DEVELOPMENT AND VALIDATION OF A RP- HPLC METHOD FOR THE QUANTITATION STUDIES OF IVERMECTIN IN SOLUTIONS DOSAGE FORMS

DEZVOLTAREA ŞI VALIDAREA METODEI RP- HPLC DE DETERMINARE CANTITATIVĂ A IVERMECTIN DIN SOLUŢII

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Rezumat

A fost dezvoltată şi validată o metodă isocratică de lichid cromatografie de performanţă înaltă pentru determinarea cantitativă a ivermectin din soluţii. Separarea HPLC a fost realizată prin cromatografie în fază inversă pe o coloana Kromasil C18 (mărimea particulelor 5 µm; 150 x 4.6 mm diametrul intern), termostatată la 25°C. Faza mobilă a fost metanol/ apă (90/10 v/v), cu cu debit de 1 ml/ min. şi detecţie UV la 254 nm. Pentru validarea metodei au fost urmăriţi următorii parametri - linearitatea (r^2=0,9999), intervalul, precizia, acurateţea, specificitatea, cantitatea minimă decelabila LOD, cantitatea minimă masurabilă LOQ. Metoda descrisă poate fi utilizată cu succes pentru analiza compusului farmaceutic activ din soluţii.

Cuvinte cheie: *ivermectin, Romivermectin, Endectocid, UV-VIS cromatografie de lichide de performanţă ridicată de fază inversă, validare*

Summary

An isocratic high-performance liquid chromatography (HPLC) procedure was developed for the quantitative determination of ivermectin in solutions. HPLC separation was carried out by reversed phase chromatography on KROMASIL C_{18} (150 mm x 4.6 mm i.e.; 5 µm particle size), kept in thermostat at 25°C. The mobile phase consisted of methanol/ water (90/ 10 v/ v), with a flow rate of 1 ml/ min and with UV detection at 254 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 solutions.

Key words: *ivermectin, Romivermectin, Endectocid, 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 ivermectin in solution, contributing to the quality and safety control of these types of pharmaceutical preparations.

Materials and methods

Reagents

The standard reference ivermectin has been provided by SIGMA (Germany).

Methanol has been provided by MERCK (Germany).

The solutions with ivermectin (Romivermectin 1%, Endectocid) 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 Kromasil C18 (5 µm particle size; 150 x 4.6 mm inner diameter). The samples have been isocratically eluate in methanol and water (90/ 10 v/ v), with flow of 1 ml/. Each sample has been filtered before injection with PVDF 0.45 µm filter (Thermo Electron). The injection volume of the sample was 5 µl, and detection was carried out at 254nm, at 25°C.

Preparing the standard reference solutions

Ivermectin standard working solution had a final concentration of 0.1 mg/ml, prepared in methanol.

The standard solutions for linearity fell within the area of 0.01-0.20 mg/ml starting from a stock solution of ivermectin of 1 mg/ml prepared in methanol. All samples have been triplicated. The stock solution of ivermectin is kept at +4°C for one week.

Preparing the test solutions

Measure an appropriate amount of solution into a 50 ml flask to obtain a concentration of 0.1 mg/ml ivermectin, dilute to volume with methanol and keep at ultrasound for 10 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 ivermectin placed between 0.01–0.2 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 ivermectin 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 0.1 mg/ml ivermectin 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.

The analysis of ivermectin in solutions

The analysis of the content in ivermectin in solutions 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 ivermectin in solutions 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 ivermectin being 13.118 min. The asymmetry of the peak was good, equal to 1.0.

The calibration curves for ivermectin 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.

By applying the linear regression for the calibration curve, a coefficient of determination $r^2 = 0.999619$.

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 ivermectin, 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.

Table1

Concentration, precision and intermediate precision in HPLC method for ivermectin

Parameter	Value
Concentration	0.1 mg/ ml
RSD% (same day)	0.809%
RSD% (different day)	1.526%

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 ivermectin, in concentration of 80, 100, 120% towards the suggested working concentration (0.08, 0.10, 0.12 mg/ ml).

Table 2

Recovery of ivermectin from samples analyzed through RP-HPLC

Theoretical amount mg/ ml	% Recovery [a]	% Accuracy
0.08	91.887%	
0.10	94.189%	95.466%
0.12	98.626%	

[a] 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 95.466% for the compound of interest - ivermectin. 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 ivermectin

Parameter	Minimum	Maximum	RSD (%)	Status
Asymmetry	1.07908	1.08763	0.291	complies
Retention time	13.055	13.152	0.261	complies
Area	11133381	11425994	1.079	complies

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

Table 4

Limit of detection and limit of quantification for ivermectin

Component	LOD	LOQ
Ivermectin	0.00002 mg/ml	0.000068 mg/ml

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 ivermectin as active substance in the solutions.
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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