SPECTROPHOTOMETRIC DETERMINATION OF ETAMSYLATE IN PHARMACEUTICALS USING FERRIC CHLORIDE BASED ON COMPLEX FORMATION REACTIONS

The present study describes two simple and selective spectrophotometric methods for the rapid determination of etamsylate (ETM) in bulk and in capsule formulations. The methods are based on the oxidation of ETM with ferric chloride in neutral medium and subsequent chelation of the resulting iron(II) with 1,10-phenanthroline (Phen) (method A) and with 2,2'-bipyridyl (Bipy) (method B). The resulting red colored chromogens are measured at 510 and 520 nm, in method A and B, respectively. In both methods, the absorbance is found to increase linearly with increasing ETM concentration. Beer’s law is obeyed over the ranges 0.5-10 and 0.8-16 µg/ml for method A and B, respectively. The calculated molar absorptivity values are $2.17 \times 10^4$ and $1.65 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ for method A and B, respectively, and the corresponding sandell sensitivities are 0.012 and 0.016 µg/cm$^2$. Intra-day and inter-day precision and accuracy of the methods were established according to ICH guidelines. The proposed methods were applied to determine ETM in dosage forms and the results were statistically compared with that of an official BP method.

Key words: etamsylate; determination; ferric chloride; complexation reactions; pharmaceuticals.

Etamsylate (ETM), chemically known as 2,5-dihydroxy benzene sulphonate acid with diethylamine [1] (Figure 1), is a haemostatic drug. It is used in the treatment of capillary hemorrhage, hematemesis, hemothysis, malena, hematuria, epistaxis, menorrhagia and post partum hemorrhage [2]. It is believed to work by increasing capillary endothelial resistance and promoting platelet adhesion.

![Figure 1. Structure of etamsylate.](image)

The literature survey reveals that high pressure liquid chromatography [3,4], high pressure thin layer chromatography [5,6], UV-spectrophotometry [7-9], capillary electrophoresis [10], irreversible biamperometry [11], adsorptive stripping voltammetry [12], flow injection potentiometry [13] and chemiluminescence spectrophotometry [14-16] have been employed for its quantification. The above-mentioned techniques, of course, are sensitive enough but are expensive. Spectrophotometry is the technique of choice even today due to its inherent simplicity. It is frequently used in the laboratories of the developing countries for the routine analytical work. In literature, only a few visible spectrophotometric methods have been reported. One kinetic spectrophotometric procedure [17] for the determination of ETM in body fluids has been described based on a catalytic acceleration of the reaction between sodium azide and iodine. The drug content in pharmaceutical preparations has also been determined spectrophotometrically [18-23] in the visible region based on different reaction schemes. The reported visible spectrophotometric methods, although a couple of them sensitive, suffer from one or other disadvantages such as the use of a costly reagent and poor sensitivity [23], a heating step [21,22] or a narrow linear range [17] (Table 1). The need for a simple, sensitive, low cost and reliable method for the determination of ETM is thus clearly recognized.
The aim of the present investigation was to develop simple, rapid, sensitive and economically viable procedures that could be used to determine ETM in bulk drug and pharmaceutical dosage forms by spectrophotometry. The methods utilize ferric chloride and 1,10-phenanthroline and 2,2’-bipyridyl as reagents. 1,10-Phenanthroline is commonly used for the determination of metal ions like iron(II) [24], cobalt and cadmium [25]. It is also used for the determination of some phenolic compounds [26-28] and drugs [29-33]. 2,2’-Bipyridyl and Fe(III) system has been employed for the estimation of several drugs by a number of workers [34-36]. The methods have the advantages of speed and simplicity besides being accurate and precise and can be adopted by the pharmaceutical laboratories for the industrial quality control.

EXPERIMENTAL DETAILS

Apparatus

All absorbance measurements were performed using a Systronics model 106 digital spectrophotometer (Ahmedabad, India) provided with 1-cm matched quartz cells.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Reagent/s used</th>
<th>Methodology</th>
<th>Linear range, µg/ml and molar absorptivity, l mol⁻¹ cm⁻¹</th>
<th>Remarks</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sodium azide and iodine</td>
<td>A decrease in the absorbance of iodine is measured at 348 nm.</td>
<td>0.3-3.0</td>
<td>Sensitive but narrow linear range and absorbance measured at shorter wavelength. The reaction rate method which demands a careful maintenance of experimental conditions.</td>
<td>17</td>
</tr>
<tr>
<td>2.</td>
<td>Iron (III) o-phenanthroline mixture</td>
<td>Tris[α-phenanthroline-iron(II)] complex measured at 510 nm.</td>
<td>0.25-30</td>
<td>Requires heating, less sensitive.</td>
<td>21</td>
</tr>
<tr>
<td>3.</td>
<td>Ammonium molybdate</td>
<td>Resulting molybdenum blue (Mo⁵⁺) possesses a characteristic λmax at 695-716 nm.</td>
<td>2.0-70</td>
<td>Requires heating in a boiling water bath.</td>
<td>22</td>
</tr>
<tr>
<td>4.</td>
<td>Ce(IV)-MBTH</td>
<td>The absorbance of the reaction product is measured at 514 nm.</td>
<td>4.0-30</td>
<td>Less sensitive, narrow linear dynamic range, costly reagent.</td>
<td>23</td>
</tr>
<tr>
<td>5.</td>
<td>Iron (III) chloride with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I) 1,10-phenanthroline</td>
<td>Red colored chromogen (ferroin) formed is measured at 510 nm.</td>
<td>0.5-10</td>
<td>Highly sensitive, wide linear dynamic ranges, inexpensive instrumental setup, use of eco friendly chemicals, and aqueous system.</td>
<td>Present methods</td>
<td></td>
</tr>
<tr>
<td>II) 2,2’-Bipyridyl</td>
<td>Red colored chromogen [Fe(II)-BPD complex] formed is measured at 520 nm.</td>
<td>0.8-16</td>
<td></td>
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</tr>
</tbody>
</table>

Materials and reagents

All chemicals and reagents used were of analytical-reagent grade and distilled water was used throughout the investigation.

Standard ETM solution

Pharmaceutical grade ETM certified to be 99.87% pure was kindly supplied by Biocon India Ltd., Bangalore, India, and used as the reference standard. Standard ETM solutions (20 and 40 µg/ml) were prepared by dissolving a calculated quantity of pure drug in distilled water.

Pharmaceutical formulations of etamsylate such as Dicynene-250 (Dr. Reddy’s Lab. Ltd., H. P., India) and K-Stat-250 (Mercury, Lab. Ltd., H. P., India) were purchased from local markets.

Ferric chloride (hydrated, 0.0033 M)

The aqueous solution of 0.05 M ferric chloride (S. D. Fine Chem., Mumbai, India) was prepared by dissolving 1.35 g of the chemical in 100 ml of distilled water and stored in a dark bottle. The stock solution was then diluted appropriately with distilled water to get 0.0033 M working concentration for both methods.
solution was prepared afresh just before the experiment.

1,10-phenanthroline (0.01 M)

The solution was prepared by dissolving 198 mg of the chemical (Qualigens Fine Chemicals, Mumbai, India, assay 100%) in distilled water and diluted to 100 ml with distilled water.

2,2'-Bipyridyl (0.01 M)

The solution was prepared by dissolving 156 mg of the chemical (Qualigens Fine Chemicals, Mumbai, India, assay 100%) in distilled water and diluted to a100 ml calibrated flask.

Orthophosphoric acid (0.02 M)

Concentrated acid (Merck, Mumbai, India) was appropriately diluted with distilled water to get the required concentration.

Methods

Method A

Different aliquots (0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml) of the standard 20 µg/ml ETM solution were accurately measured and transferred into a series of 10 ml calibrated flasks by means of a micro burette and the total volume was adjusted to 5 ml by adding water. To each flask, 2 ml of a ferric chloride (0.0033 M) and 2 ml of 1,10-phenanthroline (0.01 M) were added, followed by 1 ml of orthophosphoric acid (0.02 M), and the volume was brought to 10 ml with distilled water. The flasks were stoppered, the content mixed well and the flasks were let stand for 5 min with occasional shaking. Then, the absorbance of each solution was measured at 510 nm against the reagent blank.

Method B

Varying aliquots (0.5, 1.0, 2.0, 3.0 and 4.0 ml) of the standard ETM solution (40 µg/ml) were accurately measured into a series of 10 ml calibrated flasks by means of a micro-burette and the total volume was brought to 4 ml by adding water. To each flask 2 ml of ferric chloride (0.0033 M) and 2 ml of 2,2'-bipyridyl (0.01 M) were added followed by 1 ml of orthophosphoric acid (0.02 M), and the volume was brought to 10 ml with distilled water. The flasks were stoppered, the content mixed well and the flasks were let stand for 5 min with occasional shaking. Then, the absorbance of each solution was measured at 520 nm against reagent blank.

Procedure for tablets

An amount of finely ground tablet powder equivalent to 100 mg of ETM was accurately weighed and transferred into a 100 ml calibrated flask, 60 ml of water was added and the content shaken thoroughly for 15-20 min to extract the drug into the liquid phase; the volume was finally diluted to the mark with water, mixed well and filtered using a Whatman No. 42 filter paper. First 10 ml of the filtrate was discarded and a suitable aliquot of the filtrate (1000 µg/ml ETM) was diluted stepwise with water to get 20 and 40 µg/ml concentrations for method A and method B, respectively.

Placebo blank analysis

A placebo blank of the composition: talc (10 mg), starch (10 mg), acacia (10 mg), methyl cellulose (10 mg), sodium citrate (10 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was made and its solution was prepared as described under “Procedure for tablets”, and then subjected to analysis.

Procedure for the determination of etamsylate in synthetic mixture

To the placebo blank of the composition described above, 100 mg of ETM was added and homogenized, transferred to a 100 ml standard flask and the solution was prepared under tablets as described. The synthetic mixture solution (1000 µg/ml in ETM) was then diluted step wise with water to obtain working concentrations of 20 and 40 µg/ml in ETM for methods A and B, respectively. A convenient aliquot was then subjected to analysis by either method described above. This analysis was done to study the interference of excipients such as talc, starch, acacia, methyl cellulose, sodium citrate, magnesium stearate and sodium alginate.

RESULTS AND DISCUSSION

The proposed methods involve the oxidation of ETM with ferric chloride and subsequent complexation of resulting Fe²⁺ with 1,10-phenanthroline in method A and with 2,2'-bipyridyl in method B. The probable reaction mechanism is shown in Figure 2. Fe³⁺ oxidizes ETM and the produced Fe²⁺ forms a red colored complex, ferroin with Phen in method A, which exhibits an absorption maximum at 510 nm (Figure 3) and in method B the resulting red colored chromogen due to the formation of Fe²⁺-Bipy complex absorbs maximally at 520 nm (Figure 3). In both methods, the absorbance of the colored solution increases linearly with an increasing concentration of the ETM.
Optimization of variables

The experimental variables for the formation of the stable and sensitive colored product were optimized. When the Fe\textsuperscript{3+} concentration was increased, the absorbance value of reagent blank was found to increase. Hence, by considering the sensitivity of the reaction with a minimum blank absorbance, 2 ml of 0.0033 M ferric chloride in a total volume of 10 ml was found optimum in both methods and used throughout the experiment.

Several experiments were carried out to study the effect of Phen and Bipy concentrations on the color development. In order to determine the optimum concentration of Phen, different volumes (0.5-2.5 ml) of 0.01 M Phen solution were used with a fixed concentration of ETM (6 µg/ml) in a total volume of 10 ml and (1.5-2.5 ml) of Phen solution was found to give constant absorbance readings. Hence, 2 ml of Phen solution in a total volume of 10 ml is fixed (Figure 4). In method B, 2 ml of 0.01 M Bipy solution in a total volume of...
10 ml was found to give the maximum absorbance value (Figure 4), hence, the same volume was used.

Oxidation of ETM by Fe$^{3+}$ and subsequent chelation of Fe$^{2+}$ with either Phen or Bipy was found to occur in neutral medium, but the presence of orthophosphoric acid was necessary to increase the stability of the developed red color chelate by maintaining the desired pH. One ml 0.02 M orthophosphoric acid in a total volume of 10 ml was found adequate in both methods although 0.5-2.0 ml gave the same result. In both methods, the formation of red color complex was found to complete in 5 min and the color was found to be stable up to 60 min (Figure 5).

**Method validation**

The method was validated according to the International Conference on Harmonization (ICH) guidelines [37] for linearity and sensitivity, limit of detection and quantification, precision, accuracy, selectivity and recovery.

**Linearity and sensitivity**

Under optimum conditions, a linear relation was obtained between absorbance and concentration of ETM in the range 0.5-10 µg/ml in method A and 0.8-16 µg/ml in method B. The regression analysis of the plot using the method of least squares was made to evaluate the intercept ($a$), slope ($b$), regression coefficient ($r$) and standard deviations of slope and intercept (Table 2). The high value of the regression coefficient (close to unity) of the regression equation and the negligible value of the intercept corroborate the linearity of the calibration plot. The moderately high sensitivity of the method was indicated by the fairly high value of molar absorptivity and low values of sandell sensitivity. Sandell’s sensitivity ($S$) represents the number of micrograms of the determinant per milliliter of a solution having an absorbance ($A$) of 0.001 for a path length ($l$) of 1 cm. Thus, $S$ [µg cm$^{-2}$] = $10^{-3}/a$, where $a = \frac{(b/Molecular weight of ETM) \times 1000}{c}$, where $b$, molar absorptivity, equals $A/l$, where $c$ is the molar con-
centrations of the determinant and $l = 1$ cm is the path length.

**Limits of detection (LOD) and quantification (LOQ)**

The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae:

$$\text{LOD} = 3.3 \frac{\sigma}{s} \quad \text{and} \quad \text{LOQ} = 10 \frac{\sigma}{s}$$

where $\sigma$ is the standard deviation of five reagent blank determinations and $s$ is the slope of the calibration curve.

**Precision and accuracy**

Intra-day precision and accuracy of the proposed methods were evaluated by replicate analysis ($n = 5$) of calibration standards at three concentration levels (4.0, 6.0 and 8.0 $\mu$g/ml for method A and 4.0, 8.0 and 12.0 $\mu$g/ml for method B). Inter-day precision and accuracy were determined by assaying the calibration standards at the same concentration levels on five consecutive days. Precision and accuracy were based on the calculated relative standard deviation (RSD, %) and relative error (RE, %) of the found concentration compared to the theoretical one, respectively (Table 3).

**Robustness and ruggedness**

The method robustness was tested by making small incremental changes in FeCl₃ and Phen/Bipy concentrations ($n = 3$) and performing the experiments using 4, 6 and 8 $\mu$g/ml ETM (method A) and 4, 8 and 12 $\mu$g/ml ETM (method B). The RSD with the altered FeCl₃ and Phen/Bipy concentrations were < 1.5%. In order to demonstrate the ruggedness of the methods, a drug solution at 4, 6 and 8 $\mu$g/ml ETM (method A) and 4, 8 and 12 $\mu$g/ml ETM (method B) levels were analyzed by four different analysts, and also with three instruments by a single analyst. The inter-analysts RSD were < 1%, whereas the inter-instrumental variation expressed as RSD were < 2%. These low values of intermediate precision demonstrate the robustness and ruggedness of the proposed methods (Table 4).

**Selectivity**

A systematic study was performed to determine the effect of the matrix by analyzing the placebo blank and synthetic mixture containing ETM. The recommended procedures were applied to the analysis of placebo blank and to determine ETM in the synthetic mixture. The absorbance of the placebo blank extract was nearly the same as that of the reagent blank in

Table 2. Sensitivity and regression parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{max}$, nm</td>
<td>510</td>
<td>520</td>
</tr>
<tr>
<td>Linear range, $\mu$g/ml</td>
<td>0.5-10</td>
<td>0.8-16</td>
</tr>
<tr>
<td>Molar absorptivity ($\varepsilon$), l mol⁻¹ cm⁻¹</td>
<td>$2.17 \times 10^4$</td>
<td>$1.65 \times 10^4$</td>
</tr>
<tr>
<td>Sandell sensitivity*, $\mu$g/cm²</td>
<td>0.012</td>
<td>0.016</td>
</tr>
<tr>
<td>Limit of detection (LOD), $\mu$g/ml</td>
<td>0.06</td>
<td>0.10</td>
</tr>
<tr>
<td>Limit of quantification (LOQ), $\mu$g/ml</td>
<td>0.19</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*$\text{Limit of determination as the weight in } \mu\text{g/ml of solution, which corresponds to an absorbance of } A = 0.001 \text{ measured in a cuvette of cross-sectional area } 1 \text{ cm}^2 \text{ and } l = 1 \text{ cm}; \quad Y = a + bX \quad \text{Where } Y \text{ is the absorbance, } X \text{ is concentration in } \mu\text{g/ml, } a \text{ is intercept, } b \text{ is the slope.}$

<table>
<thead>
<tr>
<th>Regression equation, $Y$</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Intercept ($a$)</td>
<td>0.0001</td>
<td>0.004</td>
</tr>
<tr>
<td>Slope ($b$)</td>
<td>0.083</td>
<td>0.062</td>
</tr>
<tr>
<td>Regression coefficient ($r$)</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Standard deviation of $a$ ($S_a$)</td>
<td>0.009</td>
<td>0.003</td>
</tr>
<tr>
<td>Standard deviation of $b$ ($S_b$)</td>
<td>0.001</td>
<td>0.0002</td>
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</tbody>
</table>

Table 3. Evaluation of intra-day and inter-day accuracy and precision

<table>
<thead>
<tr>
<th>Method</th>
<th>ETM taken, $\mu$g/ml</th>
<th>Intra-day accuracy and precision</th>
<th>Inter-day accuracy and precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ETM found, $\mu$g/ml</td>
<td>RE, %</td>
<td>RSD, %</td>
</tr>
<tr>
<td>A</td>
<td>4.0</td>
<td>3.99</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.34</td>
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<td></td>
<td></td>
<td></td>
<td>4.03</td>
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<td></td>
<td></td>
<td>0.76</td>
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<td></td>
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<td>1.52</td>
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<tr>
<td>6.0</td>
<td>5.97</td>
<td>0.50</td>
<td>1.10</td>
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<td></td>
<td></td>
<td>6.04</td>
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<td>0.68</td>
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<td></td>
<td>0.74</td>
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<tr>
<td>8.0</td>
<td>7.97</td>
<td>0.38</td>
<td>1.24</td>
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<tr>
<td></td>
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<td></td>
<td>8.04</td>
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<td></td>
<td></td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.28</td>
</tr>
<tr>
<td>B</td>
<td>4.0</td>
<td>4.02</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
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<td></td>
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<td>4.04</td>
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<td></td>
<td></td>
<td></td>
<td>1.06</td>
</tr>
<tr>
<td>8.0</td>
<td>8.44</td>
<td>2.25</td>
<td>0.97</td>
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<td></td>
<td></td>
<td></td>
<td>8.10</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.28</td>
</tr>
<tr>
<td>12.0</td>
<td>12.6</td>
<td>1.67</td>
<td>1.16</td>
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<td></td>
<td></td>
<td></td>
<td>12.14</td>
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<td>1.47</td>
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<td></td>
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<td></td>
<td>1.08</td>
</tr>
</tbody>
</table>
both methods indicating that there was no interference by the inactive ingredients.

To assess the role of the inactive ingredients on the assay of ETM, a separate experiment was performed with the synthetic mixture. The analysis of the synthetic mixture solution prepared above yielded percent recoveries ranged between 102.8 and 111.3% with standard deviation of 1.09–1.57 (n = 5) in all the cases. The results of this study are presented in Table 5 indicating that the inactive ingredients did not interfere in the assay. These results further demonstrate the accuracy as well as the precision of the proposed methods.

Table 5. Recovery of the drug from synthetic mixture (Mean value of five determinations)

<table>
<thead>
<tr>
<th>Method</th>
<th>ETM in synthetic mixture taken, µg/ml</th>
<th>ETM recovered ± SD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.0</td>
<td>111.3 ± 1.09</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>109.3 ± 1.26</td>
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<tr>
<td></td>
<td>8.0</td>
<td>106.0 ± 1.46</td>
</tr>
<tr>
<td>B</td>
<td>4.0</td>
<td>105.5 ± 1.57</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>107.5 ± 1.34</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>102.8 ± 1.18</td>
</tr>
</tbody>
</table>

Application to analysis of tablets

The proposed methods were successfully applied to the determination of ETM in two brands of tablets and the results are summarized in Table 6. The results obtained were statistically compared with those of the official BP method [38] by applying the Student’s t test for accuracy and F test for precision. The BP method involves cerimetric titration of the drug in H₂SO₄ medium with potentiometric end point detection. As it can be seen from Table 6, the calculated t value and F value at 95% confidence level did not exceed the tabulated values of 2.77 and 6.39, respectively, for four degrees of freedom. The tests indicate that there is no difference between the proposed method and the reference method with respect to accuracy and precision.

Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery experiments through the standard addition method. Pre-analyzed tablet powder was spiked with pure ETM at three different concentration levels (50, 100 and 150% of that in tablet powder) and the total was found by the proposed methods. In all cases, the added ETM recovery percentage values ranged between 98.66 and 107.0% with standard deviation of 0.73-1.51 (Table 7) indicating that the recovery was good, and that the co-formulated substances did not interfere in the determination.

Table 4. Robustness and ruggedness expressed as intermediate precision (RSD, %)

<table>
<thead>
<tr>
<th>Method</th>
<th>ETM taken, µg/ml</th>
<th>Method robustness (parameter altered)</th>
<th>Method ruggedness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RSD, % (n = 3), with FeCl₃a</td>
<td>RSD, % (n = 3), with PTL/BPDa</td>
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<tr>
<td></td>
<td></td>
<td>Inter-analysists</td>
<td>Inter-instruments</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RSD, % (n = 4)</td>
<td>RSD, % (n = 3)</td>
</tr>
<tr>
<td>A</td>
<td>4.0</td>
<td>1.32</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>1.41</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>1.24</td>
<td>1.39</td>
</tr>
<tr>
<td>B</td>
<td>4.0</td>
<td>1.03</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>1.09</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>1.12</td>
<td>1.10</td>
</tr>
</tbody>
</table>

aFeCl₃ and PTL/BPD volumes used were 1.8, 2.0 and 2.2 ml in both methods

Table 6. Results of the analysis of tablets by the proposed methods

<table>
<thead>
<tr>
<th>Tablet brand name</th>
<th>Label claim mg/tablet</th>
<th>Reference method</th>
<th>Found ±SD, % (of label claim)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Method A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100.8 ± 0.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t = 2.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F = 3.81</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>t = 1.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F = 1.47</td>
</tr>
</tbody>
</table>

aMean value of five determinations; bMercury, Lab. Ltd., Vadodara, India; cDr. Reddy’s Lab. Ltd., Solan, India. The value of t at 95 % confidence level and for four degrees of freedom is 2.77; the value of F at 95 % confidence level and for four degrees of freedom is 6.39
CONCLUSIONS

The spectrophotometric methods developed for the determination of ETM use readily available and inexpensive chemicals compared to many reported methods [23]. The methods are selective, sensitive and reproducible. Both methods are based on well characterized complexion reactions and are free from heating or extraction step unlike the reported methods [21,22]. Although the proposed methods are less sensitive to azide-iodine method [17] in terms of linear range of applicability, they are free from stringent experimental conditions unlike the published method [17] which requires scrupulous maintenance of experimental variables. The attractive feature of the method is that it is relatively free from any interference produced from common tablet excipients. An additional advantage of the spectrophotometric methods is that the absorbance is measured at longer wavelength where the interference from excipients is less. Hence, recommended procedures are well suited for the assay and evaluation of drugs in pharmaceutical industrial quality control.

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Table 7. Accuracy assessment by recovery experiments

<table>
<thead>
<tr>
<th>Method</th>
<th>Tablet studied</th>
<th>ETM in tablet, µg/ml</th>
<th>Pure ETM added, µg/ml</th>
<th>Total found, µg/ml</th>
<th>Pure ETM recovered ± SD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Dicynene 250</td>
<td>4.02</td>
<td>2.00</td>
<td>6.03</td>
<td>100.5 ± 1.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.02</td>
<td>4.00</td>
<td>8.14</td>
<td>103.1 ± 0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.02</td>
<td>6.00</td>
<td>9.94</td>
<td>96.66 ± 1.07</td>
</tr>
<tr>
<td>B</td>
<td>Dicynene 250</td>
<td>6.15</td>
<td>3.00</td>
<td>9.13</td>
<td>99.38 ± 1.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.15</td>
<td>6.00</td>
<td>12.35</td>
<td>103.3 ± 1.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.15</td>
<td>9.00</td>
<td>15.78</td>
<td>107.0 ± 0.73</td>
</tr>
</tbody>
</table>
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CI&CEQ 16 (1) 1–9 (2010)

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KANAKAPURA
BASA VAI AH VINAY,
HOSAKERE DOD DAREVA NNA
RE VAN ASIDDAPPA,
OKRAM ZEN ITA DEVI,
KANAKAPURA BASAVAI AH

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Mysore, Manasagangotri, Mysore, India

NAUČNI RAD

SPEKTROFOTOMETRIJSKO ODREĐIVANJE ETAM-
SILATA U FARMACEUTSKIM PREPARATIMA
KOMPLEKSIRANJEM NASTALIH GVOŽĐE(II)JONA
SA 1,10-FENANTROLINOM ILI 2,2'-BIPIRIDILOM

U radu su opisane dve jednostavne i selektivne spektrofotometrijske metode za brzo
određivanje etamsilata (ETM) u rasutom stanju i u kapsulama. Metode se zasnivaju na
oksidaciji ETM gvožđe(III)-hloridom u neutralnoj sredini i naknadnim kompleksiranjem
dobijenog gvožđe(II) jona 1,10-fenantrolinom (Phen) (metoda A) i sa 2,2'-bipiridil (Bipy)
(metoda B). Intenzitet dobijenih crvenih kompleksnih jedinjenja meren je na 510 nm u
metodi A, a na 520 nm u metodi B. Za obe metode nađena je linearna zavisnost
apсорbance i koncentracije ETM-a, koja slijedi Beer-ov zakon za opseg koncentracije
0,5–10 µg/ml (metoda A) i 0,8–16 µg/ml (metoda B). Izračunati su molarni apsorpcioni
koeficijenti, i to 2,17×10^4 za metodu A i 1,65×10^4 l mol⁻¹ cm⁻¹ za metodu B i odgovarajući
Sandelovi indeksi za obe metode koji iznose 0,012 i 0,016 µg/cm ², respektivno. Odre-
dene su dnevna i međudnevna preciznost i tačnost za obe metode prema ICH uput-
stvima. Predložene metode su primenjene za određivanje ETM u dozi leka. Rezultati su
statistički obrađeni i upoređeni sa metodom po Britanskoj farmakopeji.

Ključne reči: etamsilat; određivanje; gvožđe(II)-hlorid; reakcije kompleksiranja;
farmaceutski preparati.