Validation and Application of a RP - HPLC Method with UV Detection for Loratadine Determination

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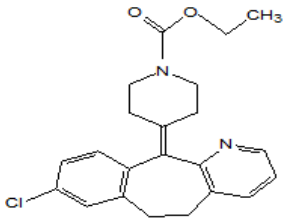
Loratadine is a second generation antihistamine with antagonistic activity on the histamine H_1 – receptor. It is found in various pharmaceutical forms such as tablets, oral solutions or syrups. This article presents the development and validation of a HPLC method used for the quantitative determination of loratadine in tablets. For this purpose the working conditions were established. A C18 Eclipse XDB column (150x4.6 mm, 5 mm) was employed. The mobile phase consisted of acetate buffer solution / methanol (15/85, v/v) and the UV detection was performed at 248 nm. All the validation parameters for linearity, detection limit (DL) and quantitation limit (QL), precision (system precision, method precision and intermediate precision) and accuracy were in agreement with ICH guidelines, which permits us to consider that the proposed method is simple, accurate, reliable, precise and suitable for the quantitative determination of loratadine in tablets.

Keywords: loratadine, HPLC, tablets

Loratadine is a tricyclic antihistamine, an important active pharmaceutical ingredient from several drugs, used for the treatment of allergy symptoms. Loratadine, ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta-[1,2-b]pyridine-11ylidene)-piperidine-1-carboxylate (fig. 1), is a second generation long-acting antihistamine drug, with selective peripheral histamine H_1-receptor antagonistic activity [1, 2].



Scheme 1. Chemical structure of loratadine

Among the second-generation antihistamines, loratadine is sedation free at recommended doses. In the literature, several methods have been reported for determination of loratadine in pharmaceutical preparations and in biological fluids, from simple colorimetric methods, with intermediate selectivity and sensitivity, to high selective and sensitive methods. These includes spectrophotometry [2], spectrofluorimetry [3], capillary electrophoresis [4, 5], polarography [6], densitometry [7], thin layer chromatography [8, 9] and liquid chromatography [10–16]. High performance liquid chromatography is the most common method used for determination of loratadine. Of these methods, different values for parameters like run time (30 min [11], 6 min [5], less than 10 min [15]), flow rate (1 mL/min [11, 15], 2 mL/min [2]), concentration range (25-150 μg/mL [11], 4-56 μg/mL [15], 5-100 μg/mL [2]) precision with intra-day and inter-day relative standard deviation values <1% [11], limit of detection (0.5 μg/mL [2], 1.96 μg/mL [5], 0.8 μg/mL [15]), were obtained.

The aim of this work was the development and validation of a rapid and simple analytical method for the quantitative estimation of loratadine in tablets. This approach was compared to literature methods and it can be considered as a simple and fast alternative to the already existing HPLC methods with applications in loratadine quantitative analysis.

Experimental part

Material and methods

Reagents

The standard of loratadine was kindly provided by AC Helcor SRL. The anhydrous sodium acetate and glacial acetic acid were purchased from Chemical Company (Iasi, Romania). All the used reagents were of analytical grade. The HPLC grade methanol was supplied by Merck (Darmstadt, Germany). Throughout the experiment, double distilled water (DDW) was used.

The pharmaceutical products that were analyzed, were purchased from different pharmacies from Iasi region, Romania. The analyzed samples included three types of tablets produced by different manufacturers, each tablet containing 10 mg loratadine per dosage form.

The loratadine stock solution (0.5 mg/mL) was prepared as follows: 0.050 g of loratadine (reference substance) were dissolved in 50 mL methanol and brought up to a final volume of 100 mL using the same solvent, after which it was stored at low temperature until use. For the preparation of subsequent dilutions, mobile phase was used.

HPLC method

For this experiment, a RP-HPLC system (Agilent 1100 equipped with a quaternary pump, DAD, thermostat and a

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degassing system) was employed. The chromatographic analysis was performed on a C18 Eclipse XDB column (150x4.6 mm, 5 mm). Different temperatures and mixtures of the mobile phase have been assessed in order to obtain the optimal conditions described below. The optimum conditions were used in order to obtain the chromatogram which is presented in figure 1. The mobile phase consisted of a 50 mM acetate buffer solution (adjusted at *p*H=3 with glacial acetic acid) / methanol (15/85, v/v) and was used at a flow rate of 1 mL/min. The injection volume was 20 µL. The temperature of the column compartment was kept at 25°C throughout the experiment. The detection was performed at 248 nm and for the identification of loratadine in different samples, the retention time of the peak in the chromatogram of the sample was compared to that obtained in the chromatogram of the reference substance. Moreover, the sample spectra was compared with the standard one.

Method validation

The proposed method was validated by studying the following parameters: linearity, detection limit (DL) and quantitation limit (QL), precision (system precision, method precision and intermediate precision) and accuracy.

Linearity: this parameter was studied over the concentration range 0.1–50 µg/mL by plotting a calibration curve. Twenty three points, each having different concentrations were prepared by dilutions from the stock solution. Each point was prepared in triplicate. The calibration curve was constructed as the average peak area *vs.* concentration (µg/mL).

Detection limit and Quantitation limit: the limit of detection and limit of quantitation for loratadine were calculated using the calibration curve. DL and QL were determined as 3.3·SE/S and 10·SE/S where SE is the standard error and S represents the slope of the calibration curve.

Precision: the precision was studied as system precision, method precision and intermediate precision. The system precision was evaluated by analyzing 10 times the same sample containing 10 µg/mL. Method precision was carried out by determining three samples at different concentrations (7 , 10 and 13 µg/mL) representing 70, 100 and 130% from the concentration of interest. The analyses were performed by the same analyst in the same day. Intermediate precision was performed by analyzing the same three concentrations (7 , 10 and 13 µg/mL) by a different analyst in different days. All the determinations were performed in triplicate.

Accuracy: in order to evaluate the accuracy of the proposed method, the standard addition assay was used. Four concentrations (4 , 7 , 10 and 13 µg/mL) were prepared and were analyzed using the proposed method. All the samples were determined in triplicate. The final concentrations were calculated using the regression equation of the calibration curve.

Application of the validated method on pharmaceutical products

For the quantification of the loratadine content in different dosage forms, twenty tablets were weighed and their average mass was calculated. Further, the dosage forms were grinded until a fine powder was obtained. Quantities equivalent to the tablets' average masses were dissolved in 50 mL mobile phase and subjected to water bath sonication for 60 min. After that, the samples were made up to 100 mL using mobile phase and filtered through Whatman filter paper. In order to fit the concentration range of the calibration curve, suitable dilutions were prepared.

Results and discussions

The separation and quantitative determination of loratadine was performed using a HPLC method, which was developed, optimized and validated according to the ICH guidelines [17].

Several mobile phase mixtures have been tested (methanol/acetonitrile/water, methanol/acetonitrile/phosphate buffer, methanol/acetonitrile/acetate buffer, methanol/phosphate buffer, methanol/acetate buffer) at different flow rates, but the best results were obtained for a mobile phase consisting of methanol/acetate buffer (*p*H=3) at a flow rate of 1 mL/min. Under the specified conditions of HPLC analysis, the retention time of loratadine was around 3.2 mijn.

The identification of the appropriate loratadine peak was performed by comparing the retention time from the chromatograms corresponding to the standard loratadine and the loratadine from the analyzed sample. In order to increase the identification quality, the spectral comparison was also carried out.

Figure 1 also presents the identification of the loratadine peak using the retention time and spectral comparison.

In order to confirm that there are no co-eluants, the chromatographic peak purity was also studied. The obtained results (fig. 2) indicate a purity higher than 99.4%.

Linearity

The analytical response was linear over the concentration range 0.1–50 µg/mL. The linear regression equation was Peak area=34.310xConcentration+0.7534. Figure 3 presents the obtained calibration curve and in table 1 is shown the statistical evaluation of the obtained results.

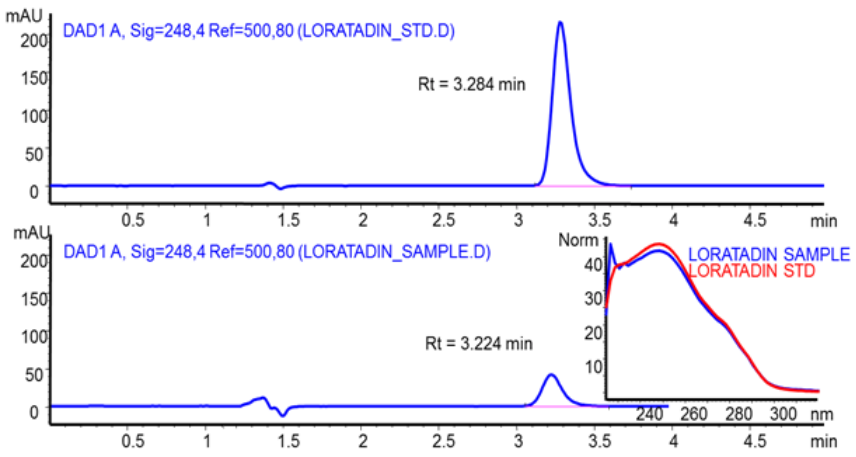

Fig. 1. Identification of loratadine after retention time and absorption spectra of standard and sample solutions respectively

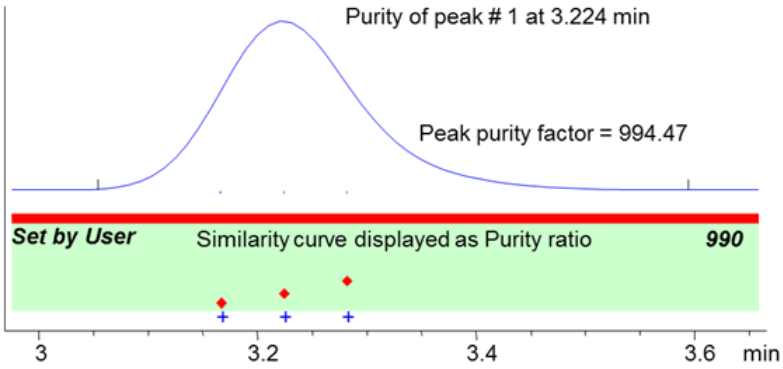

Fig. 2. Peak analysis by similarity curve vs purity ratio

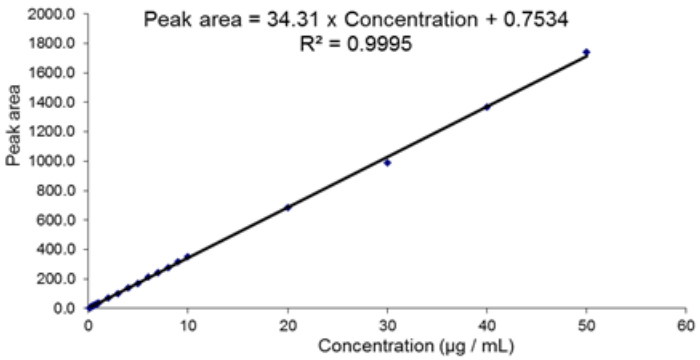

Fig. 3. Calibration curve for loratadine

Parameter	Slope	Intercept	Standard error	Regression coefficient (r^2)
Value	34.310	0.7534	11.0169	0.9995

Table 1
STATISTICAL PARAMETERS FOR THE PROPOSED METHOD

No	Peak area	No	Peak area	Statistics
1.	351.33	6.	352.502	
2.	349.073	7.	348.425	Average = 357.71
3.	353.894	8.	353.589	SD = 1.8870
4.	351.248	9.	351.593	RSD (%) = 0.5275
5.	350.419	10.	349.398	

Table 2
DATA REGARDING THE SYSTEM PRECISION (n=10)

Detection limit and quantitation limit

The calculated DL and QL were found to be 1.0596 µg/mL and 3.211 µg/mL, respectively.

Precision

System precision: the data regarding the system precision is presented in table 2 and is expressed as RSD%, representing the results of the analysis performed using the same instrument by injecting the same sample for 10 times. The obtained relative standard deviation was less than 2%.

Method precision: the results for the method precision assay, which represents the determinations carried out by the same analyst in the same analytical conditions, are shown in table 3. The concentrations were calculated using the regression equation of the calibration curve. The obtained value for the RSD% was lower than 5% so the method is considered to be precise.

Intermediate precision

In table 4 are shown the data regarding the intermediate precision. The determinations were performed in the same laboratory and the obtained results were in accordance with the international guidelines. The obtained results showed that the proposed method is precise, since all the obtained RDS% values were smaller than 5%.

Accuracy

According to the ICH guidelines, the accuracy represents the closeness degree of a measured sample to its true value [17]. The acceptable recovery can differ from the average by maximum 5%. The average recovery was 103.96 ± 0.86%. Because the mean recovery was in the range of ± 5%, the proposed method can be considered accurate.

Table 3
METHOD PRECISION RESULTS

No.	Theoretical conc. (µg/mL)	Peak area	Calculated conc. (µg/mL)	Calculated percentage	Statistics
1.	7	245.33	7.13	101.86	
2.	7	249.83	7.26	103.71	
3.	7	244.89	7.12	101.71	
4.	10	351.04	10.21	102.10	Average = 102.49 (%)
5.	10	350.08	10.19	101.80	SD = 0.8482
6.	10	349.51	10.17	101.60	RSD = 0.8276 (%)
7.	13	460.79	13.41	103.15	
8.	13	459.35	13.37	102.85	
9.	13	462.94	13.48	103.62	

Table 4
INTERMEDIATE PRECISION ASSAY RESULTS

No.	Day	Theoretical conc. (µg/mL)	Peak area	Calculated conc. (µg/mL)	Calculated percentage	Statistics
1.	1	7	251.69	7.31	104.48	
2.		10	354.70	10.32	103.16	
3.		13	461.57	13.43	103.32	
4.	2	7	251.92	7.32	104.58	Average = 103.32 (%)
5.		10	353.56	10.28	102.83	SD = 0.9240
6.		13	457.15	13.30	102.32	RSD = 0.8943 (%)
7.	3	7	251.23	7.30	104.29	
8.		10	352.98	10.27	102.66	
9.		13	456.60	13.29	102.20	

Table 5
RESULTS OF THE ACCURACY ASSAY

No.	Theoretical conc. (ug/mL)	Added conc. (ug/mL)	Peak area ± SD	Recovered conc. (ug/mL)	Recovery percentage
1.	4	-	139.06 ± 3.35	4.03 ± 0.09	100.77 ± 2.44
2.	4	3	252.34 ± 3.04	7.33 ± 0.09	104.75 ± 1.27
3.	4	6	354.29 ± 0.94	10.30 ± 0.02	103.04 ± 0.27
4.	4	9	465.10 ± 4.92	13.53 ± 0.14	104.10 ± 1.10

Application of the validated method on pharmaceutical products

The proposed RP-HPLC method was applied for the determination of loratadine from three different types of tablets, each containing 10 mg. Table 6 presents the obtained results expressed in mg loratadine / tablet and their recovery values. The data shows that the results are in accordance with the provisions of European Pharmacopoeia 8.0 [1].

No	Sample	Declared conc. of loratadine (mg/ tablet)	Peak area	Calculated conc. of loratadine (mg/ tablet)	% Recovery	Statistics
1.	Sample 1	10	326.18	9.48	94.85	Average = 92.63 (%) SD = 1.8055 RSD = 1.9490 (%)
2.			324.80	9.44	94.45	
3.			318.17	9.25	92.51	
4.	Sample 2	10	313.17	9.10	91.06	
5.			308.00	8.95	89.55	
6.			314.42	9.14	91.42	
7.	Sample 3	10	319.26	9.28	92.83	
8.			317.56	9.23	92.34	
9.			325.66	9.47	94.70	

Table 6
THE CONCENTRATIONS OF LORATADINE FOUND IN DIFFERENT TABLETS

The subject was also studied in previous papers [18, 19].

Conclusions

The proposed method was validated in terms of linearity, precision, accuracy, limit of detection and limit of quantitation. The method was found to be linear (over the concentration range 0.1–50 μg/mL, the regression coefficient was 0.9995), precise (for the system precision RSD=0.5275%, for the method precision RSD=0.8276% and for the intermediate precision RSD=0.8943%) and accurate (mean recovery=103.96%). The calculated DL and QL were found to be 1.0596 and 3.211 μg/mL. Therefore the proposed method is simple and fast and can be successfully applied for the determination of loratadine from different types of tablets.

Acknowledgements: This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/136893. The authors gratefully thank AC Helcor SRL, Maramures, for providing loratadine for this study.

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Manuscript received: 15.11.2015