namely:

(i) Reaction of DIL.HCl with an excess of NBS giving products involving oxidation.

(ii) Estimation of an excess oxidant by measuring the decrease in red color of AM spectrophotometrically at $\lambda_{\text{max}} = 521$ nm.

The various experimental parameters affecting the formation of the reaction product were optimized.

3.2 Effect of NBS concentration

To study the effect of NBS concentration, aliquots of DIL.HCl containing, 90 $\mu$g ml$^{-1}$ were transferred into a series of 10 ml volumetric flasks, 1.0 ml of 0.5 M HCl, followed by varying volumes (0.4–2.5 ml) of 100 $\mu$g ml$^{-1}$ NBS, 1.0 ml of % KBr, then 1.3 ml of $5.0 \times 10^{-4}$ M of AM and measuring the change in absorbance of the latter at 521 nm. It was found that maximum color intensity of the products was achieved in the range 1.4–1.6 ml of NBS (Fig. 2). The color intensity decreased below the lower limit and above the upper limit. Thus, the adoption of 1.5 ml of NBS (100 $\mu$g ml$^{-1}$) proved to be adequate for the maximum concentration of DIL.HCl used in the calibration curve.

3.3 Nature of acid medium and its concentration

Hydrochloric acid was the medium of choice for oxidation of DIL.HCl by NBS as well as the latter is determination with AM. The absorbance of AM was not affected in the
range (0.5–2.0 ml) of 0.5 M HCl. A 1.0 ml of 0.5 M HCl was found optimum for the oxidation of DIL.HCl and hence, the same volume was employed for the determination of NBS with AM.

3.4 Effect of time and temperature

The reaction between DIL.HCl and NBS in the presence of 1.0 % KBr was found to be completed after 5.0 min of mixing, but 10 min was sufficient to get maximum absorbance. Raising the temperature does not accelerate the oxidation process and does not give reproducible results, so maximum color intensity was obtained at room temperature (ca 25 ± 2 °C).

3.5 Effect of KBr

A 1.0 ml of KBr (1.0 %) was chosen as optimal volume in 10 ml total volume to accelerate the oxidation process.

3.6 Effect of sequence of addition

Drug – acid – NBS – KBr and then AM is the optimum sequence of addition, other sequences gave lower absorbance values under the same experimental conditions.

3.7 Effect of dye concentration

To establish the optimum concentration of the reagent, volumes (0.5–1.5 ml) of 5.0×10^{-4} M of AM dye were investigated. The optimum volume used for the production of maximum and reproducible color intensity is 1.3 ml.

3.8 Stoichiometry

In order to establish molar ratio, Job’s method of continuous variation was applied. In this method, solutions of drug and oxidant with identical molar concentrations were mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of the drug. It was found that NBS reacts with DIL.HCl with consumption of 4.0 mol of NBS for each mole of DIL.HCl giving a mixture of products. The remaining NBS reduces the intensity of red color of AM dye through disruption of the conjugation system in the dye.

3.9 Method B and C

Method B and C involve two stages:
(i) Oxidation of DIL.HCl with an excess CAS in an acid medium.

(ii) Determination of the unconsumed oxidant by measurement of the decrease in absorbance of C2R or Rh6G at a suitable $\lambda_{\text{max}} = 528$, or 525 nm for the method B or C, respectively. The influence of each of the following variables on the reaction was tested:

3.9.1 Effect of acid concentration

The most suitable acid to be used with Ce$^{4+}$ was found to be sulfuric acid of 1.0 M in which ceric ammonium sulfate was dissolved. Moreover, 1.0 ml of 1.0 M H$_2$SO$_4$ was chosen as optimum volume in a 10 ml total volume. Higher concentrations did not affect the stability of the color.

3.9.2 Effect of Ce$^{4+}$ concentration

It was found that a 3.0$\times$10$^{-3}$ M solution of Ce$^{4+}$ in the range 1.2 – 1.4 ml was necessary to achieve the maximum color intensity of the product. The color intensity decreased below the lower limit and above the upper limit, (Fig. 3). Therefore, 1.3 ml of ceric ammonium sulfate was recommended for all measurement.

3.9.3 Effect of temperature and cooling

Sample solutions containing DIL.HCl, H$_2$SO$_4$ and Ce$^{4+}$ were heated at different temperatures ranging from 30 to 100 ºC. The obtained results indicated that the reaction is catalyzed by heat, and is maximum at 100 ºC. The time required to complete the reaction is 10 min. In method B, the solution must be cooled for at least for 5.0 min before the addition of C2R. In method C, addition of Rh6G to heated solution gives maximum absorbance, so there is no need to cool the solution before addition of Rh6G.

3.9.4 Effect of dye concentration

To establish the optimum concentration of the reagent, different volumes of 1.0$\times$10$^{-3}$ M C2R or 2.0$\times$10$^{-4}$ M Rh6G solution were used. The optimum volumes used for the production of maximum and reproducible color intensity is 1.0 ml of C2R or 0.8 ml of Rh6G.

3.10 Stoichiometry

CAS reacts with DIL.HCl with consumption of 4.0 mol of CAS for each mole of DIL.HCl giving a mixture of products. The remaining CAS reduces the intensity of red color of C2R or Rh6G dye through disruption of the conjugation system in the dye.
3.11 Quantification

Beer-Lambert law limits, molar absorptivity, Sandell’s sensitivity, regression equations, and correlation coefficients obtained by linear square treatment of the results are given in Table 1. The detection and quantification limits were calculated from the standard deviation of the absorbance measurements obtained from a series of 13 blank solutions for each procedure. The limit of detection \((k = 3)\) and of quantification \((K = 10)\) were established according to the IUPAC definitions [33]. In order to determine the accuracy and precision of the methods, solution containing three different concentrations of DIL.HCl were prepared and analyzed in six replicates. The analytical results obtained from this investigation were summarized in Table 2. The performances of the methods were assessed by calculating the t- and F-values compared with the official methods [3]. The mean values were obtained in a student’s t- and F- tests at the 95 % confidence limits for five degrees of freedom [34]. The results showed that the calculated t- and F-values did not exceed the theoretical ones.

4 Analytical application

The proposed methods were successfully applied to determine diltiazem hydrochloride in tablets and capsules. The results obtained were compared statistically by student’s t- test (for accuracy) and variance ratio F- test (for precision) with officially accepted method [3], at 95 % confidence level with five degrees of freedom, as recorded in Table 3. The results showed that the t- and F- values were less than the critical value, indicating that there was no significance difference between the proposed and official methods. Because the proposed methods were more reproducible with high recoveries, they can be recommended for the routine analysis in the majority of drug quality control laboratories.

5 Interferences

The criterion of interference was an error of not more than 3.0 % in the absorbance. Experimental showed that there was no interference from additives or excipients commonly used such as glucose, lactose, fructose, calcium hydrogen phosphate, magnesium stearate and starch for the examined methods A, B and C.

6 Comparison with other methods

The performance of the proposed methods was compared other existing spectrophotometric methods (Table 4) and this comparison reveals that the proposed approaches are more sensitive, due higher molar absorptivity and low limit of detection with high accuracy and precision.
7 Conclusion

This method is simple, rapid, and offers the advantages of high sensitivity reproducibility, precision and stability of colored species for more than 24 h. Furthermore, the methods depend on simple reagents that are available, besides being less time consuming. The proposed methods may be, applied for routine analysis and in quality control laboratories for quantitative determination of DIL.HCl in raw materials, in pharmaceutical formulations depending upon the availability of the chemicals and the nature of other excipients present in the sample.

References


Fig. 1 Absorption spectra for the reaction product of 9.0 $\mu$g ml$^{-1}$ DIL.HCl with: (a) NBS-AM, (b) Ce$^{4+}$ - C2R, (c) Ce$^{4+}$ - Rh6G.
Fig. 2 Effect of ml added of 100 µg ml⁻¹ NBS on the intensity of the color produced during the reaction (9.0 µg ml⁻¹ DIL.HCl, 0.4-2.5 ml NBS).
Fig. 3 Effect of added $3 \times 10^{-3}$ M Ce$^{4+}$ on the intensity of the color produced during the reaction (7.0 $\mu$g ml$^{-1}$ DIL.HCl, 0.4-2.5 ml Ce$^{4+}$).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Media</td>
<td>HCl</td>
</tr>
<tr>
<td>(\lambda_{max}(\text{nm}))</td>
<td>521</td>
</tr>
<tr>
<td>Beer's conc. range ((\mu g\ \text{ml}^{-1}))</td>
<td>3.0-9</td>
</tr>
<tr>
<td>Detection limits ((\mu g\ \text{ml}^{-1}))</td>
<td>0.006</td>
</tr>
<tr>
<td>Quantification limits ((\mu g\ \text{ml}^{-1}))</td>
<td>0.063</td>
</tr>
<tr>
<td>Molar absorptivity (1 (\text{mol}^{-1}\text{cm}^{-1}))</td>
<td>(6.6 \times 10^4)</td>
</tr>
<tr>
<td>Sandell's sensitivity ((\mu g\ \text{cm}^{-1}))</td>
<td>6.79</td>
</tr>
<tr>
<td>Regression equation*</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.043</td>
</tr>
<tr>
<td>Slope</td>
<td>0.130</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9997</td>
</tr>
<tr>
<td>Standard deviation of slope %</td>
<td>0.005</td>
</tr>
<tr>
<td>Standard deviation of intercept %</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*A= a + bC, where C is the concentration in \(\mu g\ \text{ml}^{-1}\) and A is the absorbance.

**Table 1** Quantitative parameters for the proposed methods.
<table>
<thead>
<tr>
<th>procedure</th>
<th>Taken (µg ml(^{-1}))</th>
<th>Recovery (%)</th>
<th>RSD(^a) (%)</th>
<th>RE (%)</th>
<th>confidence limit(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.0</td>
<td>99.9</td>
<td>1.08</td>
<td>1.13</td>
<td>0.679 ± 0.0077</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>100.1</td>
<td>0.37</td>
<td>0.39</td>
<td>0.868 ± 0.0034</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>100.1</td>
<td>0.64</td>
<td>0.67</td>
<td>1.062 ± 0.0071</td>
</tr>
<tr>
<td>B</td>
<td>3.5</td>
<td>100.9</td>
<td>1.43</td>
<td>1.51</td>
<td>0.354 ± 0.0053</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>100.1</td>
<td>0.83</td>
<td>0.87</td>
<td>0.545 ± 0.0047</td>
</tr>
<tr>
<td></td>
<td>4.9</td>
<td>99.3</td>
<td>1.24</td>
<td>1.29</td>
<td>0.812 ± 0.0105</td>
</tr>
<tr>
<td>C</td>
<td>3.5</td>
<td>100.1</td>
<td>1.34</td>
<td>1.40</td>
<td>0.343 ± 0.0048</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>99.9</td>
<td>0.48</td>
<td>0.50</td>
<td>0.674 ± 0.0034</td>
</tr>
<tr>
<td></td>
<td>4.9</td>
<td>99.7</td>
<td>0.70</td>
<td>0.74</td>
<td>0.968 ± 0.0071</td>
</tr>
</tbody>
</table>

\(^a\) Relative standard deviation for five determinations.
\(^b\) 95 % confidence limits and five degree of freedom.

**Table 2** Evaluation of accuracy and precision of the proposed methods.
Commerical formulations analyzed & Supplier & Label Claim/ mg & A & B & C & Official method \\
Altiazem & E.I.P.I.CO & 60 mg & 100.5±0.42 & 99.20±0.67 & 98.8±0.78 & 98.25±1.15 \\
 & & & F = 2.85 & F = 2.44 & F = 2.50 & \\
 & & & t = 0.74 & t = 1.15 & t = 1.28 & \\
Tildiem & Amyria & 60 mg & 99.30±0.56 & 100.4±0.48 & 100.4±0.32 & 101.55±0.85 \\
 & & & F = 2.41 & F = 3.08 & F = 2.32 & \\
 & & & t = 1.08 & t = 1.43 & t = 0.89 & \\
Delay-tiazem SR & GlaxoWellcome & 90 mg & 98.9 ± 0.75 & 99.5 ± .55 & 99.3 ± .52 & 99.6 ± 0.81 \\
 & & & F = 1.16 & F = 2.16 & F = 2.42 & \\
 & & & t = 1.41 & t = 0.22 & t = 0.69 & \\

*a Average of five determinations.

**Table 3** Determination of DIL.HCl in dosage forms by the proposed methods
<table>
<thead>
<tr>
<th>No.</th>
<th>Reagent</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; nm</th>
<th>Beer’s law limit (µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Molar absorptivity l. mol&lt;sup&gt;-1&lt;/sup&gt; cm&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ferric hydroxamate</td>
<td>500</td>
<td>50 – 800</td>
<td>4.85 × 10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>28</td>
</tr>
<tr>
<td>2.</td>
<td>Cobalt thiocyanate</td>
<td>630</td>
<td>60 – 600</td>
<td>7.3 × 10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>27</td>
</tr>
<tr>
<td>3.</td>
<td>Sod. metavanadate</td>
<td>750</td>
<td>1 – 50</td>
<td>6.18 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>32</td>
</tr>
<tr>
<td>4.</td>
<td>Methyl orange</td>
<td>420</td>
<td>5 – 60</td>
<td>7.96 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>27</td>
</tr>
<tr>
<td>5.</td>
<td>Metol sulphanilamide</td>
<td>520</td>
<td>2 – 24</td>
<td>1.35 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>30</td>
</tr>
<tr>
<td>6.</td>
<td>Bromothymol blue</td>
<td>415</td>
<td>2.5 – 20</td>
<td>2.5 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>31</td>
</tr>
<tr>
<td>7.</td>
<td>Bromocresol green</td>
<td>415</td>
<td>2.5 – 12.5</td>
<td>2.53 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>31</td>
</tr>
<tr>
<td>8.</td>
<td>Bromophenol blue</td>
<td>415</td>
<td>2.5 – 10</td>
<td>2.7 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>31</td>
</tr>
<tr>
<td>9.</td>
<td>Fe II, 1,10 phenanthroline</td>
<td>495</td>
<td>1 – 10</td>
<td>2.93 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>30</td>
</tr>
<tr>
<td>10.</td>
<td>Amaranth</td>
<td>521</td>
<td>3 – 9</td>
<td>6.6 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>This work</td>
</tr>
<tr>
<td>11.</td>
<td>Rhodamine 6G</td>
<td>525</td>
<td>3.5 – 6.3</td>
<td>8.1 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>This work</td>
</tr>
<tr>
<td>12.</td>
<td>Chromtrope 2R</td>
<td>528</td>
<td>3.5 – 7</td>
<td>8.8 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>This work</td>
</tr>
</tbody>
</table>

**Table 4** Comparison of reagents of previously reported spectrophotometric methods with that of the proposed methods for DIL.HCl.