Introduction

Verapamil hydrochloride, belonging to phenylalkylamine group of calcium channel blockers, is chemically 5-[N-(3,4-dimethoxyphenylethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropyl valeronitrile hydrochloride [1]. The drug is officially listed in British, United States and Indian Pharmacopoeias [2–4]. It is a potent antihypertensive agent with significant depressant effects and has been recommended for intravenous therapy of supraventricular tachyarrhythmias [5].

The drug has been determined in biological fluids and for pharmaceutical dosage forms by a variety of analytical techniques such as high performance liquid chromatography [6–11], gas liquid chromatography [12], capillary gas chromatography [13], high performance thin layer chromatography [14], potentiometry [15] and potentiometry–conductometry [16]. The visible spectrophotometric methods are the instrumental methods of choice, which provides practical and significant economic advantages over the other methods. In the literature, only few spectrophotometric methods have been reported which are usually based on either extractable ion–pair complex formation with bromophenol blue, bromocresol purple, bromocresol green, bromothymol blue and methyl orange [17]; solochrome black–T, solochrome dark blue, solochrome cyanine R and fast sulfone FF [18]; eriogluocine and indigocarmine [19]; alizarin red–S [20] or charge transfer complex formation with polyhalo/polycyanoquinones [21–22] and azo dyes [23]. However, some of these methods suffer from one or other disadvantages such as low sensitivity, lack of selectivity and simplicity.

Chloramine–T is a strong oxidant in both acidic and alkaline media ($E_{red} = 1.138$ at pH 0.65 and 0.5 at pH 12) [24–25]. In acidic aqueous solution, chloramine–T is thought to exist
in a complex series of equilibria [26] which indicates that the probable oxidising species in acidified chloramine–T is hypochlorous acid.

Chloramine–T was initially introduced as a disinfectant and antiseptic but nowadays widely used as an oxidant for various organic functional groups [27]. It has been used as a reagent for the spectrophotometric determination of p-aminobenzenesulphonamides [28] and sulphamethoxazole, tetracycline hydrochloride, amidopyrine, nifurtimox and isoniazid [29], which involves the addition of excess chloramine–T and the determination of unreacted reagent. Chloramine–T is also utilised in the titrimetric determination where the end point is detected with either a visual indicator [28, 30–32] or potentiometrically [31].

This chapter describes the spectrophotometric determination of verapamil hydrochloride based on its oxidation with chloramine–T in acidic medium producing a yellow chromophore.

Experimental

Apparatus

Spectronic 20D+ spectrophotometer (Milton Roy, USA) with matched glass cuvettes was used for spectral runs and absorbance measurements.

Reagents

A 0.4% verapamil hydrochloride (Sigma, USA) solution was prepared in AR-grade methanol (S.D. Fine Chem. Ltd., India) and was further diluted according to the need. 1% solution of chloramine–T (E. Merck, India) and 5 M hydrochloric acid (E. Merck) were prepared in doubly distilled water.
**Recommended procedure**

Into a series of 10 mL volumetric flasks 1 mL of varying concentrations of verapamil hydrochloride, upto 340 μg mL\(^{-1}\), were pipetted out. To each flask 2.5 mL of 1% chloramine–T solution and 6 mL of 5 M HCl were added and diluted to the mark with doubly distilled water. The content was mixed well and kept at room temperature for 15 minutes. The absorbances were measured at 425 nm against the reagent blank prepared simultaneously omitting the drug. The amount of drug in each sample were calculated either from the calibration graph or regression equation.

**Analysis of verapamil hydrochloride in pharmaceutical formulations**

Ten tablets of verapamil hydrochloride, equivalent to 400 mg of pure drug, were grounded to fine powder. The whole mass was stirred in methanol and filtered through whatmann no. 42 filter paper into a 100 mL volumetric flask. The residue was washed well with methanol. The filtrate and washings were diluted to 100 mL volume. This solution was diluted according to the need and analysed by the recommended procedure.

**Results and discussion**

Chloramine–T is well known oxidant and its oxidative behaviour resembles to that of the hypohalites. Bishop and Jennings [33], Morris et al. [34] and Higuchi and Hussain [35] have studied the equilibria involved in acidified chloramine–T solution and suggested the formation of hypochlorous acid. This reacts with verapamil hydrochloride to form the relevant oxidation products. It is also known that N–bromosuccinimide reacts with tertiary amine [36]. In such studies, a methyl or methylene group attached to nitrogen was required and >N–CH\(_2\)
linkage was cleaved preferentially giving yellow coloured products. In a similar fashion it is believed that verapamil is oxidised by hypochlorous acid in which >N–CH₂ bond is cleaved resulting in the formation of aldehyde and secondary amine. The oxidation product absorbs maximally at 425 nm (Fig. 3.1). Therefore, based on the literature background and our findings, the reaction mechanism was proposed and given in Scheme 3.1.

The optimum conditions for the assay of verapamil hydrochloride were established via a number of preliminary experiments.

**Effect of time**

To investigate the effect of time on the colour development, 1 mL of 0.2% verapamil hydrochloride was pipetted in a 10 mL volumetric flask, 2.5 mL of 1% chloramine–T and 6 mL of 5 M HCl were added and diluted to the mark with doubly distilled water. The absorbance was recorded as a function of time. The results showed (Fig. 3.2) that the absorbance became constant after 13 minutes and remained unchanged upto 20 minutes. Thus absorbance was measured within the stability period.

**Effect of chloramine–T concentration**

To 1 mL of 0.2% verapamil hydrochloride, varying volumes (0.5 – 3.0 mL) of 1% chloramine–T and 6 mL of 5 M HCl were added. The coloured product was diluted to 10 mL with doubly distilled water and absorbances were measured against the corresponding reagent blanks after 15 minutes. The results (Fig. 3.3) showed that the highest absorbance was obtained with 2.25 mL, which remained constant with higher amounts of chloramine–T. Thus, 2.5 mL of 1% chloramine–T was added for colour development.
Fig. 3.1. Absorption spectra of the oxidation product of verapamil (●) and its reagent blank (○).
(1) Formation of hypochlorous acid

\[
\text{NaSO}_2\text{N—Cl} + \text{H}^+ \rightleftharpoons \text{SO}_2\text{N—Cl} + \text{Na}^+
\]

\[
\text{SO}_2\text{N—Cl} + \text{H}^+ \rightleftharpoons \text{SO}_2\text{NH}_2 + \text{SO}_2\text{NCl}_2
\]

\[
\text{SO}_2\text{N—Cl} + \text{H}_2\text{O} \rightleftharpoons \text{SO}_2\text{NH}_2 + \text{SO}_2\text{NCl}_2 + \text{H}_2\text{O}
\]

(2) Oxidation of verapamil by hypochlorous acid

\[
\text{H}_3\text{CO—CHCH}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{C—CN} + \text{HOCl}
\]

\[
\text{H}_3\text{CO—CHCH}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{C—CN} + \text{H}^+ \rightleftharpoons \text{H}_2\text{CO—CHCH}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{C—CN} + \text{H}_2\text{O}
\]

\[
\text{H}_3\text{CO—CHCH}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{C—CN} + \text{H}^+ \rightleftharpoons \text{H}_2\text{CO—CHCH}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{C—CN} + \text{H}_2\text{O}
\]

Scheme 3.1
Fig. 3.2. Effect of time on the oxidation of verapamil hydrochloride.
Fig. 3.3. Effect of volume of 1% chloramine–T on the oxidation of verapamil hydrochloride.
Effect of the concentration of hydrochloric acid

To study the effect of the concentration of hydrochloric acid, the reaction was carried out in a series of 10 mL volumetric flasks containing 200 \( \mu \)g mL\(^{-1}\) verapamil hydrochloride, 2.5 mL of 1% chloramine-T and varying volumes of 5 M HCl (2.0 – 6.5 mL). It is apparent from the Fig. 3.4 that the maximum absorbance was found with 5.8 mL of 5 M HCl, beyond which the absorbance became constant. Thus, 6 mL of 5 M HCl was used throughout the experiment.

Analytical data

Under the optimum experimental conditions, main merits of the procedure for the determination of verapamil hydrochloride have been established by least square treatment of the results. The absorbance responses at 425 nm were found to be linear in relation to the concentration of verapamil hydrochloride upto 340 \( \mu \)g mL\(^{-1}\) (Fig. 3.5) with a molar absorptivity of \( 2 \times 10^3 \) L mol\(^{-1}\) cm\(^{-1}\). Regression analysis of Beer’s law plot was made to evaluate intercept, slope and correlation coefficient \( \langle r \rangle \) and the values were found to be \( 0.29 \times 10^{-3} \), \( 4.05 \times 10^{-3} \) and 0.9999, respectively which yielded the regression equation, \( A = 0.29 \times 10^{-3} + 4.05 \times 10^{-3}C \) (where \( A \) is the absorbance and \( C \) is the concentration of verapamil hydrochloride in \( \mu \)g mL\(^{-1}\)). The detection limit for the proposed method was 0.97 \( \mu \)g mL\(^{-1}\) computed from the following equation [37].

\[
\text{Detection limit} = \sqrt{S_0^2 \frac{n - 2}{n - 1}} \frac{t}{b}
\]

where \( S_0^2 \) = variance, \( n \) = number of samples, \( t \) = Student’s \( t \)-value at 95% confidence level and \( b \) = slope of the line of regression. The high value of correlation coefficient and small value of intercept on the ordinate, which was close to zero, validated the linearity of calibration curve
Fig. 3.4. Influence of the volume of 5 M hydrochloric acid on the oxidation of verapamil hydrochloride.
Fig. 3.5. Calibration curve for the determination of verapamil hydrochloride.
whereas detection limit and slope indicated the good sensitivity of the method. Also, the small
degree of scattering of the experimental data points around the line of regression was
confirmed by the small value of variance, i.e. $5.33 \times 10^{-6}$.

There is also strong correlation between slope and intercept, which has been
established by the 95% joint confidence region drawn for them [38]. It is evident from the Fig.
3.6 that the joint confidence region is bounded by an ellipse having the point of best fit as its
centre. It can also be seen that the points with an intercept of zero fell well within the ellipse
and thus, confirmed that there is no significant deviation from the zero.

Regression analysis of the calibration data also makes it possible to evaluate the error,
$S_c$, in the determination of a given concentration of verapamil hydrochloride [39]. Fig. 3.7
shows the graph of $S_c$ against the concentration of verapamil hydrochloride. The error is
minimum when the actual absorbance become equal to the average absorbance value in the
calibration graph which corresponds to $125 \, \mu g \, mL^{-1}$. This statistical treatment may be used to
establish the confidence limit at the selected level of confidence (Fig 3.8) for the determination
of unknown concentration by using the equation [40].

$$C_i \pm \frac{t_p S_o}{b} \left[ 1 + \frac{1}{n} + \frac{(y - \bar{y})^2}{b^2 (\Sigma C - \bar{C})^2} \right]^{1/2} = C_i \pm \Delta C$$

In order to test the precision and accuracy of the proposed method ten successive
determinations of $200 \, \mu g \, mL^{-1}$ of verapamil hydrochloride were carried out. The percent
relative standard deviation (%RSD) and error (%Er) were found to be 0.24 and 0.22 respectively.
The results, therefore, indicated that the method has satisfactory precision and accuracy. The
commonly encountered excipients in the pharmaceutical dosage forms did not interfere.
Fig. 3.6. Plot of joint confidence region (at $P = 0.05$) for the slope and intercept of the line of regression for the determination of verapamil hydrochloride.
Fig. 3.7. Error ($S_C$) in the determination of the concentration of verapamil hydrochloride.
Fig. 3.8. Variation in the confidence limit at 95% confidence level for the determination of verapamil hydrochloride.
As an additional demonstration of accuracy, recovery experiments were carried out by adding a fixed amount of verapamil hydrochloride to a preanalysed tablet. The results are shown in Table 3.1. It is apparent from the table that results were reproducible with low percent relative standard deviations (0.3 – 0.82) and mean recoveries were in the range of 99.4 – 100.3%.

The method was successfully applied to the determination of verapamil hydrochloride in tablets available locally. Satisfactory results (Table 3.2) were obtained for the recovery of drug and were in good agreement with the label claimed. The results of the proposed method were statistically compared with those obtained by the reference method [22]. The calculated Student’s t- and F-values were less than the theoretical ones at 95% confidence level. The statistical evaluation indicated that there was no significant difference between the methods compared.

The proposed method is advantageous when compared to other existing visible spectrophotometric methods in wider linear range of estimation with lower value of percent relative standard deviations (Table 3.3). This is a decisive advantage since commercial dosage forms contain higher amounts. The method is sensitive enough to permit the determination of as little as 0.97 μg mL⁻¹ of the drug. The proposed method, therefore, is simple, sensitive and reproducible and can be applied for the routine analysis of verapamil hydrochloride in the quality control laboratories.
Table 3.1. Determination of verapamil hydrochloride in dosage forms by standard addition method.

<table>
<thead>
<tr>
<th>Pharmaceutical preparations</th>
<th>Amount taken (µg mL⁻¹)</th>
<th>Amount added (µg mL⁻¹)</th>
<th>Total amount found (µg mL⁻¹)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calaptin-40</td>
<td>25</td>
<td>25</td>
<td>49.85</td>
<td>99.71</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>150</td>
<td>300.20</td>
<td>100.10</td>
<td>0.30</td>
</tr>
<tr>
<td>Isoptin-40</td>
<td>25</td>
<td>25</td>
<td>50.15</td>
<td>100.30</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>150</td>
<td>300.94</td>
<td>100.30</td>
<td>0.45</td>
</tr>
<tr>
<td>Vasopten-40</td>
<td>25</td>
<td>25</td>
<td>49.10</td>
<td>99.40</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>150</td>
<td>300.2</td>
<td>100.10</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*Five independent analyses.*
Table 3.2. Analysis of pharmaceutical preparations by the proposed and reference methods.

<table>
<thead>
<tr>
<th>Pharmaceutical Preparations</th>
<th>Labelled Amount (mg)</th>
<th>Proposed method Recovery (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RSD (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference method Recovery (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RSD (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>( t_{\text{calc}}^b )</th>
<th>( F_{\text{calc}}^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calaptin</td>
<td>40</td>
<td>99.76</td>
<td>0.49</td>
<td>99.65</td>
<td>0.67</td>
<td>0.3154</td>
<td>1.9</td>
</tr>
<tr>
<td>Isoptin</td>
<td>40</td>
<td>100.12</td>
<td>0.41</td>
<td>99.88</td>
<td>0.49</td>
<td>0.8253</td>
<td>1.42</td>
</tr>
<tr>
<td>Vasopoten</td>
<td>40</td>
<td>99.63</td>
<td>0.38</td>
<td>99.88</td>
<td>0.77</td>
<td>0.6720</td>
<td>4.16</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average of five independent analyses.

<sup>b</sup> Theoretical \( t \)- and \( F \)-values at 95% confidence level are 1.86 and 6.39 respectively [41].
Table 3.3. Comparison of the proposed method with other spectrophotometric methods for the determination of verapamil hydrochloride in pharmaceutical formulations.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Beer’s law limit (µg mL$^{-1}$)</th>
<th>RSD (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eriogloucine$^a$</td>
<td>627</td>
<td>1.3 – 5.3</td>
<td>1.45</td>
<td>[19]</td>
</tr>
<tr>
<td>Indigocarmine$^a$</td>
<td>602</td>
<td>33 – 130</td>
<td>1.53</td>
<td>[19]</td>
</tr>
<tr>
<td>Bromocresol purple$^a$</td>
<td>420</td>
<td>4 – 24</td>
<td>–</td>
<td>[17]</td>
</tr>
<tr>
<td>Chromotrope 2B</td>
<td>530</td>
<td>5 – 59</td>
<td>–</td>
<td>[23]</td>
</tr>
<tr>
<td>Chromotrope 2R</td>
<td>546</td>
<td>5 – 59</td>
<td>–</td>
<td>[23]</td>
</tr>
<tr>
<td>Solochrome dark blue$^a$</td>
<td>528</td>
<td>10 – 38</td>
<td>1.53</td>
<td>[18]</td>
</tr>
<tr>
<td>Solochrome cyanine R$^a$</td>
<td>445</td>
<td>8 – 30</td>
<td>1.24</td>
<td>[18]</td>
</tr>
<tr>
<td>Chloramine–T</td>
<td>425</td>
<td>0 – 340</td>
<td>0.3 – 0.82</td>
<td>This work</td>
</tr>
</tbody>
</table>

$^a$ Extractive method.
References


[33]. E. Bishop, V.J. Jennings; Talanta 1 (1958) 197.


