

VALIDATION OF AN ANALYTICAL METHOD BASED ON HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR DETERMINATION OF LORATADINE IN PHARMACEUTICAL PREPARATIONS AND BIOLOGICAL ENVIRONMENTS

GEORGETA PAVALACHE^{1,2*}, VASILE DORNEANU¹

¹*Department of Analytical Chemistry, Faculty of Pharmacy, University
Gr. T. Popa, 16 University Street, Iasi,*

²*Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
Ovidius University, 124 Mamaia Blvd, Constanta*

**corresponding author: georgiana_pavalache@yahoo.com*

Abstract

A high-performance liquid chromatography (HPLC) method for determination of loratadine in pharmaceutical dosage forms and in biological environments was developed by us in a previous study [1]. In the present study, the method was validated in terms of linearity, accuracy, selectivity, precision, limit of detection and limit of quantification.

This method was successfully applied to synthesis process control and determination of loratadine as substance and pharmaceutical preparations.

Rezumat

Într-un studiu anterior [1] a fost prezentată o metodă HPLC pentru determinarea loratadinei din forme farmaceutice dozate și din medii biologice. În prezenta lucrare, metoda a fost validată luându-se în considerare liniaritatea, exactitatea, selectivitatea, precizia, limita de detecție și limita de cuantificare.

Această metodă a fost aplicată cu succes în controlul proceselor de sinteză și determinare a loratadinei ca substanță și din preparate farmaceutice.

Keywords: high-performance liquid chromatography, loratadine, validation

Introduction

H1 receptor antagonist loratadine (ethyl 4-(8-chloro-5,6-dihydro-11H-benzo [5.6] cyclohepta [1,2-b] pyridine -11- ylidene) piperidine -1-carboxylate) is one of the second-generation antihistamines [2].

Different HPLC methods have been published for loratadine assay in pharmaceutical preparations and biological fluids and some of them showed several disadvantages: lack of sensitivity (low limit of quantification – above 33 ng/mL), use of complex sample preparation procedures (chemical derivatization) or use of large sample volumes. The published HPLC methods use various separation modes, such as reverse phase, ion pair, cation exchange, normal phase chromatography [3,4,7,8,10].

In this paper we focused on validating an optimized HPLC method by determining its linearity, accuracy, selectivity, precision, limit of detection and limit of quantification [5,6,9]. System suitability tests were conducted to verify if resolution and reproducibility of the system were adequate for the analysis.

Materials and methods

Materials

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

Instrumentation

Experiments for HPLC method were performed with an Agilent 1200 series system including the following modules: quaternary pump, diode array detectors (DAD), thermostat, auto-sampler.

Chromatographic method

The proposed method involved liquid - phase extraction of loratadine using methanol as solvent. Separation was achieved with a C18 column (250 mm x 4.6 mm) 5 μ m XDB - C18 Agilent (Zorbax Eclipse XDB-18) employing detection λ = 264 nm. The mobile phase consisted of [0.01% triethylamine solution adjusted to pH 2.75 with orthophosphoric acid - acetonitrile (46:54, v/v)] - isopropanol (90:10, v/v) at a flow rate of 1.0 mL/min. For the substance, the standard curve was linear from 0.05 to 15.0 μ g/mL.

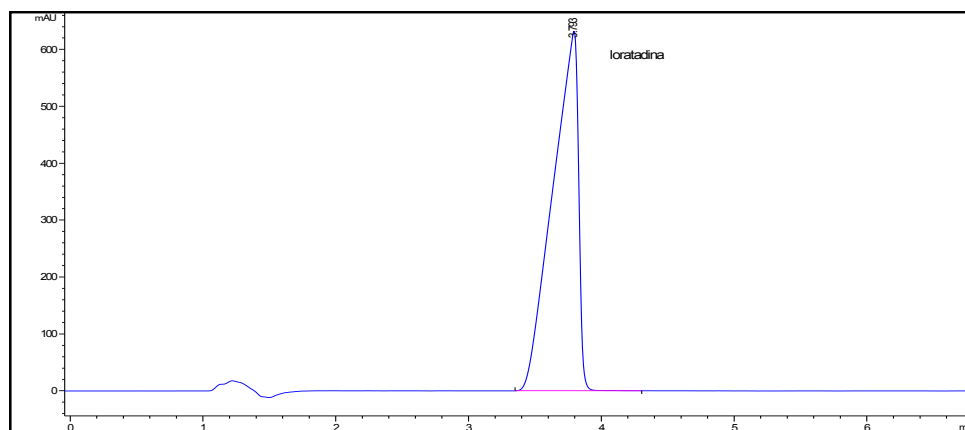
Results and discussion

Linearity

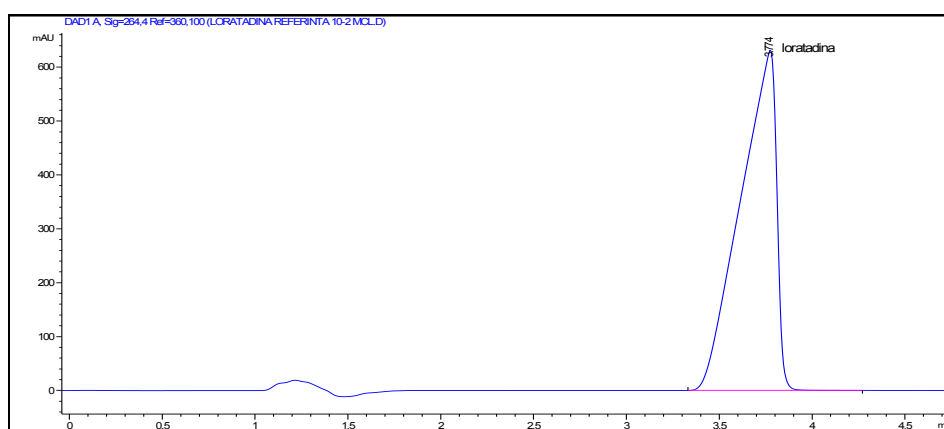
To assess linearity, we traced the regression plot. For this purpose, the results of the analytical method were mathematically provided. The method of calculating regression line used in this study is the method of least squares.

Ten solutions with different concentrations were prepared, in the range of 0.05 μ g/mL - 10.0 μ g/mL, by dissolving loratadine in methanol. Three series of determinations have been performed for each concentration. Every sample was processed with HPLC device and the retention time and the peak area were registered for loratadine.

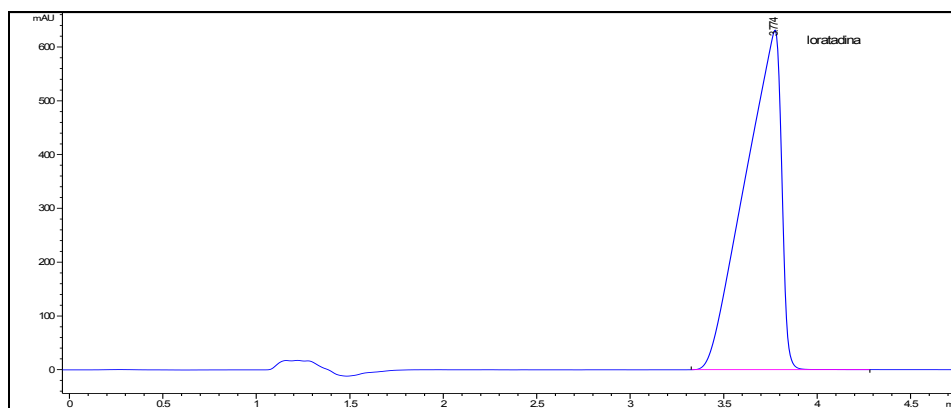
Corresponding chromatograms were recorded for each determination (Fig 1, 2, 3).

**Figure 1**

A chromatogram of loratadine standard solution 6.0 µg/mL (Series 1)

**Figure 2**

A chromatogram of loratadine standard solution 6.0 µg/mL (Series 2)

**Figure 3**

A chromatogram of loratadine standard solution 6.0 µg/mL (Series 3)

Corresponding loratadine peak area was graphically represented according to the concentration of loratadine solutions. (Figure 4)

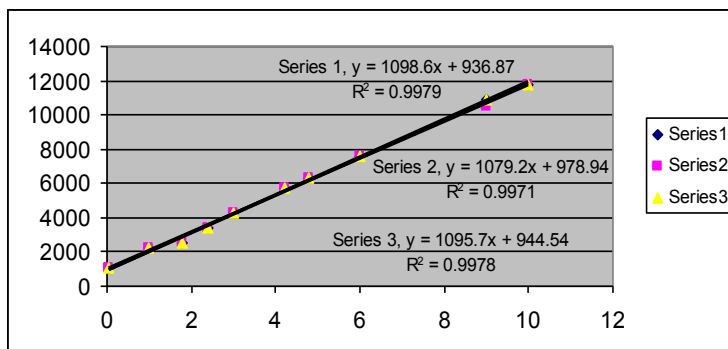


Figure 4

Curves for each series of determinations

Data obtained were statistically evaluated by determining the correlation coefficient (r), the standard deviation (s), the standard error of regression line (SE) and the corresponding equation:

$$\text{Area} = f(\text{concentration})$$

$$\text{Area} = (a \times \text{concentration}) + b$$

where a = slope; b = intercept

Variation of the average area depending on concentration was plotted and the field for which this variation is linear was determined. (Figure 5)

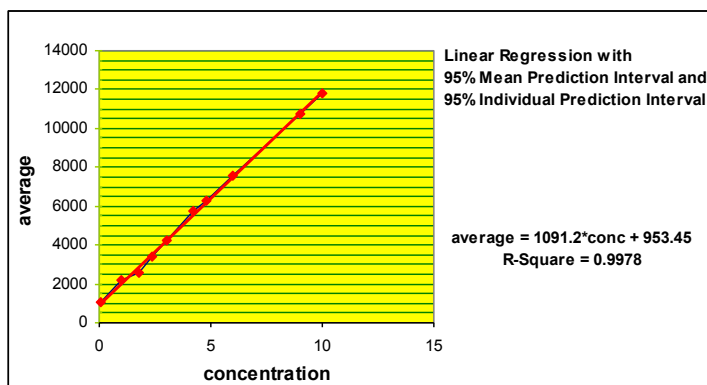


Figure 5

Calibration curve for determination of loratadine by HPLC method

The calibration equation calculated by mathematical regression is:

$$\text{Area} = (1091.2 \times \text{concentration}) + 953.45$$

Data obtained were statistically processed. The results are presented in Table I.

Table I
Statistical parameters for establishing the linearity

Nr.	Statistical Regression Calculation	
1	Correlation coefficient (r)	0.9988
2	Regression coefficient (r^2)	0.9978
3	Standard error of the regression line (SE)	4.7427
4	Intercept (b)	953.45
5	Slope (a)	1091.2

The criterion of admissibility is $r^2 \geq 0.995$, so the method was found linear in the range of 0.05 to 10.0 $\mu\text{g/mL}$.

Table II
Statistical analysis of experimental data in SPSS 10 software
Model^b Summary

Model	R	R Square	Adjusted R Square	Std. Error The Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	1.000 ^a	1.000	1.000	4.742	0.9978	11155	1	4	,000	3.52

a. Predictors: (Constant), VAR00001

b. Dependent Variable: VAR00002

Analyzing the value obtained by Fisher's test was observed that the calculated value ($F_{\text{calculated}} = 1115583.936$) is much higher than the tabulated value for a risk of 5% and five degrees of freedom ($F_{\text{tabled}} = 5.0503$), which entitles us to reject the version of "null hypothesis" where the variable factor (concentration) significantly influence the behavior of variable outcome (area) (Table II). Therefore, the correlation report is significant.

To assess the linearity of the results, we calculated the concentration found using the equation of calibration line. The obtained data were plotted (Figure 6).

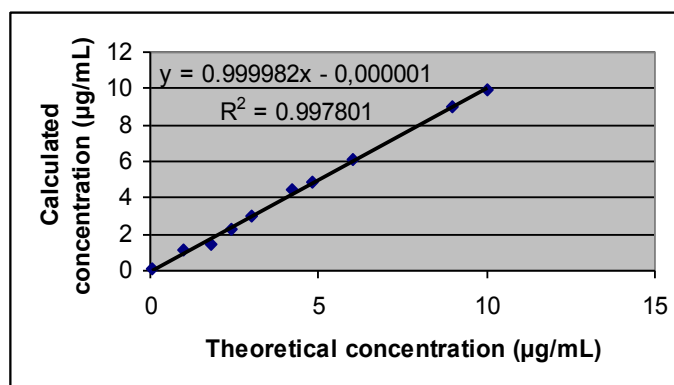


Figure 6
Linearity of HPLC results

Between the theoretical concentration and the calculated concentration (brought by using equation of calibration line) there is a linear correlation, the slope of this line being equal to 0.99982 and intercept equal to -0.0001 (a straight line which practically coincides with the first bisectrix). Correlation coefficient of this line has the value $r^2 = 0.9978$. Therefore, between introduced concentration (theoretical) and calculated one, using the equation of calibration line, the correlation is accepted. The method is linear in the chosen concentration field (0.05 to 10.0 $\mu\text{g/mL}$).

Precision

Precision of the system was demonstrated by injecting 10mL of reference solution six times and determining the corresponding peak areas of loratadine. (Table III)

Table III

Results obtained for the method's precision evaluation

Nr.	Peak area of loratadine (mAU $\times$ sec.)
1	8509.46120
2	8511.74076
3	8511.94285
4	8511.36719
5	8511.30936
6	8511.63958

In order to evaluate the precision of the system, data were statistically analyzed. (Table IV)

Table IV

Statistical data for the system precision

Average (mAU $\times$ sec.)	8511.2430
Standard deviation (SD)	0.9043
Relative standard deviation (RSD) (%)	0.3690

The system precision is high as the value RSD (0.3690%) is well below 2.

Selectivity

Selectivity of the method was determined by injecting the control sample (methanol, the solvent used to prepare solutions of loratadine) and the reference solution (containing 6.0 $\mu\text{g/mL}$ loratadine in methanol).

Solvent chromatograms (Figure 7) do not have signals that might interfere with the determination of loratadine in the reference solution (Figure 8), so the method is selective.

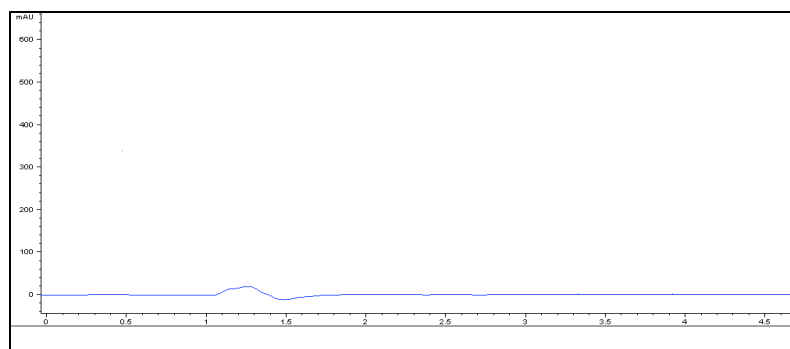


Figure 7
Solvent Chromatogram

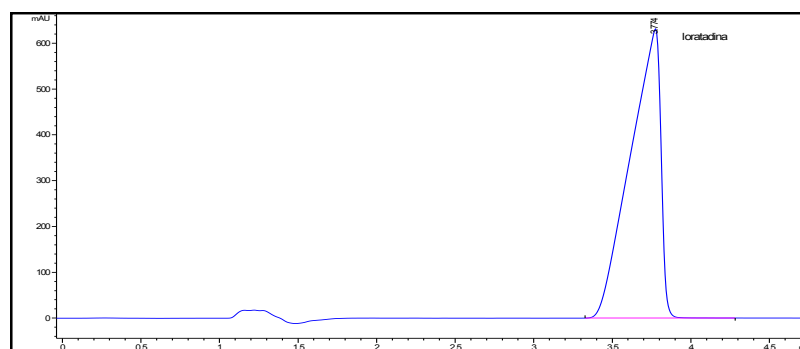


Figure 8
Chromatogram of standard solution

Recapture of the method

To determine the recapture's method percentage, six samples of loratadine solution with concentration of 6.0 mg/mL were performed. Samples were processed identically and injections volume was 10 mL. Using the values of peak areas corresponding to loratadine, the retrieval rate was calculated by analytical method according to the formula below. Statistically analyzed results are presented in Table V.

$$\text{Retrieval rate} = (\text{calculated loratadine quantity} / \text{theoretical loratadine quantity}) \times 100$$

Table V

Statistical parameters for establishing the recapture of the method

Minimum retrieval rate (%)	99.94942
Maximum retrieval rate (%)	100.0252
Average	99.99219
Standard deviation (SD)	0.026667
Relative standard deviation (RSD%)	0.010887

Accuracy

Accuracy, like precision, is affected by systematic errors (errors due to method of analysis, analyst, apparatus or reagents). Systematic errors can be eliminated or significantly reduced, while random errors are difficult to remove, because they are due to random fluctuations of the analytical signal, background noise or other factors (humidity, temperature, air pressure, etc.). Accuracy is expressed as *bias* (percentage error between measured value and target value) and must be in the range 95-105% and relative standard deviation should not exceed 2%.

$$bias = 100 \times (\text{measured value} / \text{theoretical value})$$

In order to determine the method's accuracy it was used the *addition method*: we introduced loratadine substance in standard solution in order to obtain concentrations of 50, 100 and 150% compared to the tested solution (6.0 µg/mL). The process was repeated three times, using separately prepared standard solutions.

It was calculated the concentration (µg/mL) for each sample, using the equation of the calibration line and the corresponding peak area values of loratadine. Table VI contains the results:

Table VI

Results of accuracy determination

Average (%)	100.2282
Minimum (%)	99.83953
Maximum (%)	101.6307
Standard deviation (SD)	0.609018
Relative standard deviation (RSD%)	0.203006
Field (%)	99.84 – 101.63

Limit of detection (LD) and Limit of quantification (LQ)

To determine these parameters we used the method of calculating standard error (SE) and slope:

$$LD = \frac{3 \times SE}{Slope} = \frac{3 \times 4.747}{1414.6} = 0.010 \text{ µg/mL}$$

$$LQ = \frac{10 \times SE}{Slope} = \frac{10 \times 4.7427}{1414.6} = 0.033 \text{ µg/mL}$$

Conclusions

A HPLC method for the assay of loratadine, developed in a previous study, was validated. This method showed good linearity in the concentration range of 0.05 µg/mL to 10.0 µg/mL, good precision and accuracy and similar sensitivity. The values of calculated limit of detection (0.010 µg/mL) and limit of quantification (0.033 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 substance, pharmaceutical preparations and biological samples.

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