Spectrophotometric Determination of Enalapril Using Tropeolin 00

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Rapid and sensitive spectrophotometric method has been developed for the determination of Enalapril in pharmaceutical formulations. The method is based on the complex formation in acidic media (pH 2), using the Tropeolin 00 and hydrochloric acid. The formed complex is extractable with dichloromethane and shows a maximum absorbance at 415 nm, following the Lambert–Beer law in the concentration ranging between 10 and 100 μg/mL. The developed method has been statistically validated for application in pharmaceutical quality control at the laboratory scale and shows intraday and interday precision of 1.05 % and 1.81% respectively. The recovery tests were ranged between 99.78 and 100.72 % and the detection limit was found at 2.03 μg/mL.

Keywords: Enalapril maleate, Tropeolin 00, VIS spectrophotometry, method validation

The official drug Enalapril is the maleate salt of Enalapril, the ethyl ester of a long-acting angiotensin converting enzyme inhibitor (ACE-inh), Enalaprilat. Enalapril maleate is chemically described as (1-[N-(S)-1-carboxy-3-phenylpropyl]-L-alanyl]-L-proline 1’-ethyl ester, maleate (1:1)), (fig. 1.).

Enalapril is indicated for the treatment of essential and renovascular hypertension. Since the oral absorption of Enalapril is superior to that of Enalaprilat, the former is used in oral dosage form [1]. Many publications have demonstrated that Enalapril, an ACE-inh, is able to reduce cardiovascular mortality and morbidity in patients with heart failure [2, 3].

The few reported methods in the literature for the determination of enalapril: high-performance liquid chromatography (HPLC) [4-9], FT-IR spectroscopy [10, 11], capillary electrophoresis (12, 13), nuclear magnetic resonance spectroscopy (NMR) [14, 15] and gas chromatography [16]. However, the spectrophotometric methods are among the simplest analytical tools, operating at a low cost and offering in the same time the good performances for a wide range of applications (such as: control of drugs, food, environment, etc.), when the analyte is colored or could be converted into a stable color compound [17, 18]. The spectrophotometric methods reported for the determination of the studied drug includes ternary complex formation between palladium (II) and eosin [19], between copper (II) and eosin [20], and with molybdenum (V) thiocyanate [21].

Other methods reported for the determination of Enalapril includes reaction with potassium dichromate and sulfuric acid, complex shows maximum absorption at 574 nm [22], and reaction using potassium permanganate as the oxidimetric reagent in neutral medium with λ_{max} at 340 nm and in acid medium with λ_{max} at 550 nm [23].

Have been reported, like the formation of ion pair complex between Enalapril and bromothymol blue, which is extractable into dichloromethane solvent and determined spectrophotometrically at 410 nm [24].

The aim of the present investigation was to develop accurate, rapid and reproducible method for determination of Enalapril maleate in pharmaceutical preparations. The present method is based on the formation of pale yellow colored ion pair complex between Enalapril and Tropeolin 00 at pH 2, which is easily extracted into an organic solvent such as dichloromethane and then spectrofotometricaly measured at 415 nm (λ_{max}).

Experimental part

Equipment, materials and methods

A UV-Vis Hewlett-Packard 8453 spectrophotometer with quartz cells (1=1 cm) was used for all absorbance measurements. A vibration shaker IKA-Werke type VX2 and ultrasonic bath were also used.

Materials and reagents

Enalapril maleate pur drug (purity 99.9%) as a raw material is provided from, China. The pharmaceutical products Enap, Enalapril LPH and Renitec (with uncoated tablets) have been purchased from the local pharmacies. The dosage forms in each product are as follows: Enalapril maleate per tablet: 5 mg, 10 mg, 20 mg.

All the chemicals used have been of analytical reagent grade.

Tropeolin 00: Sigma Aldrich
Hydrochloric acid 37 %: Chemical
Acetone: Promochem
Methanol chromasolv: Sigma Aldrich
Dichlormetan optigrade: Promochem

Preparation of reagent solutions

The reagent was prepared by dissolving 0.1 g of Tropeolin 00 in 100 mL of distilled water by ultrasonic bath to get 0.1% concentration.

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For a solution of hydrochloric acid $pH$ 2, was measured volume of 0.414 mL of HCl 37 % and was brought to 500 mL volumetric flask.

**Preparation of standard solution**

Stock solution of Enalapril maleate 1 mg/mL: 100 mg Enalapril was dissolved in 1 mL methanol by ultrasonic bath. After dissolution, it was brought quantitatively to 100 mL with distilled water. Standard solution in concentration range 10 - 100 μg/mL was prepared from stock solution by dilution with distilled water.

**Procedure for pharmaceutical formulation**

This stage of the experimental study was conducted according to the Romanian Pharmacopoeia Xth edition [25].

Twenty tablets have been accurately weighed and powdered. A quantity of powder containing 100 mg of Enalapril has been transferred into a 100 mL volumetric flask with 1 mL methanol by ultrasonic bath. After dissolution, was added 60 mL distilled water and then filtered. The filtrate was brought to 100 mL quantity with distilled water. Of this stock solution was made a working solution with concentration 50 μg/mL by dilution with distilled water. The amount of drug was calculated either from the calibration graph or the regression equation.

**General procedure**

For each 1 mL of the standard drug solution in concentration range 10-100 μg/mL Enalapril were added 2 mL of Tropeolin_00 solution 0.1 % followed by 0.5 mL of acetone and 0.5 mL hydrochloric acid $pH$ 2. The complex was twice extracted with 2 mL dichloromethane. The solution was shaken for 2 min each time. Absorbance of combined dichloromethane solutions was measured 20 minutes after extraction. Absorbance of the resulting solution was determined at 415 nm against blank prepared in the same manner.

**Results and discussions**

The formation of ion pair complex was confirmed by studying the absorption curves for each component under the experimental conditions, described above. In fig. 2 are shown the absorption spectra for:

- 1 mL Enalapril maleate 100 μg/mL, 0.5 mL acetone, 0.5 mL HCl $pH$ 2 and 2 mL distilled water in 4 mL dichloromethane against a blank solution containing the same constituent except drug;
- 2 mL Tropeolin_00 0.1 %, 0.5 mL acetone, 0.5 mL HCl $pH$ 2 and 1 mL distilled water in 4 mL dichloromethane against a blank solution containing the same constituent except Tropeolin_00;
- 1 mL Enalapril maleate 40 μg/mL, 2 mL Tropeolin_00 0.1 %, 0.5 mL acetone and 0.5 mL HCl $pH$ 2 in 4 mL dichloromethane against a blank solution containing the same constituent except drug (Enalapril maleate).

![Fig. 2. Absorption spectra of (1) Enalapril maleate, (2) Tropeolin_00 and (3) the complex formed between Enalapril and Tropeolin_00](image)

![Fig. 3. Influence of the pH (adjusted using hydrochloric acid) on the absorbance magnitude at $\lambda_{max} = 415$ nm](image)

**Table 1**

<table>
<thead>
<tr>
<th>No.</th>
<th>Absorbance $\lambda_{max}$ 415 nm</th>
<th>$pH$*</th>
<th>Acetone</th>
<th>Tropeolin_00 Concentration (%)</th>
<th>Tropeolin_00 Volume (mL)</th>
<th>Enalapril 300 μg/mL Chloroform Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2599</td>
<td>3</td>
<td>0</td>
<td>0.025</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.5220</td>
<td>2</td>
<td>0</td>
<td>0.025</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0.1586</td>
<td>4</td>
<td>0</td>
<td>0.025</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>0.5083</td>
<td>1</td>
<td>0</td>
<td>0.025</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>0.5132</td>
<td>1.5</td>
<td>0</td>
<td>0.025</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>0.4274</td>
<td>2.5</td>
<td>0</td>
<td>0.025</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>0.6482</td>
<td>0.25</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>0.7232</td>
<td>0.5</td>
<td>0</td>
<td>0.025</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>0.7234</td>
<td>0.75</td>
<td>0</td>
<td>0.025</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>0.1156</td>
<td>2</td>
<td>0.5</td>
<td>0.0125</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>0.8425</td>
<td>0.5</td>
<td>0</td>
<td>0.0375</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>0.9927</td>
<td>2</td>
<td>0.5</td>
<td>0.08</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>1.7692</td>
<td>0.5</td>
<td>0</td>
<td>0.1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>1.6785</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>1.7932</td>
<td>0.5</td>
<td>0</td>
<td>1.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>1.8202</td>
<td>0.5</td>
<td>0</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>1.7834</td>
<td>0.5</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>1.7785</td>
<td>0.5</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*pH values were adjusted using HCl*
The spectra revealed that organic solutions of Tropeolin easily absorbed in the ultraviolet region at $\lambda_{\text{max}} = 364$ nm and organic solutions of Enalapril do not absorb in the spectral range from 320 nm.

According to these considerations, reaction between Enalapril maleate and Tropeolin in the presence of acetone and HCl ($\text{pH} 2$) leads to the formation of a complex which shows a maximum absorption at 415 nm and is extractable in dichloromethane.

**Optimization of experimental conditions**

The experimental conditions were established by varying each experimental factor and determining the absorbance of the colored product, at maximum wavelength of 415 nm.

The planning matrix of optimization experimental and objective function values (absorbance values) is presented in table 1.

The empirical method of optimization is called "Successive variation of variables" and consists in a choice of the starting point according to previous experience (or according to some preliminary tests) followed by optimum search along each direction (i.e. each variable) performing successive displacement with a constant step, along the given direction of optimum search, keeping constant all other variables [26, 27].

When this optimum compared with first variable was achieved, it is considered as a new starting point in searching optimum, modifying the following variable, given that the other is kept constant. After completing successive search, procedure is restarted until do not differ significantly of goal function value (absorbance) from previous search or until difference between two successive values of the goal function value is less than required by the error of the method [27].

The experimental factors which were taken into consideration in this empirical optimization are:

- $\text{pH}$ of HCl solution (in the range 1 ÷ 5); the results are shown in figure 3 and found maximum absorbance at $\text{pH} 2$;
- influence of acetone (in the range 0 ÷ 0.75 mL); the results are shown in figure 4 and found maximum absorbance at 0.5 mL of acetone;
- influence of Tropeolin concentration (in the range 0.0125 ÷ 0.1 %); The results are shown in figure 5. The optimum concentration was found to be 0.1 %;
- influence of Tropeolin volume, solution with 0.1 % concentration (in the range 0.5 ÷ 3 mL). The results are shown in fig 6 and the optimum volume of Tropeolin, (with concentration of 0.1 %) was found at 2 mL;
- effect of solvent extraction.

For this phase of the study we have worked in the same conditions: at 1 mL Enalapril 300 $\mu$g/mL was added 2 mL Tropeolin 0.1 %, 0.5 mL HCl 0.01 M (coresponding to $\text{pH}$ solution of 2) and 0.5 mL acetone. The complex was extracted with 4 mL organic solvents, against a blank solution containing the same constituent except drug.

In table 2 the solvent influence involved in extraction process is pointed out. It was found that the best solvent for extraction is dichloromethane.

**Study of complex stability.**

In this step of the study, the research followed the general procedure. So, the absorbance was immediately measured after extraction.

**Table 2**

<table>
<thead>
<tr>
<th>Solvent extraction</th>
<th>Absorbance (415 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl$_3$</td>
<td>1.8312</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>1.8753</td>
</tr>
<tr>
<td>CCl$_4$</td>
<td>Not absorb</td>
</tr>
<tr>
<td>CHCl$_3$ : C$_2$H$_5$OH (1:1)</td>
<td>1.7141 Blank colour in yellow</td>
</tr>
</tbody>
</table>

Fig. 4. Influence of the acetone volume on the absorbance magnitude

Fig. 5. Influence of the Tropeolin concentration on the absorbance magnitude

Fig. 6. Influence of the Tropeolin volume (concentration of 0.1 %) on the absorbance magnitude

Fig. 7. The complex stability in time
In figure 7 it is shown the complex stability. The required time to accomplish the full reaction was 20 min and the complex is stable at least 120 min.

Validation of the proposed method

The validation of the proposed method was done according to the present ICH guidelines [28]. In order to validate the UV-Vis spectrophotometric method for Enalapril assay, the following parameters have been studied: linearity, detection and quantification limit, system and method precision, and the accuracy of the method.

Linearity and sensitivity

A linear correlation was found between absorbance at $\lambda_{\text{max}} = 415$ nm and concentration of Enalapril between 10 – 100 µg/mL with correlation coefficient ($r$) of 0.9998.

Within the Beer’s law range for this method, the graph is described by the regression equation: $A = a \cdot C - b$ where $A$ is absorbance, $C$ is concentration of drug in µg/mL, $a$ is the slope and $b$ is the intercept (fig. 8).

The method was validated for all the required parameters and the results were within accepted limits and are shown in table 3. The method has shown that the limit of detection (LOD) and the limit of quantification (LOQ) are of 2.03 µg/mL and 6.16 µg/mL, respectively.

Precision and accuracy

To determine precision, system and method precision studies were performed.

<table>
<thead>
<tr>
<th>Enalapril, µg/mL</th>
<th>Intra-day precision</th>
<th>Inter-day precision</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>found, µg/mL</td>
<td>Recovery %</td>
<td>RSD %</td>
</tr>
<tr>
<td>30</td>
<td>30.04</td>
<td>100.12</td>
<td>1.24</td>
</tr>
<tr>
<td>40</td>
<td>39.70</td>
<td>99.25</td>
<td>0.94</td>
</tr>
<tr>
<td>50</td>
<td>49.76</td>
<td>99.52</td>
<td>0.98</td>
</tr>
</tbody>
</table>

In order to evaluate the precision of the system, ten determinations of the same sample (40 µg/mL) were done under the same experimental conditions, and then the relative standard deviation (RSD = 0.78 %) was calculated, showing that the system is precise.

The precision (intra-day and inter-day) of the method was evaluated taking into consideration ± 25% of the target value (40 µg/mL). Solutions containing tree different concentration of Enalapril were prepared and analyzed in three replicates. The analytical results obtained from this investigation were summarized in table 4.

The RSD developed method has been statistically validated for application in pharmaceutical quality control laboratory and shows intra and inter-day precision of 1.05% and respectively 1.81%.

In order to determine accuracy, the addition method at concentrations of: 30, 40 and 50 µg/mL was used. Accuracy has been evaluated as percentage relative error between the measured and theoretical concentration of enalapril. The results for the precision and accuracy of the proposed method are presented in table 4.

Application on pharmaceutical formulation

The proposed method has been applied for the analysis of Enalapril as commercial tablets. For each sample were made five determinations. The results for the recovery experiment by the proposed method are listed in table 5.

The values of the active substance recovery from pharmaceutical formulation Enap tablets are between 98.05 and 100.10 %, for Enalapril LPH tablets are between

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Labeled amount, mg/tablet</th>
<th>Amount found, mg/tablet</th>
<th>RSD %</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enap</td>
<td>5</td>
<td>4.90</td>
<td>1.16</td>
<td>98.05</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.01</td>
<td>0.87</td>
<td>100.10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.79</td>
<td>1.45</td>
<td>98.96</td>
</tr>
<tr>
<td>Enalapril LPH</td>
<td>5</td>
<td>4.89</td>
<td>1.23</td>
<td>97.85</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.75</td>
<td>1.17</td>
<td>97.50</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.85</td>
<td>1.18</td>
<td>99.27</td>
</tr>
<tr>
<td>Renitec</td>
<td>5</td>
<td>4.91</td>
<td>0.94</td>
<td>98.22</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.91</td>
<td>1.56</td>
<td>99.05</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.65</td>
<td>1.26</td>
<td>98.24</td>
</tr>
</tbody>
</table>

Note:

1. trade name Enap, made by KRKA dd NOVO MAESTRO, Slovenia
2. trade name Enalapril LPH, made by LABORMED PHARMA SA, Romania
3. trade name Renitec, made by MERCK SHARP&DOHME IDEA, SUA
4. Mean value of five determination

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97.5 and 99.27 % and for Renitec tablets are between 98.22 and 99.65 %.

Results show that the commonly used excipients in the preparation of tablets (such as starch, lactose, talc and magnesium stearate) were found not to interfere in the analysis.

All results from uncoated tablets are within permissible deviations presented in Romanian Pharmacopoeia X.

Conclusions
The proposed method described in this paper is simple, rapid and applicable for routine analysis of Enalapril maleate in pharmaceutical formulations over a wide concentration range without interference from common excipients. Moreover, it exhibits the advantage of being convenient at low cost without losing accuracy. Therefore, the method should be useful for routine analytical and quality control assay of the investigated drug in bulk as well as in their tablets.

References

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