СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

Recommended by Doctor of Pharmacy, professor O.A.Yevtifeyeva

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DEVELPOMENT AND EVALUATION OF VALIDATION CHARACTERISTICS OF THE QUANTITATIVE DETERMINATION METHOD FOR LORATADINE IN THE SYRUP

A.V.Glushchenko, V.A.Georgiyants, N.Yu.Bevz

National University of Pharmacy

Key words: validation; pharmaceutical analysis; spectrophotometry; loratadine; syrup

The problem of allergic diseases growth is quite urgent in the world: up to 40% of the adult population and 10-12% of children suffer from this disease. According to the data of epidemiologic studies, the most widespread diseases are bronchial asthma, allergic rhinitis and atopic dermatitis. Among avaliable antihistamine drugs loratadine is one of the most common, it is used for elimination of allergic manifestations both in adults and children. Since for adherence to specifications of the technological process when manufacturing medicines it is necessary to have the developed and validated methods for the quality control, we have proposed a new method for quantitative determination of loratadine in the syrup by the spectrophotometric method. When studying the character of loratadine spectra in alcohol, 0.1 M solution of hydrochloric acid and 0.1 M solution of sodium hydroxide it has been found that the best solvent is 0.1 M solution of hydrochloric acid. The assay was performed by the standard method at the wavelength of 278 nm. The effect of excipients of the syrup was removed by their extraction with ether. The validation characteristics of the method (robustness, accuracy and convergence, linearity, precision) have been determined. The evaluation of such validation characteristics obtained as accuracy (0.18% ≤ 1.54%), convergence (1.6% ≤ 3.2%), intermediate precision (0.62% ≤ 4.80%) allows to control the quality of loratadine in other laboratories according to the method recommended.

In recent years the concept of pre-asthma – the state combining a number of pathological signs has been formed. According to the studies they are precoursors of development of the real asthmatic attacks. Among them there are various kinds of allergy (allergic rhinosinusopathy, enteropathy, medicamental dermatitis) and immunological disorders (complement deficiency state, lgE hyperproduction, etc.). In this regard, the medicinal effect on this state is in fact prophylaxis of bronchial asthma.

New antihistamine drugs (H_1-receptor antagonists) are referred to the medicines that are successfully used in the treatment and prevention of seasonal allergic rhinitis, allergic conjunctivitis. Unlike the medicines for treating allergic manifestations used for a long period of time, new medicines do not provide a sedative action, do not possess antiserotonin, anticholinergic and α-blocking properties; and it allows to use them more widely in a certain patient population [8].

Loratadine is one of the representatives of the group of antihistamines of the second generation that block H_1-receptors. Medicines of this group reduce the body's response to histamine, eliminate unstriated muscles spasms and prevent development of the tissue edema induced by histamine, reduce the capillary permeability, decrease the hypotensive action of histamine and allergic reactions. Under the effect of antihistamine drugs the histamine toxicity decreases.

The method of liquid chromatography is widely applied for quantitative determination of loratadine both in the pure form and in the combination with other medicinal substance in dosage forms [6, 9-11]. But, from our point of view, especially when using in industrial manufacture, spectrophotometric method for loratadine determination can be the most suitable and economical.

The aim of the work is to develop the method for spectrophotometric quantitative determination of loratadine in the syrup for the subsequent application in analysis of medicinal forms.

Materials and Methods

Claritin syrup, batch 1ANNA59001, manufactured by Schering-Plough Labo NV, Belgium; the substance of loratadine, batch LRD/0909180, manufactured by Vasudna Pharma Chem Limited were used in the research.

The following analytical equipment was used: a "Thermo scientific Evolution 60S" spectrophotometer, "AXIS" balances. The measuring glassware of class A, excipients (glycerine, polypropylene glycol, sodium benzoate) and reagents meeting the requirements of the State Pharmacopoeia of Ukraine (SPhU) were used for the work [1-5].

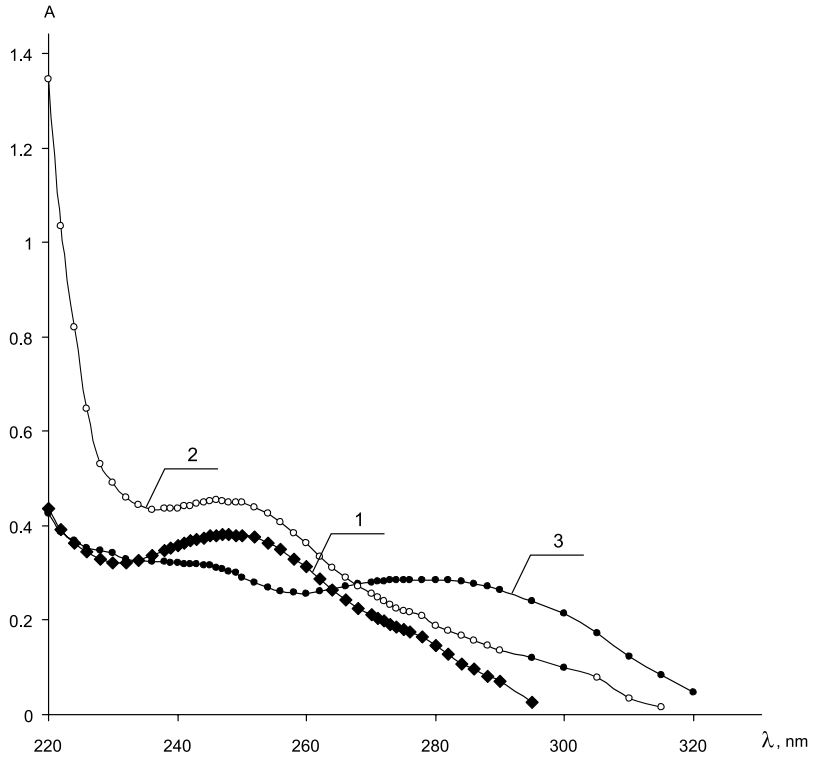

Fig. 1. The UV-spectrum of 0.001% loratadine solution in ethanol (1), in 0.1 *M* solution of sodium hydroxide (2) and in 0.1 M solution of hydrochloric acid (3).

Results and Discussion

To solve the task we recorded UV-spectra of 0.001% loratadine solution in ethanol, 0.1 M solution of hydrochloric acid and 0.1 M solution of sodium hydroxide.

The UV-spectrum of 0.001% loratadine solution in ethanol recorded in the range from 220 nm to 320 nm is given in Fig 1. As it is seen from Fig. 1, the UV-spectrum of alcohol solution has the absorption maximum at the wavelength of 248 nm, which is conditioned by the presence of the aromatic ring in the molecule of the medicine. When changing the solvent to 0.1 M solution of sodium hydroxide the character of the spectrum does not practically change and the maximum is observed at the wavelength of 246 nm. In case of using 0.1 M solution of hydrochloric acid as a solvent one can observe that the character of the spectrum changes and the maximum is observed at the wavelength of more than 278 nm (Fig. 1).

All active substances and excipients of the syrup dissolve in 0.1 M solution of hydrochloric acid. Thus, we were used exactly this solvent. It has been experimentally proven that excipients affect the intensity of the UV-spectrum of loratadine (Fig. 2).

As can be seen from Fig. 2, UV-spectra of the syrup (2) and loratadine (1) completely coincide in the given wavelength range, but the optical density of the solution of the syrup at the wavelength of 278 nm is higher ($A_2 > A_1$) than in the solution of loratadine in the same concentration. To eliminate the impact of excipients on the optical density value they were extracted from the syrup with the organic solvent (Fig. 3).

As the research results show, conformity of the optical absorption of loratadine in 0.1 M solution of hydrochloric acid to Bouguer-Lambert-Beer law is observed in the concentration range of 4.0×10^{-4}-4.0×10^{-3}%, the specific absorption indicator is from 233 to 245.

The method was metrologically standardized on the model mixtures. It has been found that the relative error of an individual determination ±2.22% does not exceed the allowable deviation interval for the active substance in the syrup (±10%). The method developed has been used for quantitative determination of loratadine in the syrup.

The method for determination of loratadine in the syrup:

Place 20.00 ml of the syrup into a 100 ml volumetric flask, dilute with 1 M solution of hydrochloric acid to the volume and mix.

Transfer 10.00 ml of the solution obtained into a separating funnel and extract each 5 ml three times with diethyl ether. Separate the organic layer. Place the aqueous layer into a 100 ml volumetric flask, add 10 ml of 1 M

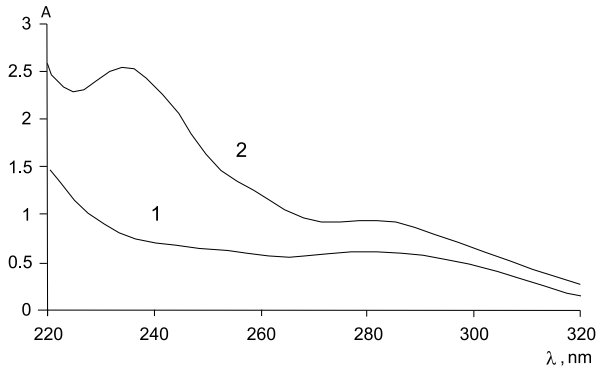

Fig. 2. The UV-spectrum of loratadine in 0.1 *M* solution of hydrochloric acid (1) and the UV-spectrum of the syrup components in 0.1 *M* solution of hydrochloric acid (2).

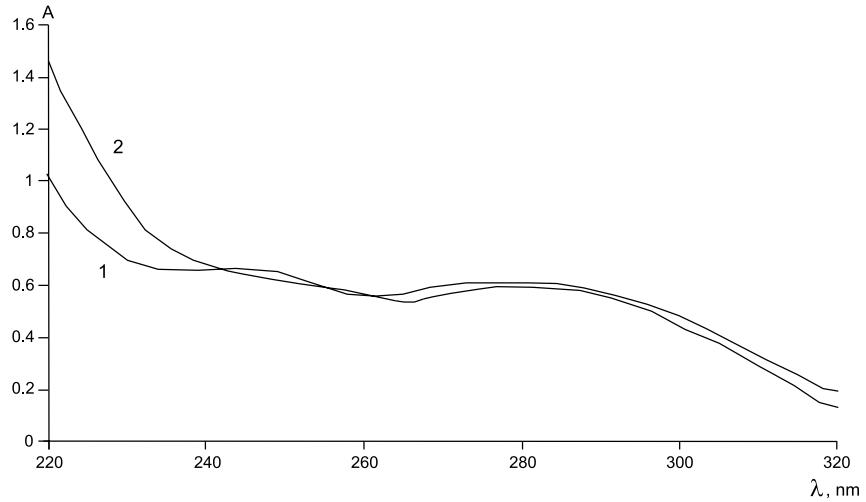

Fig. 3. The UV-spectrum in 0.1 *M* solution of hydrochloric acid: 1 – loratadine and 2 – the syrup components after extraction with ester.

Table 1

Stability of solutions in time

t, min	0	15	30	45	60	Mean	RSD_t%	Δ_t%	maxδ, %
A_o	0.5824	0.5825	0.5822	0.5828	0.5829	0.5826	0.043	0.09	1.54
A	0.5835	0.5836	0.5834	0.5835	0.5838	0.5833	0.084	0.18	

solution of hydrochloric acid and dilute to the volume with purified water.

Measure the optical density of the solution obtained at the wavelength of 278 nm in a cuvette with the layer thickness of 10 mm using 0.1 M solution of hydrochloric acid as a reference solution.

Concomitantly measure the optical density of the reference solution of loratadine.

Reference solution of loratadine. Weigh accurately approximately 0.05 g of loratadine and put into 250 ml volumetric flask, dissolve in 150 ml of 1 M solution of hydrochloric acid, dilute to the volume with the same solvent and mix.

Place 10 ml of the solution obtained into a 100 ml volumetric flask, add 10 ml of 1 M solution of hydrochloric acid and dilute to the volume with purified water.

The calculation of the loratadine content in the syrup was performed in g per 1 ml of the syrup.

Then some validation characteristics of the method were studied; they were evaluated according to the criteria given in the SPhU [1-4].

To determine robustness of the method [7], the effect of placebo on the optical density and stability of solutions over time were studied. For this purpose measure the optical density (A_{blank}) of the blank solution taking not less than three measurements with removing the cuvette. Concomitantly measure the optical density (A_{st}) of the reference solution. It was found: $A_{blank} = 0.00102$; $A_{st} = 0.5822$. The contribution of placebo in the total absorption of the medicine after extraction with ether equals $\delta_{exc} = 100 \times 0.00102/0.5822 = 0.18\%$.

When determining stability of solutions in time (Table 1) it has been found that the content of the test solution and the reference solution does not significantly change for not less than 1 h ($\Delta t \le \max\delta = 1.54\%$).

When studying linearity of the method the reference solution and the test solutions were prepared by the same scheme, actual values X_i were determined by the ratio of the mass of the actual weighted amounts of loratadine substances taken for preparing the given test solution and the reference solution.

Measurement of the optical density for each of 9 test solutions was conducted three times. The ratio of mean values of the optical density for each of 9 test solutions and the mean value of the optical density for the reference solution was calculated, the values $Y_i = (A_i/A_{cT})\times 100$ were obtained. The value $Z = 100\times(Y_i/X_i)$ representing the concentration found (%) to the concentration introduced was also calculated. The results of the calculations are shown in Table 1. The calculations of the linear dependence parameters $Y_i = b \times X_i + a$ were performed by the least square method [2]. The calculated values b=0.9989, s_b=0.01, a=0.31, s_a=0.55, s_r=0.21 (the residual standard deviation) and r=0.9999 (the correlation coefficient) meet the the requirements of the SPhU concerning the linear dependence parameters; the straight line obtained in the normalized coordinates is given in Fig. 4.

The calculations of the detection limit (DL) and the limit of the quantitative determination (LQD) for loratadine were conducted optionally by the following ratios:

$$DL = 3.3 \times s_a / b \approx 3.3 \times s_a,$$
$$LQD = 10 \times s_a / b \approx 10 \times s_a.$$

Based on values s_a and *b:* DL = $3.3 \times 0.55 = 1.82\%$ of the nominal loratadine concentration, LQD = $10 \times 0.55 = 5.51\%$ of the nominal loratadine concentration.

The given values are significantly less than the lower detection limit of concentrations (80%) and, therefore, can not influence on the accuracy of analysis.

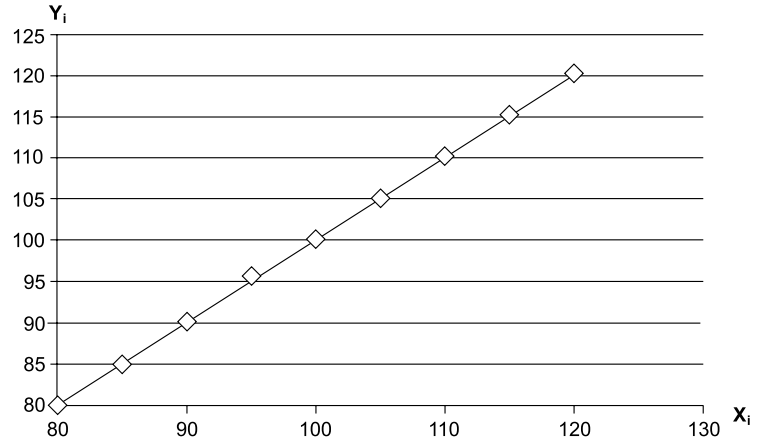

Fig. 4. The plot of linear dependence $Y_i = b \times X_i + a$ in the normalized coordinates.

Table 2

The results of analysis for test solutions and their statistical processing

No. of the test solution	Sample weight, g (m_{st} =0.02074)	Introduced in % to the concentration of the reference solution – Xi_{act} %	Average optical densities (A_i^{st} = 0.5822)	Found in % to the concentration of the reference solution – Y_i%	Found in % to the introduced $Z_i = 100 \times (Y_i / X_i)$ %
1	0.0160	80.00	0.4660	80.07	100.09
2	0.0170	85.00	0.4950	85.05	100.06
3	0.0180	90.00	0.5250	90.21	100.23
4	0.0190	95.00	0.5570	95.70	100.74
5	0.0200	100.00	0.5830	100.17	100.17
6	0.0210	105.00	0.6120	105.15	100.15
7	0.0220	110.00	0.6410	110.14	100.12
8	0.0230	115.00	0.6700	115.12	100.10
9	0.0240	120.00	0.6990	120.10	100.09
Mean, $\bar{Z}$%					100.19
Relative standard deviation, s_z%					0.21
Relative confidence interval $\Delta_{as}\% = t(95\%,8) \cdot s_z = 1.860 \cdot s_z =$					0.39
Critical value for convergence of results $\Delta_{as}\% \leq$					1.60
Systematic error $\delta = \lvert\bar{Z} - 100\rvert$					0.19
Criterion of the systematic error insignificance $\delta \leq \Delta as/(g)\text{^}0,5 = 1.07/3 = 0.36$ if it is not satisfied 1), then $\delta \leq 0.73$					 satisfied satisfied
The overall conclusion of the method:					correct

As Table 2 shows, the method of analysis is characterized by a sufficient convergence and accuracy within the range of concentrations of 80-120%.

It has been experimentally proven that the ratio $\Delta_{intra} = t[95\%,(n \times m - 1)] \times SD_{Z-intra} = 1.76 \times SD_{Z-intra} \leq max\ \Delta_{As}$ is performed 0.62 < 4.8%, i.e. the intermediate precision is confirmed.

To confirm the method's correctness when reproducing it in some other laboratory it is necessary to predict the total uncertainty of the method.

The predicted total uncertainty of the results of the quantitative determination method (0.83%) does not exceed the critical value (3.2%), i.e. the method will give correct results in other laboratories as well.

Thus, by the results obtained the possibility of the quality control of the given medicine in other laboratories according to the method developed has been confirmed.

CONCLUSIONS

1. According to the results of the study of the UV-spectral characteristics of loratadine solution in alcohol, 0.1 M sodium hydroxide solution and 0.1 M hydrochloric acid solution, the method for quantitative determination of loratadine in the syrup has been developed.

2. The validation characteristics (robustness, accuracy, convergence, linearity, precision) of the method developed for spectrophotometric quantitative determination of loratadine in the syrup have been investigated and their evaluation concerning the acceptance criteria has been carried out.

3. It has been found that the method recommended allows to control the quality of loratadine in the syrup in other laboratories.

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РОЗРОБКА ТА ОЦІНКА ВАЛІДАЦІЙНИХ ХАРАКТЕРИСТИК МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ ЛОРАТАДИНУ У СИРОПІ

Г.В.Глущенко, В.А.Георгіянц, Н.Ю.Бевз

Ключові слова: *валідація; фармацевтичний аналіз; метод спектрофотометрії; лоратадин; сироп*

Проблема зростання кількості алергічних захворювань дуже актуальна у світі: до 40% дорослого населення і 10-12% дітей страждають на алергію. Згідно з даними епідеміологічних досліджень найбільш поширеними захворюваннями є бронхіальна астма, алергічний риніт і атопічний дерматит. Серед існуючого арсеналу антигістамінних препаратів одним з найбільш поширених є лоратадин, який усуває алергічні прояви як у дорослих, так і у дітей. Оскільки для дотримання умов технологічного процесу під час виробництва лікарських препаратів необхідно мати розроблені і валідовані методики контролю їх якості, ми запропонували нову методику кількісного визначення лоратадину в сиропі спектрофотометричним методом. При вивченні характеру спектрів лоратадину у спирті, 0,1 М розчині кислоти хлористоводневої та 0,1 М розчині гідроксиду натрію встановлено, що як розчинник краще використовувати 0,1 М розчин кислоти хлористоводневої. Визначення проводили методом стандарту при довжині хвилі 278 нм. Вплив допоміжних речовин у сиропі усували екстрагуванням ефіром. Визначені валідаційні характеристики (робасність, правильність, точність, лінійність, відтворюваність) методики. Оцінка отриманих валідаційних характеристик: правильність (0.18% ≤ 1.54%), точність (1.6% ≤ 3.2%), внутрішньолабораторна прецизійність (0.62% ≤ 4.80%) дозволяють контролювати якість лікарського засобу в умовах інших лабораторій за рекомендованою методикою.

РАЗРАБОТКА И ОЦЕНКА ВАЛИДАЦИОННЫХ ХАРАКТЕРИСТИК МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ЛОРАТАДИНА В СИРОПЕ

А.В.Глущенко, В.А.Георгиянц, Н.Ю.Бевз

Ключевые слова: *валидация; фармацевтический анализ; метод спектрофотометрии; лоратадин; сироп*

Проблема роста числа аллергических заболеваний очень актуальна в мире: до 40% взрослого населения и 10-12% детей страдают аллергией. Согласно данным эпидемиологических исследований наиболее распространенными заболеваниями является бронхиальная астма, аллергический ринит и атопический дерматит. Среди существующего арсенала антигистаминных препаратов одним из наиболее распространенных является лоратадин, который устраняет аллергические проявления как у взрослых, так и у детей. Поскольку для соблюдения условий технологического процесса при производстве лекарственных препаратов необходимо иметь разработанные и валидированные методики контроля их качества, нами предложена новая методика количественного определения лоратадина в сиропе спектрофотометрическим методом. При изучении характера спектров лоратадина в спирте, 0,1 М растворе кислоты хлористоводородной и 0,1 М растворе гидроксида натрия установлено, что в качестве растворителя лучше использовать 0,1 М раствор кислоты хлористоводородной. Определение проводили методом стандарта при длине волны 278 нм. Влияние вспомогательных веществ в сиропе устраняли экстрагированием эфиром. Определены валидационные характеристики (робастность, правильность, точность, линейность, воспроизводимость) методики. Оценка полученных валидационных характеристик: правильность (0.18% ≤ 1.54%), точность (1.6% ≤ 3.2%), внутрилабораторная прецизионность (0.62% ≤ 4.80%) позволяют по рекомендованной методике контролировать качество лекарственного средства в условиях других лабораторий.