DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR THE QUANTITATION STUDIES OF FIPRONIL IN PARAKILL SUSPENSIONS

DEZVOLTAREA SI VALIDAREA METODEI RP-HPLC DE DETERMINARE CANTITATIVA A FIPRONIL DIN PARAKILL

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Summary

An isocratic high-performance liquid chromatography (HPLC) procedure was developed for the quantitative determination of fipronil in suspensions of Parakill. HPLC separation was carried out by reversed phase chromatography on Betasil C18 (250 mm x 4.6 mm i.d.; 5 µm particle size), held in thermostat at 25°C. The mobile phase consisted of Acetonitrile/ Distilled water (60/ 40 v/ v), with a flow rate of 1 ml/ min and with UV detection at 220 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 and limit of quantification. The described method can be successfully applied for the analysis of the active pharmaceutical compound in PARAKILL suspensions.

Key words: fipronil, Parakill, reversed phase high performance liquid chromatography RP–HPLC, UV–VIS, validation

Rezumat

A fost dezvoltată și validată o metodă isocratică de lichid cromatografie de performanță înaltă pentru determinarea cantitativă a fipronil din suspensie Parakill. Separarea HPLC a fost realizată prin cromatografie în fază inversă pe o coloană BETASIL C18 (mărimea particulelor 5 µm; 250 x 4,6 mm diametrul intern), termoostatată la 25°C. Faza mobilă a fost acetonitril/ apă (60/ 40 v/ v), cu debit de 1 ml/ min. și detecție UV la 220 nm. Pentru validarea metodei au fost urmăriți următorii parametri–liniaritatea ($r^2=0.9999$), intervalul, precizia, acuratețea, specificitatea, cantitatea minimă decelabilă LOD, cantitatea minimă măsurabilă LOQ. Metoda descrisă poate fi utilizată cu succes pentru analiza compusului farmaceutic activ din suspensie PARAKILL.

Cuvinte cheie: fipronil, Parakill, UV–VIS cromatografie de lichide de performanță ridicată de fază inversă, validare

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

Materials and methods

Reagents

The standard reference fipronil has been provided by SIGMA (Germany). Acetonitrile has been provided by MERCK (Germany). The suspensions of Parakill 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 µ 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 Betasil C18 (5 µm particle size; 250 x 4.6 mm inner diameter).

The samples have been isocratically eluted in acetonitrile and water (60/ 40 v/ v), with flow of 1 ml/ min. Each sample has been filtered before injection with PVDF 0.45 µm filter (THERMO ELECTRON). The injection volume of the sample was 6 µl, and detection was carried out at 220 nm, at 25°C.

Preparing the standard reference solutions

Fipronil standard working solution had a final concentration of 0.1 mg/ ml, prepared in mixture of acetonitrile/ water 3/ 2. The
standard solutions for linearity fell within the area of 0.05 – 0.15 mg/ml starting from a stock solution of fipronil of 1 mg/ml prepared in mixture of acetonitrile/water 3/2. All samples have been triplicate. The stock solution of fipronil 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 1 mg/ml fipronil, dilute to volume with mixture of acetonitrile/water 3/2 and keep at ultrasound for 20 minutes (stock solution). Prepare the working solution daily, by diluting the stock solution in mixture of acetonitrile/water 3/2, in order to obtain working concentrations of fipronil of 0.1 mg/ml. 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 5 different concentrations of fipronil placed between 0.05–0.15 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 fipronil at a volume of 6 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 intermediate precision (different days). The repeatability has been determined through 12 repeated analyses of the same test sample of Parakill, 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 0.1 mg/ml fipronil have been injected on this purpose at a volume of 6 µl. The evaluation of the system compliance has been carried out with the ChromQuest soft, by analyzing the parameters – area, retention time and asymmetry.

The analysis of fipronil in suspension
The analysis of the content in fipronil in the suspension of PARAKILL 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 fipronil in the suspension of PARAKILL 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 16.518 min. The asymmetry of the peak was good, equal to 1.0.

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

The linearity has been observed in the selected reference field. The concentration range was 50 – 200% towards the working concentration.

By applying the linear regression for the calibration curve, a coefficient of determination \( r^2 = 0.995708 \) has been established.
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 0.1 mg/ml fipronil, 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
Concentration, precision and intermediate precision in HPLC method for fipronil

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0.1 mg/ml</td>
</tr>
<tr>
<td>RSD% (same day)</td>
<td>0.187%</td>
</tr>
<tr>
<td>RSD% (different day)</td>
<td>0.170%</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 fipronil, in concentration of 80, 100, 120% towards the suggested working concentration (0.08, 0.10, 0.12 mg/ml).

Table 2
Recovery of fipronil from samples analyzed through RP-HPLC

<table>
<thead>
<tr>
<th>Theoretical amount mg/ml</th>
<th>% Recovery</th>
<th>% Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>112.500%</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>110.318%</td>
<td>119.052%</td>
</tr>
<tr>
<td>0.12</td>
<td>127.785%</td>
<td></td>
</tr>
</tbody>
</table>

* mean of three replicates

The analysis of data presented in Table 2 reveals that the method is accurate within the recommended range, the average recovery rate being 119.052% for the compound of interest - fipronil.

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 3
Results of the system compliance test for fipronil

<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>1.02859</td>
<td>1.03278</td>
<td>0.305</td>
<td>complies</td>
</tr>
<tr>
<td>Retention time</td>
<td>16.590</td>
<td>16.629</td>
<td>0.185</td>
<td>complies</td>
</tr>
<tr>
<td>Area</td>
<td>4554254</td>
<td>4566563</td>
<td>0.094</td>
<td>complies</td>
</tr>
</tbody>
</table>

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

Table 4
Limit of detection and limit of quantification for fipronil

<table>
<thead>
<tr>
<th>Component</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fipronil</td>
<td>0.003083 mg/ml</td>
<td>0.010275 mg/ml</td>
</tr>
</tbody>
</table>

Concluzii

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 fipronil as active substance in 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.

Bibliography

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