

Development and Validation of Methods of Quantitative Determination of Loratadine in Tablets

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Abstract

Methods for the quantitative determination of loratadine tablets by differential spectrophotometry and photo-colorimetry have been developed. During the procedure parameters (range of application methods, stability, specificity, robustness, convergence, internal laboratory accuracy (precision), accuracy, reproducibility) have been considered. The results indicate that the method photo-colorimetry has not met modern criteria for analytical methods for quantitative determination of parameters such as stability, accuracy and linearity, so other validation parameters (robustness, convergence and inter-laboratory precision) have been determined only by spectrophotometric methods. It is found that the technique of quantitative determination of loratadine by differential spectrophotometry is accurate, correct, reproducible and linear, to determine its suitability for use in pharmaceutical analysis.

Keywords: Differential Spectrophotometry, Loratadine Tablets, Photo-Colorimetry, Quantitative Detection, Validation

1. Introduction

Loratadine - H₁-antihistamines second generation anti-histamine non sedative effective long-term action to rapid and severe allergic effect. It does not affect the central nervous system, does not exhibit anticholinergic and sedative action does not cause addiction. Also detects and anti vasoconstrictive effect on the nasal mucosa, prevents spasm of the bronchi, has a membrane stabilizing effect and so on. Its use to quickly achieve remission and relapse prevention spending allergic diseases, especially in children¹ and in treatment of complex allergic disease, such as asthma and allergic rhinitis, allergic conjunctivitis, atopic dermatitis, etc². Pharmaceutical market in Ukraine has many mono-drugs and some combination drugs that contain loratadine³.

The problem of the development of new methods for the quantitative determination of active pharmaceutical ingredients in the finished product was and remains relevant and at present there. Due to the large quantities of organic solvents for the test, complexity and sustainability sample preparation and methods in general, the high cost of reagents and instruments - will increase the costs for analyzing both the laboratory quality control of medicines and in the Central Laboratory of pharmaceutical companies. Therefore, the development of new, more advanced and simple in execution methods of analysis of drugs remains a topical issue of modern pharmacy.

As we know from literature sources⁴, the substance is loratadine sufficiently studied in terms of pharmaceutical analysis: for its developed methods of identification and quantitative determination. Quantitative determination

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of loratadine in medicines and methods of differential spectrophotometry and photo-colorimetry literature practically are not described. Therefore, the above mentioned methods we have chosen to quantify loratadine tablets.

1.1 The Purpose of the Work

Development and validation of quantitative determination of loratadine tablets by photo-colorimetry and differential spectrophotometry.

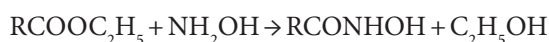
2. Experimental Section

2.1 Research Methods

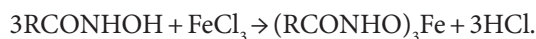
The study was conducted on five samples loratadine tablets domestic producers «Zdorovja» (series 31012), «Lekchim» (series 100512), «Farmak» (series 330912), «Arterium» (series 40612), «Darnitsa» (series 200712). In this series using analytical equipment: UV Carry-spectrophotometer 50, electronic scales AVT-120-5DM; measuring vessel class, appropriate reagents that meet the requirements of SPU and pharmacopoeia standard sample (CRS) loratadine (certificate number 11 / 1-243 of 02.12.13), acquired in the State enterprise «Ukrainian scientific center pharmacopoeia quality medicines».

2.2 Sample Preparation

Preliminary tests we have developed methods of identification of loratadine in tablets, one of which was based on qualitative reactions - the formation of hydroxamic acids^{5,6}. Loratadine as the ester can form hydroxamic acid by reaction equation:



Hydroxamic acid ions Fe^{3+} forms a complex compound of blue-red color, whose intensity can be measured spectrophotometrically:



Spectrophotometric method of quantitative determination of loratadine tablets by differential spectrophotometry below:

2.2.1 Test Solution

The exact weight powdered tablets equivalent to 0.00104 millimole loratadine is dissolved in *methanol R* and filtered

into volumetric flask, add 0.5 ml of 2 mole *hydroxylamine hydrochloride R* in *methanol R*, 0.5 ml solution of 0.09 millimole *potassium hydroxide R* in *alcohol R*, heated and cooled with shaking. The resulting solution is acidified with 6 drops of 2 mole *hydrochloric acid R*, add 0.2 ml of 0.26 millimole *iron (III) chloride R1* diluted 10 times; appears bluish-red color. The resulting solution is adjusted *methanol R* to the mark and immediately measure the optical density.

2.2.2 Reference Solution

0.00104 millimole loratadine CRS dissolved in *methanol R* in a volumetric flask, add 0.5 ml of 2 mole *hydroxylamine hydrochloride R* in *methanol R*, 0.5 ml solution of 0.09 millimole *potassium hydroxide R* in *alcohol R* is heated and cooled with shaking. The resulting solution is acidified with 6 drops of 2 mole *hydrochloric acid R*, add 0.2 ml of 0.26 millimole *iron (III) chloride R1* diluted 10 times; appears bluish-red color. The resulting solution is adjusted methanol to the mark and immediately measure the optical density.

2.2.3 Compensation Solution

0.00104 millimole loratadine CRS dissolved in *methanol R* in a volumetric flask, add 0.5 ml solution of 0.09 millimole *potassium hydroxide R* in *alcohol R*, heated and cooled with shaking. The resulting solution is acidified with 6 drops of 2 mole *hydrochloric acid R*. The resulting solution is adjusted methanol to the mark and immediately measure the optical density. Absorbance of the test solution and reference solution is measured at 530 nm wavelength regarding compensation solution. Calculate the content of loratadine in a single tablet, in g, based on the average tablet weight, based on the declared loratadine content of CRS loratadine. Content loratadine tablets, g, calculated by formula (3.1).

$$X = \frac{A_x \cdot m_o \cdot m_{av}}{A_o \cdot m}$$

Validation methods performed in accordance with the requirements of HFCs under the standard procedure of validation techniques quantification of medicines [7].

3. Results

During the procedure considered the following parameters: range of application methods, stability, specificity,

Table 1. The study of the stability analytical solution for the quantitative determination by photo-colorimetry of loratadine in tablets

№	Term stability studies, t, min					Average	RSDt, %	Δt , %	max δ , %
	2,5	5	10	15	30				
Ast	0,4840	0,4817	0,4753	0,4613	0,4437	0,4678	3,416	7,28	1,52
Ax	0,4787	0,4712	0,4660	0,4557	0,4393	0,4641	3,336	7,11	

Table 2. The study of the stability analytical solution for the quantitative determination by spectrophotometry of loratadine in tablets

№	Term stability studies, t, min					Average	RSDt, %	max δ , %
	0	15	30	45	60			
Ast	0,4117	0,4117	0,4130	0,4130	0,4140	0,4123	0,311	1,52
Ax	0,4060	0,4053	0,4087	0,4053	0,4053	0,4057	0,299	

Table 3. Statistical indicators metrological characteristics of methods

Metrological Characteristics	Photo-colorimetric technique	Spectrophotometric technique
Convergence and accuracy		
Gotten result, Z, %	101,17	99,63
relative standard deviation, Szi, %	4,53	0,83
The relative confidence interval, Δz , %	8,20	1,46
Critical for convergence results, Δas , %	4,80	4,80
Systematic error, δ	1,07	-0,37
Criterion uncertainty bias	1,52	1,52
Linearity		
The angular coefficient of linear dependence, b	1,1932	0,9691
Sb	0,0617	0,0129
Free Member linear relationship, a	-17,3389	2,5450
The critical value for free member, a	5,1200	5,1200
Sa	6,3161	1,3228
The correlation coefficient methods, r	0,9950	0,9997
Criterion linear correlation coefficient, Rc	0,9914	0,9914
Conclusion:	does not comply	Comply or correct

Table 4. Effect of pH on the absorption of optical density analytical solutions

№	Optical density testing			Sr_{pH} , %	RSD_{pH} , %	ΔpH , %	max δ , %
	Diluted hydrochloric acid R	Without adding	100 g / l potassium hydroxide R				
1	0,315	0,220	0,325	0,0049	0,484	1,40	1,52
2	0,375	0,270	0,388	0,0047	0,467	1,36	
3	0,450	0,320	0,460	0,0045	0,451	1,32	

Table 5. The results determine the internal laboratory accuracy

Number of model solution	Added, % $X_i, \%$	Received result, % $Y_i, \%$	Received to Added $Z_i = 100 (Y_i / X_i)$
1	70,00	71,78	102,55
2		70,79	101,13
3		71,29	101,84
4	86,00	86,88	101,02
5		86,63	100,74
6		88,88	101,02
7	100,00	98,76	98,76
8		98,71	98,51
9		99,26	99,26
10	115,00	115,84	100,73
11		116,34	101,16
12		116,09	100,95
13	130,00	129,70	99,77
14		129,46	99,58
15		130,69	100,53
The relative standard deviation, $S_r, \%$			1,1199
The relative confidence interval, $\Delta z, \%$			0,1315
Systematic error, δ			-0,185
Criterion uncertainty bias and error			1,52

robustness, convergence, internal laboratory accuracy (precision), accuracy, reproducibility.

4. Discussion

To assess the model error of sample preparation solutions and standard working solution were calculated theoretical values of uncertainty of analytical operations. Complete uncertainty for sample preparation photo-colorimetric and spectrophotometric methods are: $\Delta_{sp} = 1,02\%$, $\Delta_{sp} = 1,30\%$ respectively. If validation photometric method of assay validation are first considered parameters such as stability analytical solution at the time, linearity, accuracy and convergence. The research methodology was carried out under the same conditions: in one day, in one device, by the same analyst. Check the stability of solutions was conducted for 30 minutes to 60 minutes and photo-colorimetric spectrophotometric method for analyzing the working standard solution and sample solutions. These tables indicate that the analytical solution is only stable for about 5 minutes, causing difficulties in conducting the experiment, so all the results obtained by this method for the first five minutes of cooking. Instability of analytical solutions can lead to deviations from the Beer-Lambert law and getting incorrect results. In this case, the stabil-

ity of the solution is influenced by such factors: the rate of flow of a colorimetric reaction; conditions in which we determine the extent of coverage and working rooms; pH, concentration of loratadine and reagents; Properties hydroxamate iron; change the degree of dissociation of the compound in solution dilution. So to avoid errors when using this technique has important way to prepare analytical solutions.

Analytical solution spectrophotometric method stable for hours, which is enough to determine the absorbance. To confirm the specificity of the technique relied systematic error, which made solvent and reagents, that contribution placebo. For each method measured the optical density of the solutions placebo and working standards. The values of optical densities placebo solution number 1 and standard methods for photo-colorimetric: $A_{blank} = 0.001$; $A_{st} = 0,389$, hence $\delta_{exc} = 100 \cdot 0,001 / 0,389 = 0,26\%$. Effect placebo solution number 2 results spectrophotometric method is: $A_{blank} = 0.001$; $A_{st} = 0,468$, $\delta_{exc} = 0,21\%$, confirming the absence of a meaningful impact on the measurement results for both techniques. To assess the linearity and accuracy were obtained 15 values of optical densities of model solutions and 4 of the optical densities for solution standard. The results are presented in the table. Calculated ratio of average

values of optical densities for each of the 15 solutions to the mean absorbance reference solution to give value $= S_i \text{ Hui} / S_{st} \cdot 100\%$, $Y_i = (A_i / A_{st}) \cdot 100$. Worked in normalized coordinates, giving the concentration and analytical signal as a percentage of the nominal value. They found as the value of $Z = 100 \cdot (Y_i / X_i)$, which is found concentrations as a percentage of the administered.

The results indicate that the method photo-colorimetric do not meet modern criteria for analytical methods for quantitative determination of parameters such as stability, accuracy and linearity, so other validation parameters (robustness, convergence and inter-laboratory precision) determined only spectrophotometric methods.

Robustness evaluation was performed taking into account the type of methodology, in this case spectrophotometric method for studying the following parameters: stability over time analytic solutions prepared by the method (described above); effect of pH on the stability of the optical absorption solution ethacridine. The results of studying the stability of analytical solutions spectrophotometric method for hours in the table below and demonstrate the stability of analytical solutions, inequality $\Delta t\% \leq 0,32 \cdot \max \Delta A_s = \max \delta$ performed. To study the effect of pH for the test solution (85%, 100%, 115%) was added to 1-2 drops of HCl or KOH solution to play fluctuation pH of $\pm 10\%$. For model solutions obtained measured optical density at the selected wavelength. Internal laboratory accuracy (precision) was assessed by the analysis of 15 samples of various model series, which was carried out in two different days and different analysts in one laboratory conditions. To prepare two different series were taken in our various sample concentration range. To prepare dilutions of these series use different measuring laboratory dishes. During the preparation of solutions and of technique was possible fluctuations in temperature, humidity, atmospheric pressure and other factors influencing the internal laboratory analysis. The technique is correct, because ΔZ , calculated by the ratio, the requirements: $\Delta Z\% \leq \max \Delta A_s = 4,8\%$. The method has significant bias. To examine the reproducibility of this method in terms of other laboratories, we measured the

optical density of the solutions of a series of model solutions loratadine on different hardware, different days in three different laboratories. The results are presented in the table and are the results of comparing statistical deviations of three different experiments, associations and the average value of a single relative standard deviation. The resulting metrological data show that this technique can be replicated in other laboratories with confidence probability 95% deviation of the unit value of $100 \pm 1,23\%$. The results of analysis show that this technique can be used to quantify the loratadine tablets and can be included in the quality control methods for loratadine in tablets.

5. Conclusion

In conclusion, we developed spectrophotometric and photo-colorimetric methods of quantitative determination of loratadine in tablets. These methods are showed good following parameters: specificity, linearity, range, accuracy (convergence) and accuracy, robustness, the results of which revealed that spectrophotometric method can be used for the determination of loratadine in tablets and can be used for quality control of tablets.

6. References

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