

Development and validation of a RP- HPLC method for the quantitation studies of praziquantel and pyrantel pamoate

Dezvoltarea și validarea metodei RP- HPLC de determinare cantitativă a praziquantelului și pyrantelului pamoat din produsul total

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Abstract

An isocratic high-performance liquid chromatography (HPLC) procedure was developed for quantitative determination of praziquantel and pyrantel pamoate in tablet dosage forms of TOTAL. HPLC separation was carried out by reversed phase chromatography Betasil C₁₈ (250 mm x 4,6 mm i.d.; 5 μm particle size), held at 25°C respective Kromasil 60-5SIL. (250 mm x 4,6 mm i.d.; 5 μm particle size), held at 25°C. The mobile phase consisted of acetonitrile/distilled water (60/ 40 v/ v), run at flow rate of 1 mL/ min and with UV detection at 210 nm, respective acetonitrile/ 0,1% phosphoric acid aq. (60/40 v/v), run at flow rate of 1 mL/ min and with UV detection at 240 nm. Method validation investigated parameters such as linearity ($r^2=0,9999$), range, precision, accuracy and specificity. The described method can be successfully applied for the analysis of TOTAL tablets.

Key words: praziquantel, pyrantel pamoate, 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 praziquantel și pyrantel pamoate din comprimate TOTAL. Separarea HPLC a fost realizată prin cromatografie în fază inversă BETASIL C18 (mărimea particulelor 5 μm; 250 x 4.6 mm diametrul intern), respectiv Kromasil 60-5SIL (mărimea particulelor 5 μm; 250 x 4.6 mm diametrul intern), termostată la 25°C. Faza mobilă a fost acetonitril/ apa (60/40 v/v), cu un debit de 1 ml/ min. și detecție UV la 210 nm, respectiv acetonitril/ soluție apoasă 0.1% acid fosforic (60/ 40), cu un debit de 1 ml/ min și detecție UV la 240 nm. Pentru validarea metodei au fost urmăriți următorii parametri - linearitatea ($r^2=0,9999$), intervalul, precizia, acuratețea și specificitatea. Metoda descrisă poate fi utilizată cu succes pentru analiza compușilor farmaceutici activi din comprimate TOTAL.

Cuvinte cheie: praziquantel, pyrantel pamoate, UV-VIS cromatografie de lichide de performanță ridicată de fază inversă, validare

Purpose of this paper was to develop and validate a sensitive HPLC procedure, applicable for determining the amount of praziquantel/ pyrantel in tablet dosage forms TOTAL, contributing to the quality control and safety of this type of pharmaceutical preparation.

Materials and methods

Reagents

Praziquantel and pyrantel used as reference standards were provided by Sigma (Germany). Acetonitrile, phosphoric acid, glacial acetic acid and diethylamine were provided by Merck (Germany).

TOTAL tablet dosage forms, were provided by Romvac Company and were used during the period of validity.

All chemicals used were of pharmaceutical grade or analytical.

Double distilled water was used, filtered by membranes 0.45 μ.

Chromatographic system and conditions

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

Integration of chromatographic peaks was made with ChromQuest software^(Thermo Electron).

Analyses were performed by using a column Betasil C18 (250mm x 4,6mm i.d.; 5μm particle size) to separate praziquantel, respectively Kromasil^(Akzo Nobel) 60-5SIL (250 mm x 4,6 mm i.d.; 5μm particle size), specific for pyrantel.

Samples were isocratic eluted with acetonitrile and water (60/40 v/v), run at flow rate of 1 ml/ min - praziquantel, respective acetonitrile/0,1% phosphoric acid aq. (60/40 v/v), run at flow rate of 1 ml/ min - pyrantel.

Each sample was filtered before injection through a PVDF filter 0.45 μm^(Thermo Electron).

Injection volume of samples, was 5 μL , and detection was performed at 210 (praziquantel)/ 240 (pyrantel) nm, at 25°C.

Preparation of reference standard solutions

Standard working solution praziquantel had a final concentration of 0.08 mg/ml, prepared in acetonitrile/water 3/2.

Standard solutions for linearity were included in 0.01 – 0.15 mg/ mL domain, from a stock solution of praziquantel 0.8 mg/ ml prepared in ethanol. All samples were performed in triplicate. Stock solution of praziquantel is kept at -20°C for 3 months.

Standard working solution pyrantel pamoate had a final concentration of 0.01 mg/ ml, prepared in acetonitrile.

Standard solutions for linearity were included in 0.001 – 0.020 mg/ ml domain, from a stock solution of pyrantel 1 mg/ ml prepared in glacial acetic acid/ water/ diethylamine 5/5/2. All samples were performed in triplicate.

Stock solution of pyrantel is kept at -20°C for 3 months.

Sample preparation solutions

There have been increased about 20 tablets dosage forms of TOTAL with a praziquantel content 25 mg/ 250 mg, respective pyrantel 72 mg/250 mg.

In a 50ml volumetric flask weigh an appropriate amount of powder, to obtain a concentration of 1 mg/mL praziquantel, dilute to volume with acetonitrile/water 3/2 and is sonicated for 20 minutes (stock solution).

To prepare the sample stock solution, to determine pyrantel pamoate, weigh an appropriate amount of powder, to obtain a concentration of 1 mg/ml pyrantel, which is transferred into 50 ml volumetric flask, bring to volume with glacial acetic acid/ water/ diethylamine 5/5/2 and is sonicated for 20 minutes.

Working solutions are prepared daily by diluting stock solutions with acetonitrile/ water 3/ 2, to obtain the working solutions concentration of praziquantel 0.08 mg/ mL, respective with acetonitrile, to obtain the working solution concentration of pyrantel 0.01 mg/ mL. Before injection, solutions are filtered through a PVDF filter 0.45 μm .

Chromatographic method validation

After determining the chromatographic conditions the procedure was validated by following the following parameters: *linearity*,

working range, precision, accuracy, specificity and system suitability, using ICH Guide.

Linearity and working range

Analytical curve was obtained by means of 6 different concentrations of praziquantel in the range of 0.01-0.15 mg/ml, prepared in triplicate. Linearity was assessed by linear regression analysis. For pyrantel, analytical curve was obtained by means of 5 different concentrations in the range of 0.005 – 0.020 mg/ml, prepared in triplicate. Linearity was assessed by linear regression analysis. System has been balanced for at least 30 minutes. There were injected every 3 replicates of each standard concentration of praziquantel/ pyrantel in a volume of 5 μL , to verify the reproducibility of detector response at each level of concentration.

Precision

The precision of the method was determined by repeatability (same day) and between-day precision (between several days). Repeatability was determined by 12 repeated analysis of the same sample solution TOTAL (praziquantel, respective pyrantel), on the same day, in the same experimental conditions. Between-day precision of the method was determined by analyzing on 2 days (between days), respective and by another analyst in the same laboratory (among analysts).

Accuracy

To confirm the accuracy of the proposed method, 9 samples were analyzed using 3 levels of concentration which covers working range.

System's suitability

To ensure the validity of the analytical method was developed the system suitability test. For this purpose, 5 samples were injected containing 0.08 mg/ml praziquantel, respective 0.01 mg/ ml pyrantel, injected in a volume of 5 μL . System suitability assessment was developed using ChromQuest software, by evaluating parameters – area, retention time, asymmetry.

Analysis of praziquantel and pyrantel from tablet dosage forms

Content analysis in praziquantel/ pyrantel, in tablet dosage forms of TOTAL, was made based on the procedure developed and proposed for validation using the 2 reference standards.

Results and discussion

To determine the amount of praziquantel/pyrantel in tablet dosage forms TOTAL was proposed a method HPLC by reversed phase, being selected the optimum conditions for chromatographic separation.

The analysis of chromatograms it is found that the 2 compounds do not interfere, retention time of praziquantel was 5.473 minutes, and of pyrantel was 3.178 minutes.

Peak asymmetry's good for both compounds, equal to 1.0.

Praziquantel/ pyrantel calibration curves were constructed by representing the area's peak against concentration.

For both compounds was observed linearity in the chosen field. Concentration range was 10-200% compared to the working concentration.

Applying linear regression for the 2 calibration curves, has been determined a coefficient of determination $r^2 = 0.9998$ (pyrantel), respectively 0.997 (praziquantel).

Precision of the method is the degree of suitability between single test results, the repeated application of the method on multiple sample of a homologous series.

Repeatability was studied by calculating the relative standard deviation (RSD) of 12 samples with a concentration of 0.08 mg/ml praziquantel, respective 0.01 mg/ml pyrantel, performed on the same day and in the same experimental conditions.

Between-day precision includes estimating variability analysis when the method is used in different laboratories, on different days, by different analysts and different equipment.

The results are presented in Table 1 and Table 2.

Table 1
Concentration, intra-day precision and between-day precision data of RP-HPLC for praziquantel

Parameter	Value
Concentration	0,083 mg/ ml
RSD% (same day)	1,651%
RSD% (different day)	1,303%

Table 2
Concentration, intra-day precision and between-day precision data of RP-HPLC for pyrantel

Parameter	Value
Concentration	0,01 mg/ ml
RSD% (intra-day)	0,357%
RSD% (between - day)	0,288%

Accuracy of the procedure is the closeness of the results obtained practically

using the method, compared with the theoretical value.

Accuracy was determined by analysis of every 9 samples containing praziquantel, respective pyrantel, with concentration 80, 100, 120% compared to the working concentration proposed (0.064; 0.08; 0.096 mg/ ml – praziquantel; 0.008; 0.01; 0.012 mg/ ml – pyrantel).

Table 3
Recovery of praziquantel from samples analyzed by RP-HPLC

Theoretical amount mg/ ml	% Purity ^a	% Accuracy
0,064	94,792%	108,764%
0,080	98,584%	
0,096	118,944%	

^amean of three replicates analyses

Table 4
Recovery of pyrantel pamoate from samples analyzed by RP-HPLC

Theoretical amount mg/ ml	% Purity ^a	% Accuracy
0,008	91,666%	96,489%
0,010	99,526%	
0,012	93,451%	

^amean of three replicates analyses

The data analysis presented in Table 3 and 4 we find that the method shows accuracy in the proposed range, the average recovery was 108.764% for praziquantel and 96.489% for pyrantel.

To assess the resolution and reproducibility analysis system, proposed, were performed suitability tests.

The results presented in Table 5 and 6 show that the parameters are within the limits of suitability.

Table 5
Results of the system suitability test for praziquantel

Parameter	Minimum	Maximum	RSD(%)	Status
Asymmetry	1,06827	1,08245	0,491	passed
Retention time	5,473	5,482	0,063	passed
Area	2467661	2562232	1,651	Passed

Table 6
Results of the system suitability test for pyrantel

Parameter	Minimum	Maximum	RSD(%)	Status
Asymmetry	0,96673	1,08506	0,471	passed
Retention time	3,168	3,173	0,054	passed
Area	2024128	2037562	0,288	passed

Detection and quantification limits were calculated, obtaining these values:

Table 7

Limit of detection and limit of quantification for praziquantel/ pyrantel

Component	LOD	LOQ
Praziquantel	0,013915 mg/ml	0,046382 mg/ml
Pyrantel	0,000014 mg/ml	0,000046 mg/ml

Conclusions

1. The results presented in the validation of RP –HPLC method show *the accuracy the linearity, the precision of and the limits of detection and quantification.*
2. The method can be successfully applied to quantify praziquantel and pyrantel as active ingredients in tablet dosage forms.
3. The proposed procedure has the advantage of using convenient analytical method that requires a simple preparation of samples. In this way, the method can be used for routine analysis.

Bibliography

1. <http://www.ich.org>
2. <http://www.waters.com>
3. <http://www.ncbi.nlm.gov.com>
4. **Mainardes, Rubiana M., Cinto, Priscila O., Gremiao, Maria P.D., (2006).** High performance liquid chromatography determination of praziquantel in tablets and raw materials. *Acta Farm. Bonaerense*, **25(4)**, 567-570.
5. <http://www.thermoscientific.com>
6. **Bialecka, Wanda, Kulick, Anna (2010).** Determination of active substances in multicomponent veterinary preparations of antiparasitic action by HPLC method. *Acta Poloniae Pharmaceutica*, **67(5)**, 463-468.
7. <http://onlinelibrary.wiley.com>
8. <http://findarticles.com>
9. <http://medind.nic.in>
10. <http://www.inchem.org>