ORIGINAL ARTICLE

VALIDATION AND APPLICATION OF A NEW DAD-HPLC METHOD FOR DETERMINATION OF LORATADINE FROM PHARMACEUTICALS

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Abstract

In our previous research, we developed a DAD-HPLC method for the quantitative determination of loratadine in different samples [1]. The proposed method was internally validated and its performance parameters were established: linearity, working range, selectivity, recovery test, accuracy and precision. Validation results showed a good accuracy of the method, the optimum repeatability and reproducibility. *Bias* for the accuracy of loratadine assay by HPLC is 100.20%, being in the recovery range of 95-105%, which shows that the method is accurate. The applicability of the method was verified by analysing samples from pharmaceutical products containing loratadine (Roletra® Tablets, Clarinase® modified-release Tablets and Symphoral® syrup).

Rezumat

În cercetările noastre anterioare a fost dezvoltată o metodă DAD-HPLC pentru determinarea cantitativă a loratadinei din diferite probe [1]. Metoda propusă a fost validată, stabilindu-se parametrii săi de performanţă: liniaritate, domeniul optim de concentraţie, selectivitate, testul de regăsire, acurateţe şi precizie. Rezultatele validării au demonstrat o bună precizie a metodei, repetabilitate şi reproductibilitate optime. *Bias*-ul pentru precizia metodei de determinare a loratadinei prin HPLC are valoarea 100,20%, încadrându-se în intervalul de regăsire 95-105%, ceea ce demonstrează că metoda este precisă. S-a verificat aplicabilitatea metodei prin analiza unor probe din forme farmaceutice cu loratadină.

Keywords: loratadine, validation, pharmaceuticals, HPLC

Introduction

Loratadine is a tricyclic piperidine derivative, orally effective, long-acting, non-sedating, second-generation antihistaminic with no significant antimuscarinic activity. It is used for the symptomatic relief of allergic conditions including rhinitis and chronic urticaria [2]. In allergic manifestations were especially important the effects of histamine obtained by H1 receptor activation. These effects can be controlled by using H1-receptor antagonists (e.g. loratadine) [2].

The pharmaceutical industry has strict regulations on activities development and control analytical laboratories. Thus, special attention is given to optimize and use methods of analysis and control of raw materials, intermediates and finished products [3-6].

The specialized literature indicates a series of HPLC methods for active substances determination from pharmaceuticals [8-12].

In this context, researches of the present paper were directed on validation of a sensitive HPLC method for determination of loratadine in pharmaceuticals (tablets, syrups). Our previous research developed the method, setting parameters and working conditions [1]. System suitability tests were conducted to verify if resolution and reproducibility of the system were adequate for the analysis [6].

Materials and Methods

Materials

All solvents (HPLC grade) and reagents were from Merck. Loratadine was used as standard reference compound according to European Pharmacopoeia [7].

Chromatographic method

Experiments for HPLC method were performed using an Agilent 1200 series system including the following modules: quaternary pump, DAD, thermostat, auto-sampler.

The principle is states the separation and quantification of loratadine using XDB-C8 column and mobile phase, phosphate buffer pH = 2.9 with acetonitrile, 15/11 (v / v), with detection at 280 nm.

The method involved is a liquid - phase extraction of loratadine using methanol as solvent. Separation was achieved with a type-C8 chromatography column (Zorbax Eclipse XDB-C8 double related ends, 4.6 mm x 150 mm, particle size 5 µm, t_{limit} = 60°C, pH = 2-9); flow rate: 1.5 mL/min; Column temperature: 27°C; injection volume: 10 µL; mobile phase: acetonitrile / (aqueous solution of ammonium

dehydrogenate phosphate 10 g/L was added to 5 mL of phosphoric acid): 11/15 (v/v); detection: 280 nm. For the drug, the standard curve was linear between 0.5 to 9.0 µg/mL.

The chromatographic column was equilibrated using the mobile phase for 60 minutes; further 10 µL sample were injected and the chromatogram with UV detection at 280 nm was recorded.

Results and Discussion

Linearity

In order to assess the method's linearity, the regression curve was traced and for the analysis method results were mathematical by processed. The method of calculating the regression line used in this study is the method of the least squares [4, 5].

Six solutions with different concentrations were prepared, in the concentration range 0.12 µg/mL - 0.60 µg/mL, by diluting the standard solution of loratadine. There were assessed three series of determinations for each concentration. The samples were carried out by HPLC/DAD. Corresponding chromatograms were recorded and peak areas were calculated for each determination.

Peak area was plotted against the concentration of loratadine in loratadine solutions (Figure 1).

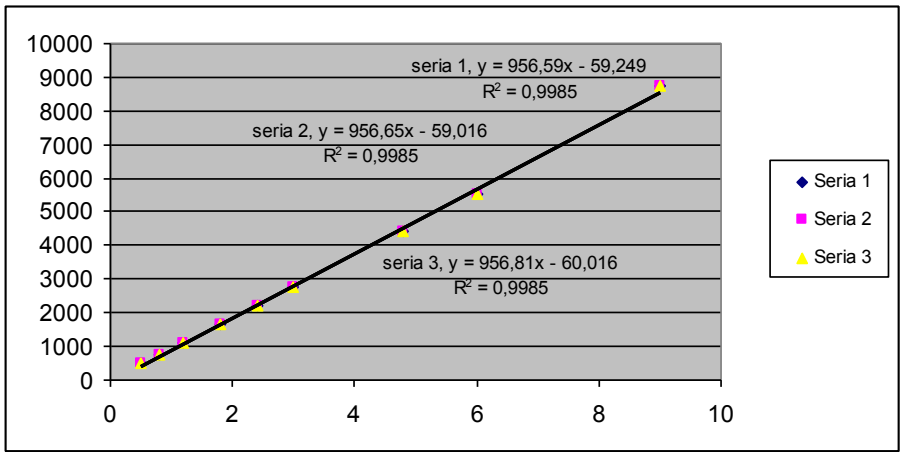

Figure 1.
Three series of measurements chart

The obtained data were statistically evaluated by determining the correlation coefficient (r), regression coefficient (r^2) and standard error of regression line (SE). The results are listed in Table I.

Calibration line equation, calculated by mathematical regression is:

$$\text{Area} = 956.68 * \text{concentration} - 59.427$$

The criterion of admissibility is $r^2 \geq 0.995$, so the method was found linear in the concentrations range 0.5 to 9.0 µg/mL.

Table I
Linearity evaluation of the HPLC method

No. crt.	Statistical Regression Calculation	
1	Correlation coefficient (r)	0.9992
2	Regression coefficient (r^2)	0.9985
3	Standard error of the regression line (SE)	95.668
4	Intercept (b)	-59.428
5	Slope (a)	856.68

Table II
Statistical analysis of experimental data in SPSS 10 (ANOVA test)

Model	Sum of squares (SS)	Degrees of freedom (df)	Average sum of squares (MS)	Variation found for the group average/variation expected for the group average Test – Raport F	Sig.
Regression	104000000	1	104093465.0	1231.536	.000[a]
1 Residual	676186.5	8	84523.313		
Total	105000000	9			

The analysis of value obtained by Fisher's test has shown that the calculated value ($F_{calculated}$ = 1231.536) is much higher than the tabulated value for a risk of 5% and 8 degrees of freedom (F_{tabled} = 5.0503), which entitles us to reject the version of "null hypothesis" where the variable factor (concentration) significantly influence the behaviour of variable outcome (area) (Table II). Therefore, the correlation report is significant.

To assess the linearity of the results, it was calculated the concentration, using the equation of calibration line. Between theoretical concentration and calculated concentration there is a linear

correlation, the slope of this line being equal to 1.000 and the intercept equal to 0.000001 (a straight line which practically coincides with the first bisectrix). The correlation coefficient of this line has the value $r^2 = 0.998$. The method is linear in the chosen concentration range (0.5 to 9.0 μg/mL).

Precision

The precision of the system was demonstrated by injecting 10μL of reference solution, six times and determining the corresponding peak areas of loratadine. In order to evaluate the precision of the system, data were statistically analysed (Table III).

Table III

Statistical data for precision determination

Average peak area (mAU x sec.)	5516.742
Standard deviation (SD)	4.702229
Relative standard deviation (RSD%)	1.919677

The system precision is high as the value RSD (1.92%) is less than 5% [2].

Selectivity

The selectivity of the method was determined by injecting the blank sample (methanol, the solvent used to prepare solutions of loratadine) and the reference solution (containing 6.0 μg/mL loratadine in methanol). Solvent chromatograms do not have answers that might interfere with the determination of loratadine in the reference solution, so the method is selective.

Recovery of the method

In order to determine the recovery method, six samples of loratadine solution with a concentration of 6.0 μg/mL were assessed. Samples were processed identically and the injections volume was 10 μL. By using the values of peak areas corresponding to loratadine, the recovery rate of the analytical method was calculated according to the formula [4]:

Recovery% = calculated loratadine quantity / theoretical loratadine quantity x 100

Statistically analysed results are listed in Table IV.

Table IV

Recovery evaluation for the HPLC method

Minimum recovery (%)	99.88548
Maximum recovery (%)	101.9879
Average recovery (%)	100.6862
Standard deviation (SD)	0.942082
Relative standard deviation (RSD%)	0.384603

Table V

Statistical data for total recovery of the HPLC method

Minimum recovery (%)	99.88122
Maximum recovery (%)	100.8635
Average recovery (%)	100.2838
Standard deviation(SD)	0.553655
Relative standard deviation (RSD%)	0.319653
Recovery factor	0.997777

Total percentage of recovery

Three samples of working solution with different concentrations of loratadine were analysed and the data obtained were statistically processed (Table V). The method complies within the requirements validation rules having a recovery factor of 0.9977.

Accuracy

Accuracy, like precision, is affected by systematic errors (errors due to method of analysis, analyst, apparatus or reagents) [6]. Systematic errors can be eliminated or significantly reduced, while random errors are difficult to remove, they are due to random fluctuations of the analytical signal, background noise or other factors (humidity, temperature, air pressure, etc.) [4, 6]. In order to determine the method's accuracy it was used the *addition method:* loratadine substance was introduced in standard solution in order to obtain concentrations of 75, 100 and 125% in comparison with the tested solution (6.0 μg/mL). The process was repeated three times, using separately prepared standard solutions.

The recovery was calculated as a percentage of the theoretical concentration, average recovery for each sample and the field of recovery variation. Table VI presents the results.

Table VI

Accuracy of HPLC assay method for loratadine

Bias (%)	100.2006
Minimum recovery (%)	99.68855
Maximum recovery (%)	100.9366
Standard deviation (SD)	0.41341
Relative standard deviation (RSD%)	0.137803
Field (%)	99.68 – 100.93

To sum, the *bias* in this study of accuracy for the method was set to 100.20% and the recovery is within the concentrations range 95-105%, which proves that the method is accurate.

Limit of detection and Limit of quantification

To determine these parameters it was used the method of calculating standard error and the slope:

$$LOD = \frac{3 \times SE}{Slope} = \frac{3 \times 95.668}{956.68} = 0.3 \ \mu g/mL$$

$$LQ = \frac{10 \times SE}{Slope} = \frac{10 \times 95.668}{956.68} = 1.0 \ \mu g/mL$$

Method applications on pharmaceutical forms

The following pharmaceuticals were studied: Roletra® Tablets (RANBAXY LABORATORIES) containing 10 mg loratadine; Clarinase® product, modified-release tablets (SCHERING PLOUGH LABO NV) containing 5 mg loratadine and pseudoephedrine sulfate 120 mg and Symphoral®

syrup (GEDEON RICHTER) containing 1 mg/mL loratadine. All samples obtained from tablets (Roletra®, Clarinase®) were prepared as follows: average weight of a unit was determined by using the analytical balance for 20 tablets. The weighed tablets were triturated to obtain a fine, homogeneous powder. A quantity equivalent to 10 mg loratadine was weighed. The powder weighed and 20 mL of chloroform were brought into a 100 mL flask. After vigorous stirring for 10 minutes, the volume was completed with the same solvent. The resulting suspension was filtered through quantitative filter paper and the filtrate was brought to the evaporator to completely remove the solvent.

A syrup volume corresponding to 5 mg loratadine (5 mL syrup, the usual dose for 24 hours for children aged 2 to 12 years) and 10 mL of chloroform were brought into the extraction funnel. First, the sample was vigorous by stirred for 10 minutes, and rested till the total separation of the two phases and then the chloroform component was collected in a flask with a stopper. The operation was repeated 4-5 times and then the solvent was removed using an evaporator.

The substance thus separated was used to prepare a methanol solution of theoretical concentration 0.5 µg/mL. It was used chloroform for extraction from pharmaceuticals because loratadine is slightly soluble and excipients cited in the prospectus are poorly soluble in this solvent.

A number of 6 solutions for each form of pharmaceutical were prepared and analysed by the HPLC method as described. The chromatograms obtained show the selectivity of the method: peak of the loratadine is well evidenced and excipients traces do not interfere with the analyte (Figure 2).

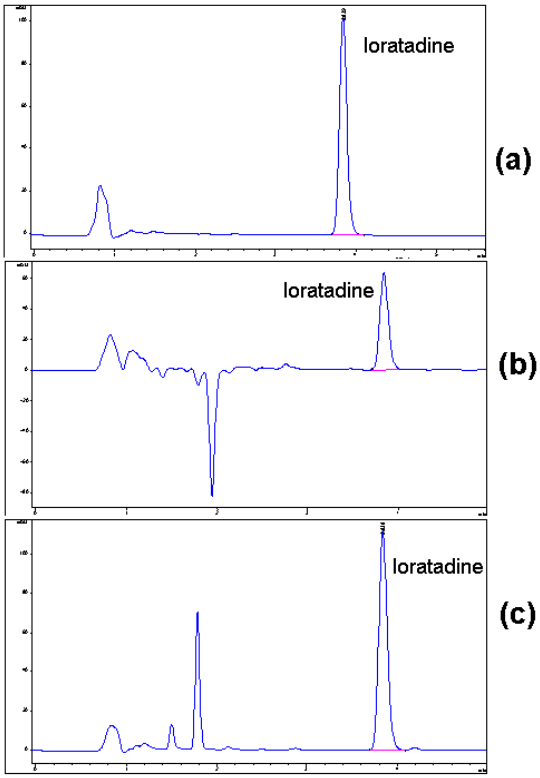

Figure 2.
Chromatograms of loratadine in pharmaceutical samples: (a) Roletra, (b) Clarinase, (c) Symphoral

The concentration of the samples was calculated using the equation of the calibration. The results were statistically processed (Table VII).

Table VII
Statistical data for loratadine determination in pharmaceuticals formulations

	Roletra® tablets	Clarinase® tablets	Symphoral® syrup
Average recovery (%)	99.50795	96.91266	101.7412
Minimum recovery (%)	97.90296	96.09399	100.2256
Maximum recovery (%)	100.7668	99.76586	102.6759
SD	1.211626	1.405741	0.886604
RSD%	0.4946	0.57389	0.36195
Area	97.90 – 100.76	96.09 – 99.76	100.22 – 102.67

The active substance content per unit of pharmaceutical form was determined. According to the declared content, some deviations were detected: 0.49% for Roletra®, 3.08% for Clarinase® and 1.74% for Symphoral®, values which fall within the permissible deviation of ± 5% [7].

Conclusions

A HPLC method was developed and validated for the assay of loratadine. This method showed good linearity in the concentration range of 0.5 µg/mL to 9.0 µg/mL, good precision and accuracy and similar sensitivity. The values of calculated limit of detection (0.3 µg/mL) and limit of quantification (1.0 µg/mL) showed that the method can be extended to concentrations of loratadine below their chosen field. The method is useful for synthesis process control and determination of loratadine in drug substance, and pharmaceutical preparations. The applications have shown that the method proved to be sensitive and suitable for the quality control and evaluation of pharmaceutical products loratadine and bioequivalence studies.

References

1. Pavalache G., Dorneanu V., Popescu A., A new method for the separation and quantitative

determination of loratadine. *Rev. Chim.*, 2016; 67(1): in press.
2. Song M., Hang T.J., Shen J.P., Tao P., Zhang Y.D., Zhang Z.X., Study of pharmacokinetics and relative bioavailability of loratadine tablets by HPLC-MS method. *Yaowu Fenxi Zazhi*, 2004; 24(4): 374-377.
3. European Directorate for the Quality of Medicines & Health Care, Council of Europe. OMCL Guideline on Validation of Analytical Procedures. PA/PH/OMCL (05) 47 DEF. Concil of Europe, 2005; URL:http://www.edqm.eu/site/Validation_of_ Analytical_ Procedures pdf-en-826-2.html
4. Bojiţă M., Roman L., Săndulescu R., Oprean R., Analiza şi controlul medicamentelor. Bazele teoretice şi practice. Analysis and control of drugs. Theoretical and practical. Volumul I, Cluj-Napoca, Editura Intelcredo, 2003: 89-148.
5. Bojiţă M., Roman L., Săndulescu R., Oprean R., Analiza şi controlul medicamentelor. Metode instrumentale în analiza şi control. Analysis and control of drugs. Instrumental methods in analysis and control. Volumul II, Cluj-Napoca, Editura Intelcredo, 2003: 173-217.
6. Oprean R., Rozet E., Dewe W., Boulanger B., Hubert P., Ghid de validare a procedurilor analitice cantitative. Validation user of quantitative analytical procedures. Cluj-Napoca, Editura Medicală Universitară „ Iuliu Haţieganu", 2007: 50-61, 85-90.
7. Council of Europe. European Pharmacopoeia, 8th edition, Strasbourg: Council of Europe, 2013.
8. Palade L., Popovici I., Popa G., Cojocaru I., Elaboration and validation of an HPLC Method for the quantitative assay of 5-Fluorouracil. *Farmacia*, 2013; 61(3): 526-532.
9. Bîrsan M., Cojocaru I.C., Scutariu M.M., Popovici I., Validation of a chromatographic method for miconazole assay from oral sustained release mucoadhesive tablets. *Farmacia*, 2014; 62(3): 555-563.
10. Khan I.U., Ashfaq M., Razzaq S.N., Mariam I., Simultaneous determination of piroxicam and paracetamol in pharmaceutical formulations using stability indicating HPLC method. *J. Liq. Chrom. Relat. Tech.*, 2013; 36(10): 1437-1450.
11. Srivatsan V., Dasgupta A.K., Kale P., Datla R.R., Soni D., Patel M., Patel R., Mavadhiya C., Simultaneous determination of itraconazole and hydroxyitraconazole in human plasma by high-performance liquid chromatography. *J. Chrom.*, 2004; 1031(1-2): 307-313.
12. Zhong D., Blume H., HPLC determination of loratadine and its active metabolite descarboethoxyloratadine in human plasma. *Pharmazie*, 1994; 49(10): 736-739.