

O. O. Mamina¹, V. I. Kabachny¹, N. Yu. Bondarenko¹, O. V. Lozova²¹ National University of Pharmacy of the Ministry of Health of Ukraine, Ukraine
53, Pushkinska str., Kharkiv, 61002. E-mail: a_mamina@ukr.net² Private Higher Educational Institution “Kyiv Medical University”, Ukraine

The identification and the quantitative determination of loratadine by the HPLC method

Aim. To develop the unified method of the HPLC analysis of loratadine, which can allow obtaining reliable and reproducible results of the studies of pharmaceuticals and biological matrices for monitoring the treatment effectiveness.

Materials and methods. The HPLC analysis was performed on a “Milichrome A-02” microcolumn liquid chromatograph under the following conditions: a reversed-phase variant, 2 × 75 mm column filled with a non-polar sorbent Prontosil 120-5 C₁₈ AQ, 5 μm; the mobile phase in the mode of a linear gradient – from eluent A (5% of acetonitrile and 95% of a buffer solution) to eluent B (100% of acetonitrile) for 40 min. The flow rate of the mobile phase was 100 μL/min; the injection volume was 4 μL. The multichannel detection of the substance was carried out using an UV-detector at 210, 220, 230, 240, 250, 260, 280 and 300 nm; the optimal value of the column temperature was 37–40 °C, and the pump pressure was 2.8–3.2 MPa.

Results and discussion. As a result of the studies performed, the retention parameters of loratadine and spectral relationships were determined using the unified HPLC method. This made it possible to include the results obtained in the database for the identification of antihistamines in the therapeutic monitoring of the treatment with an individual drug or in the complex treatment of allergic reactions. The development of the quantitative determination of loratadine by HPLC on model solutions using various concentrations of the drug was carried out. The content of loratadine was determined by the equation $S = 1.14 \times 10^{-3}C - 0.50 \times 10^{-4}$; the correlation coefficient was 0.9998. It was found that the relative standard deviation *RSD* did not exceed 0.93% when analyzing loratadine in the model solutions by HPLC.

Conclusions. The identification and the quantitative determination of loratadine by the unified HPLC method have been conducted. The method allows obtaining reliable and reproducible research results. The results of the studies can be recommended for implementation in the practice of forensic bureaus, toxicological centers, and clinical laboratories.

Key words: loratadine; identification; quantitative determination; high-performance liquid chromatography

O. O. Маміна¹, В. І. Кабачний¹, Н. Ю. Бондаренко¹, О. В. Лозова²¹ Національний фармацевтичний університет Міністерства охорони здоров'я України, Україна² Приватний вищий навчальний заклад «Київський медичний університет», Україна

Ідентифікація та кількісне визначення лоратадину методом ВЕРХ

Мета. Розробити уніфікований метод ВЕРХ-аналізу лоратадину, що може дозволити отримувати достовірні та відтворювані результати досліджень фармацевтичних препаратів і біологічних матриць для моніторингу ефективності лікування.

Матеріали та методи. ВЕРХ-аналіз проводили на мікроколонковому рідинному хроматографі «Міліхром А-02» у таких умовах: обернено-фазовий варіант, колонка 2 × 75 мм з неполярним сорбентом Prontosil 120-5 C₁₈ AQ, 5 мкм; рухлива фаза в режимі лінійного градієнта – від елюента А (5% ацетонітрилу і 95% буферного розчину) до елюента В (100% ацетонітрилу) протягом 40 хв. Швидкість потоку рухомої фази складала 100 мкл/хв, об'єм проби – 4 мкл. Багатоканальне детектування речовини проводили з використанням УФ-детектора за 210, 220, 230, 240, 250, 260, 280 і 300 нм; оптимальне значення температури колонки – 37–40 °C, тиск насоса – 2,8–3,2 МПа.

Результати та їх обговорення. У результаті досліджень з використанням уніфікованого методу ВЕРХ визначено параметри утримання лоратадину та спектральні відношення, що дозволило внести одержані результати в базу даних для ідентифікації антигістамінних препаратів за терапевтичного моніторингу лікування індивідуальним препаратом або за комплексного лікування алергічних реакцій. Проведено розробку кількісного визначення лоратадину методом ВЕРХ на модельних розчинах з використанням різних концентрацій препарату. Вміст лоратадину визначали за рівнянням $S = 1,14 \times 10^{-3}C - 0,50 \times 10^{-4}$; коефіцієнт кореляції становив 0,9998. Виявлено, що значення відносного стандартного відхилення результатів *RSD* не перевищувало 0,93% за ВЕРХ-аналізу лоратадину в модельних розчинах.

Висновки. Проведено ідентифікацію та кількісне визначення лоратадину за допомогою уніфікованого методу ВЕРХ. Метод дозволяє отримувати достовірні та відтворювані результати досліджень. Результати досліджень можуть бути рекомендовані для впровадження в практику бюро судово-медичних експертів, токсикологічних центрів, клінічних лабораторій.

Ключові слова: лоратадин; ідентифікація; кількісне визначення; високоефективна рідинна хроматографія

Introduction

The pharmacological treatment is the mainstay of allergy therapy, and many people use over-the-counter antihistamines to cure symptoms of seasonal allergic rhinitis.

Loratadine sold under the brand name Claritin is used as the second-generation antihistamine for the symptomatic treatment of allergic rhinitis and chronic idiopathic urticaria and has no clinically significant sedative and anticholinergic effects [1, 2]. By the chemical structure it is 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidene)-1-piperidinecarboxylic acid ethyl ester; thus, it belongs to the group of tricyclic selective blockers of peripheral H₁-histamine receptors (Fig. 1).

Loratadine is also widely used as a drug of the adjunctive therapy for the topical treatment of eczema in adults and children. *Matterne et al.* found that the use of loratadine reduced itching, improved the sleep of patients and enhanced the effect of the treatment [3]. *Fritzand et al.* observed that the well-being of patients with malignant skin diseases (melanoma) could be improved by application of loratadine and its metabolite [4]. Another research group of *Yamprasert et al.* determined that the combination of loratadine with the ginger extract had a positive effect on reducing nasal symptoms when treating allergic rhinitis [5]. *Nayak et al.* evaluated the efficacy and safety of the loratadine syrup compared with the cetirizine syrup and made a conclusion that cetirizine was more effective, but loratadine had fewer side effects [6].

In case of overdose and self-medication, loratadine can cause intoxication, which is accompanied by anaphylaxis, tachycardia, dizziness, seizures, pathological changes in the liver function [1, 2]. *Cobb et al.* reported about the deliberate intake of 300 mg of loratadine in a 6-year-old child, which resulted in an increase in blood pressure and the heart rate [7].

Motola et al. conducted a comparative analysis of the safety profiles of H₁-antihistamines using data from the WHO database (VigiBase) [8]. The authors found that cetirizine (18%), loratadine (16%) and diphenhydramine (10%) were the most demanded medications used for the treatment of children. Taking into ac-

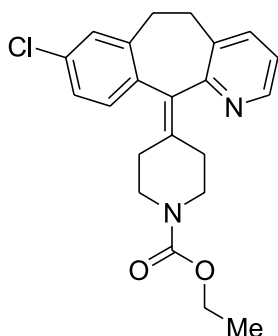


Fig. 1. The structure of loratadine

count the possibility of adverse reactions of the drugs it is necessary to carefully monitor their use.

Considering the widespread use of loratadine in medical practice and its toxic effect the development of effective and economical methods of its analysis is a topical task. Among modern methods for the analysis of drugs, one of the most highly sensitive and selective is the method of high-performance liquid chromatography (HPLC), which is widely used to diagnose intoxication with antihistamines and monitor the effectiveness of the treatment.

The literature review provides information about HPLC methods for the determination of loratadine under various conditions (the use of isocratic and gradient elution modes, the use of different compositions of the mobile phase, sorbents, buffer solutions), which are based on the individual properties of the drug, but they do not take into account the possibility of the treatment with mixtures of different drugs and combined intoxications.

Kanthiah in collaboration with *Kannappan* developed a method for the simultaneous determination of hydroxyzine, loratadine, terfenadine, rupatadine and their main active metabolites, namely cetirizine, desloratadine and fexofenadine in the serum and urine [9]. The bioanalytical method developed was approved in accordance with the FDA recommendations. The solid-phase extraction method for the sample purification and the analyte concentration was carried out using Phenomenex Strata-X-C and Strata X polymer cartridges. The chromatographic analysis was performed on a Phenomenex cyano analytical column (150×4.6 mm, 5 μm). The mobile phase was a mixture of acetonitrile-methanol-ammonium acetate (18:36:46). The flow rate was 1.5 mL/min. The analysis of substances with a UV spectrophotometric detector was carried out only at one wavelength of 222 nm. The detection limit of drugs was 0.06–0.15 μg/mL in the serum and urine samples.

Kunitski developed the method for the determination of loratadine in the blood plasma using HPLC-UV [10]. After the liquid-liquid extraction with the 2-methylbutane/hexane mixture (2:1) and evaporation of the organic phase the compounds were redissolved in 0.01 M HCl, followed by another evaporation and separation on a Supelcosil LC-18-DB column. The analyses were carried out at ambient temperature under isocratic conditions using the mobile phase of acetonitrile/water/0.5 M solution of potassium dihydrogen phosphate/phosphoric acid (440:480:80:1). The UV-detection was performed at 200 nm with a quantification limit of 0.5 ng/mL.

Li et al. elaborated and validated the method of high-performance liquid chromatography in tandem with mass spectrometry for the quantitative analysis of loratadine in samples of dry human blood stains [11]. The spots were extracted with methanol and

chromatographed using a Waters XSelect C18 column and isocratic elution to detect loratadine by MS/MS.

According to the procedure designed by *Pisareva et al.* chromatography was carried out in a reversed-phase version using a 4.0×150.0 mm steel column filled with a 3 μm Reprosil-Pur Basic C18 sorbent [12]. Elution was carried out in the isocratic mode using the mobile phase of the phosphate buffer pH 2.5/acetonitrile (30:23). The flow rate was 0.8 mL/min. The analysis of the substance was performed at the only wavelength of 220 nm.

The chromatographic analysis of loratadine by *Polyak* was conducted on a “Hewlett Packard” liquid chromatograph (USA) using an Exsclipse XDB C8 column [13]. Elution was carried out in the isocratic mode using the mobile phase of methanol/phosphate buffer pH 3.0 (70:30). The flow rate was 1.5 mL/min. The UV-detection of the substance was carried out at only one wavelength of 220 nm.

For the analysis of loratadine in biological objects *Moffat et al.* recommended different chromatographic conditions [2]. The investigation was carried out in a reversed-phase variant using a 4.6 × 250.0 mm steel column filled with a Symmetry C₈, 5 μm of the sorbent with a Symmetry C₁₈ pre-column (20 mm). Elution was carried out in the gradient mode using eluent A (phosphate buffer (pH 3.8)) and eluent B (acetonitrile). The elution program was as follows: from (85:15) to (65:35) for 6.5 min, from (65:35) to (20:80) for 25 min, hold (20:80) for 3 min and back to the initial conditions for 7 min. The flow rate was 1 mL/min for 6.5 min, then the linear increase to 1.5 mL/min within the time range of 6.5-25 min and holding for 3 min. The UV-diode-array detection was used in the research. The retention time of loratadine under the conditions mentioned above was 22.9 min.

For the analysis of loratadine in the isocratic mode, the following procedure was recommended [2]. Chromatography was carried out in a reversed-phase variant using a 4.0×125.0 mm steel column filled with a LiChrospher 100 RP-18e, 5 μm of the sorbent. The mobile phase was prepared according to the following procedure: 146 μL of triethylamine and 750 μL of phosphoric acid were added to 530 mL of water, pH was adjusted to 3.3 using 10% solution of potassium hydroxide, and finally 470 mL of acetonitrile was added. The flow rate was 0.6 mL/min. The UV diode-array detection was applied. The retention time of loratadine in the method was 14.6 min.

It is worth noting that the methods of the HPLC analysis of loratadine mentioned above have certain disadvantages. The use of an isocratic elution mode limits the possibility of all sample components leaving the column in narrow zones and efficient separation of drug mixtures [2, 10, 12]. The use of a nonlinear gradient during the elution of loratadine complicates

the chromatography process [2]. The detection of a drug at one wavelength reduces the reliability of the results obtained during the identification since it allows using only retention parameters without taking into account spectral ratios [9, 10, 12]. Moreover, HPLC methods for the analysis of loratadine described have limitations in their application to the study of mixtures containing other drugs.

Taking into account the possibility of the complex treatment of allergic reactions with various drugs simultaneously the analysis of loratadine by the unified HPLC method is an urgent task. Thus, the aim of this work was to develop the unified method of the HPLC analysis of loratadine, which can allow obtaining reliable and reproducible results of the studies of pharmaceuticals and biological matrices for monitoring the treatment effectiveness.

The presented study includes the following stages: determination of loratadine retention parameters, spectral ratios and limits of drug detection in the sample; elaboration of the HPLC method of the quantitative determination of loratadine in model solutions using different concentrations of the drug; calculation of validation characteristics of the HPLC method for determining loratadine: the range of linearity, limits of the quantitative determination, accuracy and precision based on the results of the quantitative determination of the drug by the HPLC method in model solutions.

Materials and methods

Acetonitrile (Sigma-Aldrich Laborchemikallen, GmbH), methanol (Merk, Germany) and double-distilled water (Merk, Germany) were of the purity grade “for HPLC”. The following reagents were used: lithium perchlorate trihydrate (Sigma-Aldrich, USA), acid perchloric 70% (Chimmed, Russia).

Loratadine was isolated from “Loratadine” tablets, 10 mg, (10 pcs) (Astrafarm, Ukraine) as follows. 20 Tablets containing 200 mg of loratadine were transferred to a porcelain mortar and triturated to a homogeneous state. Methanol (100.0 mL) was added to the mixture and mixed thoroughly. The resulting mixture was filtered through a paper filter in a porcelain cup, and the solvent was evaporated on a water bath at a temperature not higher than 40 °C followed by drying the residue in air at room temperature. The quality assurance was determined in accordance with the requirements of the pharmacopoeial monograph [14].

A standard methanol solution of loratadine with the concentration of 1.0 mg/mL obtained from Kharkiv Regional Bureau of Forensic Medicine was used. The chromatographic analysis was performed on a “Milichrome A-02” microcolumn liquid chromatograph (EkoNova, Russia) according to the unified HPLC methodology developed by *Baram G. Y.* [15, 16].

The studies were conducted in a reversed-phase variant on a 2×75 mm column filled with a nonpolar sorbent Prontosil 120-5 C_{18} AQ, $5 \mu\text{m}$. The mobile phase included an organic solvent and a buffer solution. Acetonitrile was filtered through the MPA-MA-N-2 (TU 6-05-1909-81) membrane with a particle size of $0.15\text{--}0.25 \mu\text{m}$ and degassed using vacuum. The buffer solution contained an ion-pair agent (0.2 M solution of lithium perchlorate in 0.005 M solution of perchloric acid), which prior to use was 25 times diluted with the potentiometric setting of the pH value at 3.0 by adding 0.005 M solution of perchloric acid.

The gradient elution with mixtures of solvents was performed with a linear gradient – from eluent A (5% of acetonitrile and 95% of a buffer solution) to eluent B (100% of acetonitrile) for 40 min. The gradient mode provided a decrease in the eluent polarity with the addition of a less polar solvent (acetonitrile) and reduced the retention time of the components. The gradient mode created the conditions for the exit from the column of loratadine in the form of a narrow zone. The final stage of the gradient corresponded to the phase with a high content of acetonitrile. The column regeneration was performed for 2 min with a mixture of solvents (2% of acetonitrile and 98% of the buffer solution).

The optimum pressure of the pump was 2.8–3.2 MPa; the flow rate of the mobile phase was $100 \mu\text{L}/\text{min}$; the optimum temperature value – $37\text{--}40^\circ\text{C}$. The volume of the samples for injection was $4 \mu\text{L}$.

The detection of the substance after it left the column was carried out with the use of a dual-beam multi-wave UV spectrophotometer in the wavelength range of 190–360 nm, the accuracy of the wavelength was 0.5 nm. For the multichannel detection of substances the wavelengths of 210, 220, 230, 240, 250, 260, 280 and 300 nm are recommended. For each value of the wavelength on the chromatogram of the substances an appropriate peak with the same retention time, but with different amplitudes was observed [15, 16].

Symmetrical, acute peaks on chromatograms were obtained by applying the unified HPLC method for the analysis of loratadine. This allowed us to calculate the required characteristics using the “MultiChrom” software (“AmperSend”, Russia), which was a standard software of the chromatograph (Fig. 2).

Results and discussion

The identification of loratadine was conducted using absolute parameters of retention time (t_R) and retention volume (V_R) (Table 1).

The suitability of the chromatographic system for HPLC studies of loratadine was confirmed by the determination of the symmetry coefficients (K_s) of the substance (not exceeding the optimal values of 0.8–1.5); coefficients of the capacity ratio (k') (were not less than 2.0) [17].

The spectral ratio values of S_λ/S_{210} are given in Table 2.

The comparative assessment of spectral ratios makes it possible to obtain more reliable and reproducible

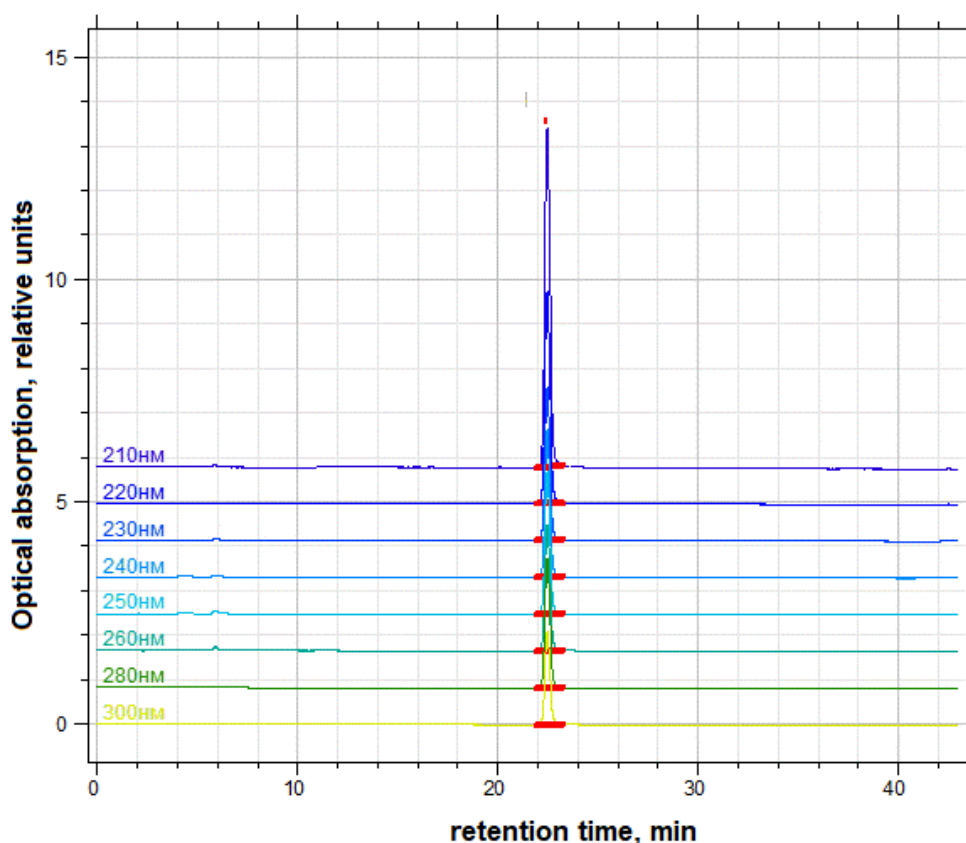


Fig. 2. The chromatogram of the methanol solution of loratadine ($C = 1.0 \text{ mg}/\text{mL}$)

Table 1

Retention parameters of loratadine obtained as the result of the HPLC analysis
($n = 5, P = 95\%$)

Parameter	Parameter values	Metrological characteristics					
		\bar{x}	S^2	$S\bar{x}$	$\Delta\bar{x}$	RSD	$\bar{\epsilon}, \%$
t_{abs}, min	22.18–23.28	22.73	0.19	0.20	0.55	0.87	2.42
$V_{abs}, \mu\text{L}$	2218.5–2328.3	2273.4	1944.8	19.76	54.94	0.87	2.42
Coefficient of the peak symmetry, Ks	0.88–1.00	0.95	0.0026	0.023	0.064	2.41	6.74
Coefficient of the capacity ratio, k'	13.70–14.53	14.16	0.09	0.13	0.37	0.95	2.59

Table 2

The spectral ratios (S_λ / S_{210}) of loratadine ($n = 5$)

Substance	S_{220}/S_{210}	S_{230}/S_{210}	S_{240}/S_{210}	S_{250}/S_{210}	S_{260}/S_{210}	S_{280}/S_{210}	S_{300}/S_{210}
Loratadine	0.617 ± 0.005	0.440 ± 0.004	0.424 ± 0.004	0.409 ± 0.004	0.363 ± 0.003	0.373 ± 0.004	0.268 ± 0.003

Note: S – is the area under the peak on the chromatogram

results, as well as to identify drugs with similar values of retention parameters.

The HPLC method for the loratadine determination was validated by such parameters as the linearity range, detection limit (LOD), quantification limit (LOQ), accuracy and precision in the regions of low, medium and high concentrations of the substances [17]. The method of absolute calibration was used for the quantitative determination of loratadine by the HPLC method.

To determine the range of linearity, a calibration graph was constructed in the coordinates: S, mm^2 (area under the peaks) – $C, \mu\text{g/mL}$ (the concentration of loratadine solutions) (Fig. 3).

For the HPLC analysis of loratadine, 100.00 mL volumetric flasks were poured with 0.50; 1.00; 2.00; 4.00; 6.00; 8.00 and 10.00 mL of the standard loratadine solution (1 mg/mL) using a pipette. The volumes of solutions were diluted to the volume with the ap-

propriate solvent; thus, the working standard solutions 1-7 had concentrations of 5.00; 10.00; 20.00; 40.00; 60.00; 80.00 and 100.00 $\mu\text{g/mL}$, respectively).

The HPLC studies were performed with the application of the method proposed, the volume of the sample was 4.0 μL . Five parallel HPLC measurements were performed for each concentration.

The main validation characteristics of the quantitative determination of loratadine (coefficients of regression of the calibration graph, the correlation coefficient, the linearity interval of the calibration graph) are presented in Table 3. The regression coefficients of the calibration graph equation were calculated using the least squares method.

It was found that the linearity of the calibration graph of loratadine in the coordinates $S, \text{mm}^2 - C, \mu\text{g/mL}$ was observed in the interval of concentrations of 5.0–100.0 $\mu\text{g/mL}$, which corresponded to the content of loratadine in the sample (4.0 μL) from 20.0 ng to 400.0 ng, respectively. The equation of the calibration curve was $S = 1.14 \times 10^{-3}C - 0.50 \times 10^{-4}$. After verifying the significance of the coefficient a in the equation it was concluded that the transition to its shortened form $S = 1.14 \times 10^{-3}C$ was possible. The correlation coefficient (r) was equal to 0.9998.

The values LOD and LOQ were calculated based on the parameters of the calibration line (the standard deviation of a free term of the equation – Sa and the tangent of slope – b) according to the equations: $LOD = 3,3Sa/b$; $LOQ = 10Sa/b$ [17].

It was determined that the detection limit (LOD) and the quantification limit (LOQ) of loratadine by the HPLC method were equal to 0.16 $\mu\text{g/mL}$ or 0.64 ng in the sample and 0.47 $\mu\text{g/mL}$ or 1.88 ng in the sample, respectively.

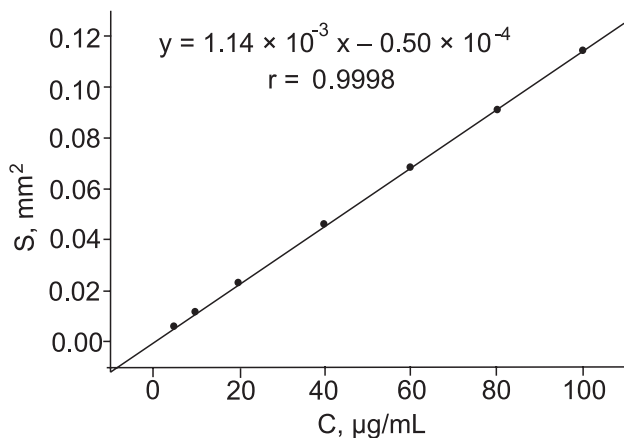


Fig. 3. The calibration graph of the quantitative determination of loratadine by the HPLC method

Table 3

The coefficients of regression of the calibration graph $S = bC + a$ of the quantitative determination of loratadine by the HPLC method ($P = 95\%$, $n = 7$)

The coefficients of regression of the calibration graph		Confidence intervals of coefficients of regression		Correlation coefficient (r)	The interval of the linearity of the calibration graph, $\mu\text{g/mL}$
a	b	Δa	Δb		
$-0.50 \cdot 10^{-4}$	$1.14 \cdot 10^{-3}$	$1.30 \cdot 10^{-4}$	$2.23 \cdot 10^{-6}$	0.9998	5.0–100.0

Table 4

The results of the quantitative determination of loratadine in model solutions by the HPLC method ($n = 5$, $P = 95\%$)

Concentration of the solution, $\mu\text{g/mL}$	S , mm^2	Concentration of the substance determined		Metrological characteristics					
		$\mu\text{g/mL}$	%	\bar{x}	S^2	$S\bar{x}$	$\Delta\bar{x}$	RSD	$\bar{\epsilon}$, %
5.0	0.0057	5.01	100.2	100.2	2.83	0.75	2.09	0.75	2.09
10.0	0.0113	9.95	99.5	99.5	3.69	0.86	2.39	0.86	2.40
20.0	0.0229	20.10	100.4	100.4	2.93	0.76	2.13	0.76	2.12
40.0	0.0447	39.28	98.2	98.2	2.45	0.70	1.94	0.71	1.98
60.0	0.0689	60.79	101.3	101.3	2.08	0.64	1.79	0.64	1.77
80.0	0.0911	79.76	99.7	99.7	2.85	0.75	2.10	0.76	2.10
100.0	0.1169	102.50	102.5	102.5	3.88	0.88	2.45	0.86	2.39

The results of the quantitative determination of loratadine in model solutions by the HPLC method are presented in Table 4.

The accuracy and precision of the HPLC method for the quantification of loratadine were determined from the values of the relative standard deviation RSD for various loratadine concentrations in the model solutions using the calibration curve or the equation. It was found that the values of the relative standard deviation were close in magnitude and were in the range of 0.71–0.86%. It indicates that the results of the analysis are close to the true value.

The studies were performed on samples of one batch of drugs by one analyst under identical condi-

tions (reagents, equipment, laboratory) for a short period of time, which confirmed the convergence of the results.

For comparative evaluation of the reproducibility of the loratadine analysis by the HPLC method, the studies were conducted with the change of certain conditions (the analysis at different times) in the regions of low (10.0 $\mu\text{g/mL}$), medium (60.0 $\mu\text{g/mL}$) and high concentrations (100.0 $\mu\text{g/mL}$) of the substance studied: during one day of the investigation (*intra-day*) and during the next day (*inter-day*). The results of the accuracy and precision analysis of the loratadine quantitative determination in model solutions by the HPLC method are presented in Table 5.

Table 5

Accuracy and precision of the quantitative determination of loratadine in the model solutions by the HPLC method ($n = 5$, $P = 95\%$)

Concentration of the solution, $\mu\text{g/mL}$	Concentration of substance determined, %	Metrological characteristics					
		\bar{x}	S^2	$S\bar{x}$	$\Delta\bar{x}$	RSD	$\bar{\epsilon}$, %
<i>Intra-day</i>							
10.0	98.2–101.7	99.92	2.02	0.64	1.77	0.64	1.77
60.0	98.1–102.0	100.06	2.56	0.71	1.98	0.71	1.98
100.0	98.1–102.4	100.22	2.96	0.77	2.14	0.77	2.14
<i>Inter-day</i>							
10.0	99.0–102.0	99.82	3.20	0.80	2.22	0.80	2.22
60.0	97.4–102.5	99.94	4.33	0.93	2.58	0.93	2.59
100.0	97.8–103.0	100.40	4.24	0.92	2.56	0.92	2.55

The precision of the quantitative determination of loratadine in the model solutions by the HPLC method was studied as a result of the *intra-day* and *inter-day* research.

It was found that the values of the relative standard deviation of the results of the analysis of loratadine during one day (*intra-day*) in the area of low, medium and high concentrations of *RSD* were in the range of 0.64–0.77%.

The values of the relative standard deviation of the results of the analysis of loratadine during the next day (*inter-day*) in the area of low, medium and high concentrations of *RSD* were in the close range of 0.80–0.93%, confirming the convergence of the analysis results. Some change in the *RSD* value may be due to the volatility of the solvent in methanol solutions.

The HPLC method developed for the analysis of loratadine can be recommended for implementation in the practice of the Bureau of Forensic Medicine when conducting research on drugs found near the corpse.

At the same time, the procedure developed can be part of the algorithm for studying loratadine in biological objects, including isolation, purification,

identification, and the quantitative determination of the substance.

Conclusions

1. The identification and the quantitative determination of loratadine by the unified HPLC method have been conducted. The method allows obtaining reliable and reproducible research results.

2. Such validation characteristics of the HPLC method for determining loratadine as the range of linearity, the limits of detection and quantification, the accuracy and precision of the results of the quantitative determination of the drug by HPLC in model solutions have been calculated.

3. After conducting the experimental studies on the biological material using the HPLC method for the analysis of loratadine the data obtained can be recommended for implementation in the practice of forensic bureaus, toxicological centers, and clinical laboratories for the study of drugs in biological objects.

Conflict of Interests: the authors have no conflict of interests to declare.

References

- Mashkovskiy, M. D. *Lekarstvennye sredstva* [Medicines, in Russian], 16th ed.; Novaya Volna: Moscow, 2012.
- Moffat, A. C.; Osselton, M. D.; Widdop, B. *Clarke's Analysis of Drugs and Poisons in Pharmaceuticals, Body Fluids and Postmortem Material*, 4th ed.; Pharmaceutical Press: London, 2011.
- Matterne, U.; Böhmer, M. M.; Weisshaar, E.; Jupiter, A.; Carter, B.; Apfelmacher, C. J. Oral H1 antihistamines as 'add-on' therapy to topical treatment for eczema. *Cochrane Database of Systematic Reviews* 2019, 1. <https://doi.org/10.1002/14651858.CD012167.pub2>.
- Fritz, I.; Wagner, P.; Bottai, M.; Eriksson, H.; Ingvar, C.; Krakowski, I.; Nielsen, K.; Olsson, H. Desloratadine and loratadine use associated with improved melanoma survival. *Allergy* 2020, 75 (8), 2096–2099. <https://doi.org/10.1111/all.14273>.
- Yamprasert, R.; Chanvimalueng, W.; Mukkasombut, N.; Itharat, A. Ginger extract versus Loratadine in the treatment of allergic rhinitis: a randomized controlled trial. *BMC Complementary Medicine and Therapies* 2020, 20 (1), 119. <https://doi.org/10.1186/s12906-020-2875-z>.
- Nayak, A. S.; Berger, W. E.; LaForce, C. F.; Urdaneta, E. R.; Patel, M. K.; Franklin, K. B.; Wu, M.-M. Randomized, placebo-controlled study of cetirizine and loratadine in children with seasonal allergic rhinitis. *Allergy and Asthma Proceedings* 2017, 38 (3), 222–230. <https://doi.org/10.2500/aap.2017.38.4050>.
- Cobb, D. B.; Watson, W. A.; Fernández, M. C. High-dose loratadine exposure in a six-year-old child. *Veterinary and human toxicology* 2001, 43 (3), 163–164.
- Motola, D.; Donati, M.; Biagi, C.; Calamelli, E.; Cipriani, F.; Melis, M.; Monaco, L.; Vaccheri, A.; Ricci, G. Safety profile of H1-antihistamines in pediatrics: an analysis based on data from VigiBase. *Pharmacoepidemiology and Drug Safety* 2017, 26 (10), 1164–1171. <https://doi.org/10.1002/pds.4246>.
- Kanthiah, S.; Kannappan, V. D-Optimal mixture design optimization of an HPLC method for simultaneous determination of commonly used antihistaminic parent molecules and their active metabolites in human serum and urine. *Biomed. Chromatogr.* 2017, 31 (8), e3932. <https://doi.org/10.1002/bmc.3932>.
- Kunicki, P. K. Determination of loratadine in human plasma by high-performance liquid chromatographic method with ultraviolet detection. *Journal of Chromatography B: Biomedical Sciences and Applications* 2001, 755 (1), 331–335. [https://doi.org/10.1016/S0378-4347\(01\)00053-6](https://doi.org/10.1016/S0378-4347(01)00053-6).
- Li, W.; Doherty, J.; Moench, P.; Flarakos, J.; Tse, F. L. LC-MS/MS bioanalysis of loratadine (Claritin) in dried blood spot (DBS) samples collected by subjects in a clinical research study. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* 2015, 983–984, 117–124. <https://doi.org/10.1016/j.jchromb.2014.12.033>.
- Pisareva, N. A.; Novikov, O. O.; Pisarev, D. I.; Zhilyakova, E. T.; Sabelnikova, N. N. Razrabotka metodik kolichestvennogo opredeleniya loratadina v substansii [Development of the method for quantitative determination of loratadine in a substance, in Russian]. *Belgorod State University Scientific bulletin. Medicine. Pharmacy* 2012, 22, 161–164.
- Polyak, O. B.; Bondar, V. S. Rozrobka metodiv identyfikatsii loratadynu [The elaboration of methods for loratadine identification, in Ukrainian]. *News of Pharmacy* 2003, 4 (36), 40–44.
- European Pharmacopoeia*, 6th Ed.; European Department for the Quality of Medicines: Strasbourg, 2008, 2286–2288.
- Baram, G. I. Portable liquid chromatograph for mobile laboratories I. *Aims. J. Chromatogr. A* 1996, 728 (1), 387–399. [https://doi.org/10.1016/0021-9673\(95\)01271-0](https://doi.org/10.1016/0021-9673(95)01271-0).
- Baram, G. I. Hromatograf "Milihrom A-02". Opredelenie veshchestv s primeneniem baz danyh "VEZhKh-UF" [Chromatograph "Milichrom A-02". Determination of substances using databases "HPLC-UV", in Russian] https://new.econova.ru/wp-content/uploads/2021/01/publication_collections_2.pdf (accessed Aug 10, 2021).
- Derzhavna farmakopeia Ukrainy*, 1 vydannia, dopovnennia 2 [The State Pharmacopoeia of Ukraine, 1st ed., suppl. 2, in Ukrainian]; State Enterprise "Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines": Kharkiv, 2008.

Received: 14. 07. 2021

Revised: 17. 08. 2021

Accepted: 25. 08. 2021

The work is a part of the researches of the National University of Pharmacy on the topic "Organic synthesis and analysis of biologically active compounds, drug development on the basis of synthetic substances" (the state registration No. 01144000943; the research period of 2019–2024)