DEVELOPMENT AND VALIDATION OF A RP–HPLC METHOD FOR QUANTIZATION STUDIES OF ALBENDAZOLE SUSPENSION DOSAGE FORMS OF ROMBENDAZOL

DEZVOLȚAREA ȘI VALIDAREA METODEI RP–HPLC DE DETERMINARE CANTITATIVĂ A ALBENDAZOL DIN SUSPENZII ROMBENDAZOL

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Rezumat

A fost dezvoltată și validată o metodă isocrată de lichid cromatografie de performanță înaltă pentru determinarea cantitativă a albendazol din suspensii Rombendazol. Separarea HPLC a fost realizată prin cromatografie în fază inversă pe o coloană Kromasil C18 (mărimea particulelor 5 µm; 150 x 4.6 mm diametrul intern), termostată la 25°C. Faza mobilă a fost metanol/apa (65/35 v/v), cu debit de 1,2 ml/min. și detecție UV la 308 nm. Pentru validarea metodei au fost urmăriți următorii parametri - linearitatea ($r^2=0,9999$), intervalul, precizia, acuratețea, specificitatea, cantitatea minima decelabilă LOD, cantitatea minimă măsurabilă LOQ. Metoda descrisă poate fi utilizată cu succes pentru analiza compusului farmaceutic activ din suspensii

Cuvinte cheie: albendazol, suspensie, UV-VIS cromatografie de lichide de performanță ridicată de fază inversă, validare

Summary

An isocratic high-performance liquid chromatography (HPLC) procedure was developed and validated for the quantitative determination of albendazole in suspension of Rombendazole. HPLC separation was carried out by reversed phase chromatography on Kromasil C18 (150 mm x 4.6 mm i.e.; 5 µm particle size), held in thermostat at 25°C. The mobile phase consisted of Methanol/ Distilled water (65/35 v/v), with a flow rate of 1.2 ml/min and UV detection at 308 nm. In order to validate the method, the following parameters have been investigated - linearity ($r^2=0.9999$), range, precision, accuracy, specificity, limit of detection LOD and limit of quantification LOQ. The described method can be successfully applied for the analysis of the active pharmaceutical compound in suspensions.

Key words: albendazole, suspension, reversed phase high performance liquid chromatography RP-HPLC UV-VIS, validation

This paper aimed to develop and validate an HPLC sensitive applicable method to determine the quantity of albendazole in suspensions of Rombendazole, contributing to the quality and safety control of these types of pharmaceutical preparations.

Materials and methods

Reagents

The standard reference albendazole has been provided by SIGMA (Germany). Methanol and hydrochloric acid have been provided by MERCK (Germany).

The suspensions of albendazole (Rombendazole 2.5%, Rombendazole 5%, Rombendazole 10%, Rombendazole plus) have been provided by Romvac Company and used during shelf-life.

All the chemical substances used had pharmaceutical or analytical degree. Double distilled water, filtered on 0.45 µm membrane was used.

System and chromatographic conditions

HPLC method was carried out on a LC Surveyor (Thermo Electron Corporation, USA) provided with quaternary pump, auto sampler, 25 µl loop and UV-VIS detector – diode array (Thermo Electron Corporation, USA).

The integration of chromatographic peaks has been carried out with the ChromQuest soft (Thermo Electron).

The analyses have been performed by using a Kromasil C18 (5 µm particle size; 150 x 4.6 mm inner diameter).

The samples have been isocratically eluate in methanol and water (65/35 v/v), with flow of 1.2 ml/.

Each sample has been filtered before injection with PVDF 0.45 µm filter (Thermo Electron).
The injection volume of the sample was 5µl, and detection was carried out at 308 nm, at 25°C.

Preparing the standard reference solutions
Albendazole standard working solution had a final concentration of 0.2 mg/ml, prepared in mobile phase.

The standard solutions for linearity fell within the area of 0.02-0.40 mg/ml starting from a stock solution of albendazole of 1 mg/ml prepared in 1% hydrochloric acid in methanol. All samples have been triplicated. The stock solution of albendazole is kept at +4°C for one week.

Preparing the test solutions
Weigh an appropriate amount of suspension into a 50 ml flask to obtain a concentration of 0.2 mg/ml albendazole, dilute to volume with methanol and keep at ultrasound for 20 minutes. Before injection, filter the solutions through a 0.45 µm PVDF filter.

Chromatographic method validation
After establishing the chromatographic conditions, the method has been validated by observing the following parameters: linearity, working range, precision, accuracy, limit of detection, limit of quantification, specificity and system compliance, using ICH guide.

Linearity and working range
The analytical curve has been obtained with 6 different concentrations of albendazole placed between 0.02–0.4 mg/ml, prepared in triplicate.

The linearity was evaluated by linear regression analysis.

The system has been balanced for minimum 30 minutes. 3 replicates have been injected from each concentration of standard albendazole at a volume of 5 µl, in order to verify the reproducibility of the detector response at each level of concentration.

Precision
The method precision has been determined through repeatability (same day) and the intermediate precision (different days).

The repeatability has been determined through 12 repeated analyses of the same test sample, on the same day, in the same experimental conditions.

The intermediate precision of the method has been determined through the analysis during 2 days (same day), and by other analyst within the same laboratory (different analysts).

Accuracy
In order to certify the accuracy of the recommended method, 9 samples have been analyzed using 3 levels of concentration which cover the working range.

System compliance
In order to ensure the validity of the analytical method, the test of system compliance has been carried out. 6 samples with 0.2 mg/ml albendazole have been injected on this purpose at a volume of 5 µl.

The evaluation of the system compliance has been carried out with the ChromQuest soft, by analyzing the parameters – area, retention time and asymmetry.

Analysis of albendazole in suspension
The analysis of the content in albendazole in suspensions of Rombendazole has been carried out under the developed method recommended for validation, using the reference standard.

Results and discussions
In order to determine the quantity of albendazole in Rombendazole suspensions, a HPLC method of reversed phase has been suggested, choosing the optimum conditions of chromatographic separation.

The analysis of the chromatograms reveals that there are no interferences between the compound of interest and the rest of the matrix constituents, the retention time for albendazole being 8.115 min.

The asymmetry of the peak was good, equal to 1.0.

The calibration curves for albendazole have been formed by representing the peak area towards concentration.

The linearity has been observed in the selected reference field.

The concentration range was 10 – 200% towards the working concentration.

The method precision represents the degree of compliance between the results of the individual tests, through repeated
application of the method on multiple samples of a homologue batch.

Repeatability has been studied by calculating the relative standard deviation (RSD) of 12 samples with a concentration of 0.2 mg/ml albendazole, carried out on the same day and experimental conditions.

The intermediate precision involves the estimation of the variability of analysis when the method is used in different laboratories, on different days, by different analysts or with different equipment.

The results are detailed in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0.2 mg/ml</td>
</tr>
<tr>
<td>RSD% (same day)</td>
<td>0.219%</td>
</tr>
<tr>
<td>RSD% (different day)</td>
<td>0.574%</td>
</tr>
</tbody>
</table>

The accuracy of method is the degree of similarity between the results practically obtained with the method, compared to the theoretical value.

The accuracy has been determined by analyzing 9 samples with albendazole, in concentration of 80, 100, 120% towards the suggested working concentration (0.16, 0.20, 0.24 mg/ml).

**Table 2**

<table>
<thead>
<tr>
<th>Theoretical amount mg/ml</th>
<th>% Recovery a</th>
<th>% Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16</td>
<td>104.04%</td>
<td>90.08%</td>
</tr>
<tr>
<td>0.20</td>
<td>85.961%</td>
<td></td>
</tr>
<tr>
<td>0.24</td>
<td>80.238%</td>
<td></td>
</tr>
</tbody>
</table>

a mean of 3 replicates

The analysis of data presented in Table 2 reveals that the method is accurate within the recommended range, the average recovery rate being 90.08% for the compound of interest- albendazole.

In order to evaluate the resolution and reproducibility of the recommended system of analysis, compliance tests have been carried out.

The results presented in Table 3 prove that the parameters are within the limits of compliance.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>RSD (%)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymmetry</td>
<td>1.07941</td>
<td>1.08978</td>
<td>0.371</td>
<td>complies</td>
</tr>
<tr>
<td>Retention time Area</td>
<td>8.083</td>
<td>8.135</td>
<td>0.254</td>
<td>complies</td>
</tr>
</tbody>
</table>

The limits of detection and quantification have been calculated, reaching the following values:

**Table 4**

<table>
<thead>
<tr>
<th>Component</th>
<th>LOD mg/ml</th>
<th>LOQ mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>0.000061</td>
<td>0.000203</td>
</tr>
</tbody>
</table>

**Conclusions**

1. The results presented for the validation of RP–HPLC method prove its accuracy, linearity and precision and show the limits of detection and quantification.
2. The method can be successfully used for the quantification of albendazole as active substance in the suspension.
3. The recommended method provides the advantage of using a comfortable analytical method, which requires a simple preparation of samples. Therefore, the method can be used for the routine analysis.

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