Analytical method validation of eperisone hydrochloride in tablet dosage form by tlc–densitometry

Vinda Aisyah Vira¹, Nia Kristiningrum¹*, Aisyah Rahmatullah¹

¹Faculty of Pharmacy, Jember University, Indonesia.

*Corresponding author: (Nia Kristiningrum)
Email: niakristiningrum.farmasi@unej.ac.id

Abstract.

Context: Eperisone HCl is a muscle relaxant and vasodilator that is related to how the drug works on the central nervous system and vascular smooth muscle, works on peripheral centers and has analgesic action. One method of analyzing single substances from Eperisone HCl is Thin Layer Chromatography (TLC)–Densitometry. Aims: The purpose of this research to determinate the Eperisone hydrochloride level in tablet dosage form. Methods and Material: Using Thin Layer Chromatographic (TLC)–densitometry method with methanol as solvents, methanol p.a: ammonium p.a (100:1) as the mobile phase, pre–coated TLC Silica Gel 60 F254 plate as the stationary phase and followed by densitometric measurements of their spots at 267 nm. Statistical analysis used: The parameters that used for the interpretation of analytical method validation results are the calculation of retention factor (Rf) value, correlation coefficient (r), relative standard deviation (RSD), and % recovery. Results: This method shows good selectivity/specificity with Rf value 0.6 ± 0.02. The linearity signal using 10 standard concentrations of 50 mg L⁻¹ to 500 mg L⁻¹ gives a good linear relationship with r = 0.994 04. Limit of detection and limit of quantitation are (40.545, 121.637) mg L⁻¹ respectively. Precision are tested for three different days and it’s found that the RSD value is 0.8 %. Accuracy with the results of the % recovery value in various additions is 99.3 % ± 0.9 %. Conclusions: This method was found to be fast, specific, precise and accurate also can be successfully applied for the analysis of Eperisone HCl in marketed dosage forms.

Keywords: Antispasmodic drugs, chromatography, accuracy, precision

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Introduction

Eperisone HCl is a new generation of antispasmodic drugs,[1] generally prescribed as an analgesic for acute lower back muscle pain with effective therapeutic doses of 300 mg d–1[2] and lower back pain in outpatients at the Poliklinik Rumah Sakit Umum Sragen (Sragen Public Hospital Polyclinic).[3] Eperisone HCl level determination test is still hasn’t been listed in the official reference book or doesn’t have a published level determination method yet, so it’s necessary to make an Eperisone HCl analysis method. A new analytical method before being proposed and published replaces the previous method as a standard analysis method must be proven its validity with the validation[4].

In this research, a new validation method will be developed to analyze the determination of Eperisone HCl levels in tablet dosage forms using the TLC–densitometry method. The TLC method has more advantages if compared to the UV/Vis spectrophotometry method, which are easier sample separation, can be done rapidly, has a higher level of precision, has a wider scope of analysis, also can separate and analyze samples (identification and separation). Whereas when compared with the HPLC method, the TLC method give a relatively simple methodology, the cost-effectiveness and the low amounts of solvents used[5].

Materials and Methods

Materials and reagents

Standard of Eperisone HCl, ammonium p.a (Merck), methanol p.a (Merck), technical methanol, and tablets on the market containing Eperisone HCl (sample A and B).

Methods

Optimization of analysis conditions

The conditions of analysis that need to be optimized are solvents for sample preparation, mobile/eluent phase, observation wavelength, and analyte concentration.

i. Solvent optimization for sample preparation
   Solvent selection was carried out with the composition: Methanol, 70 % Ethanol, and Water. Observation of analyte solubility is done visually.

ii. Eluent optimization
   Eluents were selected with the following compositions: p.a 100 % methanol, Methanol p.a: ammonium p.a (100:1), and Ethanol p.a: ammonium p.a (100:1).
   The most optimum eluent assessment is based on the chromatogram efficiency parameter, such as the largest theoretical plate value, the smallest HETP (height equivalent of theoretical plate) value, and Resolution value is ≥ 1.5.

iii. Wavelength optimization (λ)
   Wavelength optimization was done by scanning the analyte spot on Camag TLC Scanner 3 (Densitoscanner) and Wincats program software. Initial scanning was carried out at wavelength of 200 nm to 400 nm. The optimum wavelength
evaluation was obtained by looking at the spectrum of analytes that are read at the maximum wavelength.

iv. Analytical concentration optimization
Optimization was done by selecting analytes at (100, 150, 200, 250, 300) mg L$^{-1}$. The most optimal assessment of analyte concentration is based on the chromatogram parameters, which are the largest theoretical plate value, HETP value, and the Rf value.

Validation of analysis methods

i. Specificity or selectivity
The selectivity or specificity of an analytical method is its ability to measure the levels of specific analytes accurately in addition to other components contained in the sample matrix$^{[4]}$. Specificity/selectivity in this research was determined by using samples and standards in selected concentration which was spotted as much as 2 µL on Silica Gel F$_{254}$ TLC plate. In TLC–densitometry, specificity can be measured by comparing the correlation of purity and identity through the spectral peak of samples and standards observation. Correlations were assessed by comparing spectra at three different levels, which are peak–start (S), peak–apex (M) and peak–end (E) positions$^{[6]}$.

ii. Linearity
The linearity of a method can be tested using several Eperisone HCl standard concentrations of (50, 100, 150, 200, 250, 300, 350, 400, 450, 500) mg L$^{-1}$, then each standard was spotted as much as 2 µL on the Silica Gel F$_{254}$ plate using selected conditions and finally the standard data area was generated. Area and concentration data were analyzed using a validation program. The parameters assessed in the validation program for linearity testing are correlation coefficients, which show a proportional relationship between concentration and area. Scanning results by TLC–densitometry produce linearity between concentrations and areas of Eperisone HCl standard$^{[6]}$.

iii. Limit of detection (LOD) and limit of quantitation (LOQ)
The limit of detection was used to determine the limits of the concentration of analytes that are still detected by the instrument, while the limit of quantitation is the concentration of analytes that can be quantified on precision and accuracy. The determination of LOD and LOQ in this research was evaluated through 10 standard concentrations of (110, 120, 130, 150, 160, 170, 180, 190, 200) mg L$^{-1}$. Furthermore, it was spotted as much as 2 µL on the Silica Gel F$_{254}$ TLC plate. The TLC plate was analyzed and the detection limit parameters and quantitation limits can be calculated from the scanning data with a validation program$^{[6]}$.

iv. Precision
Precision indicates the compatibility between each test result if the analysis procedure is applied repeatedly in a number of samples taken from a homogeneous sample and determined by calculating the relative standard deviation (RSD) value. In this research, repeatability was carried out with the accuracy of the methods performed on the same sample, repeated by the same analyst, the same conditions and in short time intervals$^{[6]}$. To test the precision of tablet preparations, samples were made with 250 mg L$^{-1}$ of Eperisone HCl (replication six times) and tested for 3 d. From the scanning data with the validation program, the value of precision, SD and RSD can be calculated where the RSD value cannot be > 2 %$^{[6]}$. 
v. Accuracy

Accuracy is the closeness of the results of analyte measurements in samples obtained from a method (experimental results) compared to the actual analyte levels\(^6\). Accuracy is often expressed in percent recovery (% recovery). Calculation of accuracy in this research using the addition sample method, then calculating the % recovery of experimental concentration compared to theoretical concentration.

Determination of accuracy was performed by making additive samples of tablet preparations in certain batch numbers with the addition of active compound (Eperisone HCl standard) of (30, 45, 60) % (6 × replication) and Eperisone HCl standard in methanol with a concentration of (150, 200, 250, 300, 350) mg L\(^{-1}\). Furthermore, it was spotted as much as 2 µL on the Silica Gel F\(_{254}\) TLC plate.

Value of accuracy was obtained by analyzing and calculating the scanning data of TLC plate with a validation program\(^6\).

*Determinations of eperisone–HCl levels in tablets*

Standard preparation was done by making Eperisone HCl standard in methanol at the concentration of (150, 200, 250, 300, 350) mg L\(^{-1}\). Furthermore, eluent preparation was carried out by mixing the selected eluent with a measure pipette. Weighed the sample containing 25 mg eperisone HCl (2 × replication), dissolved and diluted it with methanol so that it contains a concentration of 250 mg L\(^{-1}\) for sample preparation. After that, the vessel of TLC or chamber was saturated by eluent. Then each standard solution and sample was spotted to the TLC Silica Gel F\(_{254}\) plate as much as 2 µL with micropipette. TLC plate was developed by inserting the plate into a saturated vessel, wait the eluation until it reaches the boundary mark, then dry and observe the spots formed under UV light. Finally, KLT was analyzed with the help of computers and Densitometer Scanner Camag 3 devices at selected wavelengths of optimization result. From the comatogram, the levels of % w/w eperisone HCl can be calculated in the sample.

*Statistic analysis*

The parameters that used for the interpretation of analytical method validation results are the calculation of retention factor (Rf) value, correlation coefficient (r), relative standard deviation (RSD), and % recovery.

*Result*

*Optimization of analysis conditions*

From the optimization results, the most optimum analysis conditions for Eperisone HCl analysis include:

i. Solvents: methanol
ii. Eluent or mobile phase: methanol p.a: ammonium p.a (100:1)
iii. Stationary phase: Silica Gel F\(_{254}\) TLC Plate.
iv. Wavelength: 267 nm
v. Test concentration: 250.0 mg L\(^{-1}\)
Validation of the analysis method

Selectivity or specificity

Specificity can be obtained through purity and identity test which can be seen in Fig 1 (a) and Fig 1 (b). Based on the picture, it can be seen that spectra of standards and samples at wavelengths of 200 nm to 400 nm have the same spectra. It means that in the sample there is an Eperisone HCl compound. Calculation of spectra correlation in purity and identity test was performed in Table 1. The values of $r(s, m)$ and $r(m, e)$. The $r(s, m)$ is the correlation of spectra taken at the initial peak of the slope with the maximum peak. Whereas $r(m, e)$ is the correlation of spectra taken at the maximum peak at the peak of the end slope, so that it can be seen that the spectra of values $r(s, m)$ and $r(m, e)$ from densitometry data are more than 0.990. It shows this method is selective for Eperisone HCl.

**Fig 1.** (a) sample and standard spectra in the purity test and (b) sample and standard spectra in the identity test

- red = spectra of Eperisone HCl standard
- green = spectra of sample containing Eperisone HCl
Table 1. Scanning results of the spectrum from densitometry

<table>
<thead>
<tr>
<th>Trac k</th>
<th>Rf</th>
<th>Compound</th>
<th>R(s,m)</th>
<th>R(m,e)</th>
<th>Conclusion</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.62</td>
<td>Eperisone HCl</td>
<td>0.999 232 0.999 436</td>
<td></td>
<td>Purity</td>
<td>Eperisone HCl</td>
</tr>
<tr>
<td>2</td>
<td>0.60</td>
<td>Eperisone HCl</td>
<td>0.999 640 0.999 525</td>
<td></td>
<td>Purity</td>
<td>Eperisone HCl</td>
</tr>
</tbody>
</table>

**Linearity**

The parameters that assessed in the validation program for linearity testing is correlation coefficients, which show a proportional relationship between concentration and area. The results of TLC densitometry scanning produced concentrations and areas of Eperisone HCl standard which can be seen in Table 2 and the proportional (linear) relationship between the concentration and standard area of Eperisone HCl can be seen in Fig 2. Based on the data in Table 2 and Fig 2, the correlation coefficient value generated from the method has met the requirements for linearity with a linear regression value \((r) > 0.99\) and value of \(V \times 0 < 5\%\)\(^b\).

Table 2. Correlation coefficients between concentrations and areas of Eperisone HCl standard in linearity experiments.

<table>
<thead>
<tr>
<th>Concentration (mg L(^{-1}))</th>
<th>Mass (ng)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>200.0</td>
<td>400.0</td>
<td>2 667.42</td>
</tr>
<tr>
<td>250.0</td>
<td>500.0</td>
<td>3 284.38</td>
</tr>
<tr>
<td>300.0</td>
<td>600.0</td>
<td>3 922.24</td>
</tr>
<tr>
<td>350.0</td>
<td>700.0</td>
<td>4 235.07</td>
</tr>
<tr>
<td>400.0</td>
<td>800.0</td>
<td>4 775.54</td>
</tr>
</tbody>
</table>

Linear Regression

\[(r) > 0.99\]

\[
\text{Area} = 676.8 + 5.167\times \\
(r) = 0.99404
\]

Fig 2. The linearity curve of the concentration and area of Eperisone HCl standard.
Limit of detection (LOD) and limit of quantitation (LOQ)

The results of scanning by TLC densitometry show the concentration and area data of the Eperison HCl standart as in Table 3. Areas and concentrations data were analyzed using a validation program. Concentration and area data were analyzed using validation programs. Based on analysis data from Table 3 with using a validation program, the limit detection and quantitation values of Eperisone HCl were obtained with a LOD of 40.545 mg L$^{-1}$ and a LOQ of 121.637 mg L$^{-1}$. The LOD and LOQ on precision and accuracy can be seen in Fig 3.

Table 3. Correlation coefficients between concentrations and areas of Eperisone HCl standard in the LOD and LOQ experiments.

<table>
<thead>
<tr>
<th>Concentration (mg L$^{-1}$)</th>
<th>Mass (ng)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>140.0</td>
<td>280.0</td>
<td>4 221.78</td>
</tr>
<tr>
<td>150.0</td>
<td>300.0</td>
<td>4 533.95</td>
</tr>
<tr>
<td>160.0</td>
<td>320.0</td>
<td>4 904.08</td>
</tr>
<tr>
<td>168.0</td>
<td>336.0</td>
<td>5 035.04</td>
</tr>
<tr>
<td>180.0</td>
<td>360.0</td>
<td>5 361.46</td>
</tr>
</tbody>
</table>

Linear Regression

\[ \text{Area} = 278.280 4 + 14.201 07x \]

\( (r) > 0.99 \)

\( (r) = 0.994 \)

Fig 3. Graphic of LOD and LOQ

Precision

In the precision test of tablet dosage form, samples were made with Eperisone HCl in 250 mg L$^{-1}$ (replication six times) and tested for 3 d and obtained the results as in Table 4. Precision data for 3 d of experimentation giving the mean of relative
standard deviation is 0.8 %. It can be concluded that this method has fulfilled the precision requirements with an RSD value of less than 2 %\[^6\].

**Table 4. Three–day precision data of experiments with n= 6**

<table>
<thead>
<tr>
<th>Day</th>
<th>RSD (n= 6)</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9 %</td>
<td>100.4 %</td>
</tr>
<tr>
<td>2</td>
<td>0.9 %</td>
<td>99.7 %</td>
</tr>
<tr>
<td>3</td>
<td>0.7 %</td>
<td>99.2 %</td>
</tr>
<tr>
<td>Mean</td>
<td>0.8 % &lt; 2 %</td>
<td>99.7 %</td>
</tr>
</tbody>
</table>

**Accuracy**

Accuracy calculations in this research used addition sample method, then calculated % recovery of experimental concentration compared to theoretical concentration. Measurements were made with three replications to obtain data such as Table 5. Based on the table it is known that % recovery in various additions is 99.3 % ± 0.9 %. This value is in the permissible range of 98 % to 102 %\[^7\], so this method meets the requirements for accuracy.

**Table 5. Results of meanaccuracy**

<table>
<thead>
<tr>
<th>Addition (%)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>99.8 %</td>
</tr>
<tr>
<td>45</td>
<td>98.8 %</td>
</tr>
<tr>
<td>60</td>
<td>101.4 %</td>
</tr>
<tr>
<td>Mean</td>
<td>99.3 % ± 0.9 %</td>
</tr>
</tbody>
</table>

**Determination of Eperisone HCl Levels**

After validating the analysis method, the last stage of this research was the application of Eperisone HCl analysis methods in tablet dosage form on the market. The samples used were samples A and B. The samples were determined by validated TLC–Densitometry method, so that the data of Eperisone HCl levels in samples A and B were obtained as shown in Table 6. The mean concentration value of tablet A is 100.6 % while in tablet B it is 98.8 %. Because monographs of Eperisone HCl tablets has not been listed in any official reference book, for reference of the concentration range values are allowed to use the requirements of other drug preparations which have the same therapeutic effect\[^8\] as antispasmodics and skeletal muscle relaxants such as Tizanidine HCl tablets which has requirements for the range of 90.00 % to 110.00 %\[^9\].
Table 6. Determination of Eperison HCl levels in tablet dosage form

<table>
<thead>
<tr>
<th>Sample</th>
<th>Theoretical Concentration (mg L(^{-1}))</th>
<th>Experimental Concentration (mg L(^{-1}))</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>250.0</td>
<td>251.5 ± 0.6</td>
<td>100.6 % ± 0.2 %</td>
</tr>
<tr>
<td>B</td>
<td>250.0</td>
<td>247.2 ± 1.4</td>
<td>98.8 % ± 0.5 %</td>
</tr>
</tbody>
</table>

Discussion

In this research, the method of analysis and determination of Eperisone HCl level in tablet using TLC densitometry was validated. The first step is to optimize the analysis conditions, then proceed with the validation of analysis method and determination of Eperisone HCl level in tablet samples on the market. The conditions of analysis that need to be optimized are solvents for sample preparation, mobile/eluent phase, observation wavelength, and analyte concentration. For the solvent optimization, methanol was chosen because according to the existing literature, eperison HCl spectra can be detected in methanol solvents\(^{[10]}\). In addition, methanol is also cheaper than ethanol. Eluent optimization performed methanol pa: ammonium pa (100:1) as eluent which produces a chromatogram according to the chromatogram efficiency parameter with the greatest theoretical plate value (N), the smallest H (plate height) value with duration of saturation and eluation for 20 min. Optimization of wavelength showed the wavelength of 267 nm Eperison HCl gives maximum absorbance. Based on the results of the test concentration optimization, it known that the most optimum test concentration which can be seen from the chromatogram efficiency is 250 mg L\(^{-1}\), so that concentration value is used as the optimum concentration. Based on the results of testing the parameters of specificity, linearity, sensitivity, precision and accuracy, it is known that the method of analyzing Eperisone HCl in tablet dosage form by TLC–Densitometry produces valid data. Determination of Eperison HCl levels using validated TLC–Densitometry in tablet samples in the market has met the requirements of the range of 90.00 % to 110.00 %, as in sample A of 100.6 % ± 0.2 % and Sample B of 98.8 % ± 0.5 %. So that this method is found to be fast, specific, precise, accurate and can be successfully applied to the analysis of Eperisone HCl in marketed dosage forms.

Conclusion

This method was found to be fast, specific, precise and accurate also can be successfully applied for the analysis of Eperisone HCl in marketed dosage forms.

Acknowledgement

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References


