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Validation of Quantitative Determination Methods for Fexofenadine Hydrochloride and Cyanocobalamine in Separate Ophthalmological Dosage Forms Using UV-Spectrophotometry Instrumentation

Elena Zhilyakova
Pharmaceutical technology department, Belgorod State National Research University
Belgorod, Russia
EZhilyakova@bsu.edu.ru

Nabel Mohamad
Pharmaceutical technology department, Belgorod State National Research University
Belgorod, Russia
nabilmohamad1945@gmail.com

Abdulhadi Bakri
Pharmaceutical technology department, Belgorod State National Research University
Belgorod, Russia
Hadiolya4@gmail.com

Denis Naplekov
Pharmaceutical technology department, Belgorod State National Research University
Belgorod, Russia
d.naplekov@ya.ru

Diana Martseva
Pharmaceutical technology department, Belgorod State National Research University
Belgorod, Russia
dianamartceva@mail.com

Abstract—Aim of this study is validation of quantitative determination methods for Fexofenadine hydrochloride, Cyanocobalamine in separate ophthalmological dosage forms using UV-spectrophotometry instrumentation. Assay tests of the studied ophthalmological dosage forms were carried out using the APIs and reagents of pharmaceutical grade. As APIs, Fexofenadine hydrochloride, Cyanocobalamine were chosen. For dilution of Fexofenadine hydrochloride dosage form, 5% solution of sodium hydroxide and purified water were used. Solution of cyanocobalamine was diluted by purified water. Spectrophotometer SF-104 was used in this study, manufactured in Russia by Akvilon SZ Company. Spectrophotometric cells with optical path length of 10 mm were used for all the measurements. By the obtained experimental data it was established that the proposed assay methods for Fexofenadine hydrochloride and Cyanocobalamine ophthalmological solutions are reliable and valid. The new methods for Fexofenadine hydrochloride and Cyanocobalamine ophthalmological solutions were proposed and successfully validated in from the perspective of specificity, linearity, analytical range, correctness and repeatability.

Keywords—Fexofenadine hydrochloride, assay, ophthalmology, UV-spectrophotometry, cyanocobalamine.

I. INTRODUCTION

At the present moment the problem of necessity of developing the new dosage forms for treatment of various ophthalmological disorders is continuing to become more relevant year by year. This fact is explained by modern living and working conditions of people, in particular, the most valuable contribution to development of eye disorders are brought by overuse of portable electronic device, personal computers and etc. This is why such disorder of eyes can be developed as dry eye syndrome.

From the other side, another eye disease is the same spread all over the world as glaucoma and dry eye syndrome [1]. It is called allergic conjunctivitis. Its frequent development is not explained by the modern lifestyle of population, but by absence of adequate dosage forms on Russian pharmaceutical market. As it's been highlighted in previous studies already, currently such active pharmaceutical substances (APIs) are used as either representatives of H_1-hystamine blockers from previous generations or substances of steroid molecular structure. In the first case, the use of H_1-hystamine blockers is not preferable due to their side effects, large list of contraindications and not sufficient efficacy. In the second case, the use of steroid-structured APIs is not preferable either due to their severe suppressive effect on both local and general immune system, which might lead to various clinical complications and hence, development of other diseases.

This is why the development of new dosage forms for treatment of dry eye syndrome and allergic conjunctivitis is a relevant goal for pharmaceutical development field. For this purpose the new dosage forms were proposed. For treatment of allergic conjunctivitis, Fexofenadine hydrochloride is an adequate modern H_1-hystamine blocker of the last generation to be included into the content of eye drops. For treatment of dry eye syndrome, Cyanocobalamin in combination with Hyaluronic acid is

included in the second dosage form in order to normalize the intraocular metabolism and lubricate the eye surface [2, 3].

Although the methods for listed APIs are described in various regulatory documentation, e.g., United States Pharmacopoeia, National Pharmacopoeia of Russian Federation and etc., the validation of the assay methods is required since all the proposed dosage forms include various polymeric substance with the purpose of expanding the drug residence on the eye surface.

The most rational assay method is chosen to be UV-spectrophotometry, by which it will be proved that the presence of polymeric substances does not affect the quantitative determination nor for Fexofenadine hydrochloride, or Cyanocobalamine.

Aim of this study is validation of quantitative determination methods for Fexofenadine hydrochloride, Cyanocobalamine in separate ophthalmological dosage forms using UV-spectrophotometry instrumentation.

II. EXPERIMENTAL

Reagents and chemicals

Assay tests of the studied ophthalmological dosage forms were carried out using the APIs and reagents of pharmaceutical grade. As APIs, Fexofenadine hydrochloride, Cyanocobalamine were chosen.

For dilution of Fexofenadine hydrochloride dosage form, 5% solution of sodium hydroxide and purified water were used. Solution of cyanocobalamine was diluted by purified water.

UV-spectrophotometric instrumentation

Spectrophotometer SF-104 was used in this study, manufactured in Russia by Akvilon SZ Company. Spectrophotometric cells with optical path length of 10 mm were used for all the measurements.

Assay method for Fexofenadine hydrochloride ophthalmological solution

1 ml of Fexofenadine hydrochloride solution is transferred into 50 ml volumetric flask, the volume is lead up to 50 ml using the 5% sodium hydroxide solution. The sample is shaken for no less than 5 minutes. Optical density is measured at wavelengths range of 200-240 nm at absorbance maximum of 228 nm. 5% sodium hydroxide solution is used as a reference solution.

The concentration is calculated with the help of specific absorbance coefficient.

Assay method for Cyanocobalamine ophthalmological solution

1 ml of Cyanocobalamine solution is transferred into 25 ml volumetric flask, the volume is lead up to 25 ml using the purified water. The sample is shaken for no less than 5 minutes. Optical density is measured at wavelengths range of 340-390 nm at absorbance maximum of 361 nm. Purified water is used as a reference solution.

The concentration is calculated with the help of specific absorbance coefficient.

Approach to validation of the studied dosage forms

The same approach to validation was chosen for all three dosage forms and complied with to monograph 1.1.0012.15 «Validation of analytical methods». The assay methods were validated for such parameters as analytical range, linearity, repeatability and correctness.

Mathematical methods

The processing of the obtained results was performed with the help of the preset for data analysis in MS Excel 2010

III. RESULTS

As the first step of validation, the specificities of assay methods of Fexofenadine hydrochloride and Cyanocobalamine were confirmed first. The graphs of UV-absorption of Fexofenadine hydrochloride and Cyanocobalamine are shown on figures 1 and 2.

As it can be seen from pictures above, the assay methods are specific both for Fexofenadine hydrochloride and Cyanocobalamine dosage forms.

This statement is based upon the fact that spectra of APIs look the same according to the ones provided in scientific data bases [4,5]. Analytical range and linearity. It was found that the method withstands the requirements for the quantitative determination of Fexofenadine hydrochloride and Cyanocobalamine in the concentration

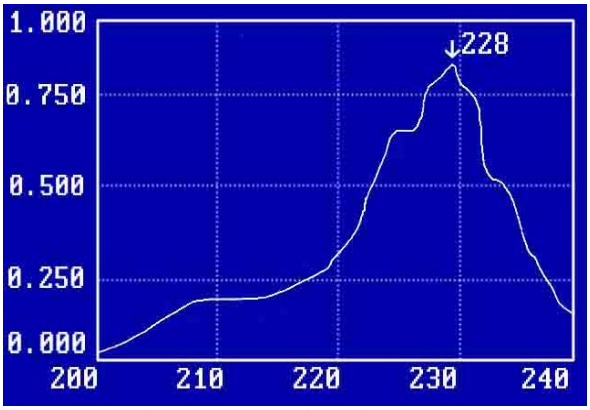

Fig. 1. Dynamics of UV-absorption of Fexofenadine hydrochloride solution.

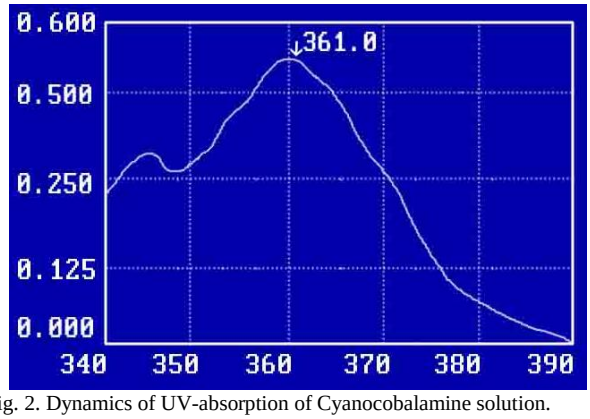

Fig. 2. Dynamics of UV-absorption of Cyanocobalamine solution.

range from 80 to 120% of the nominal value of the content of the active substance in the solution of the model sample. The basis for confirming the analytical area was the approach according to which 5 model solutions were prepared with Fexofenadine and Cyanocobalamine contents from 80 to 120% of the nominal each, in 10% increments. Then, the optical densities of the model solutions were measured, their concentrations were calculated, and a linear dependence of the optical density on the concentration of Fexofenaine hydrochloride and Cyanocobalamine were plotted. The results are shown in table I and figures 3 and 4.

As follows from the data above, all the experimental points lie on the trend line, which indicates the presence of a linear dependence of the optical density of the solution on the concentration of Fexofenadine hydrochloride and Cyanocobalamine, while the generalized dynamics of UV absorption by samples of ophthalmic solutions of different concentrations look like shown in figures 5 and 6.

As can be seen from pictures 5 and 6, with each subsequent decrease in the concentration of the solution, a decrease in the optical density is also observed.

Correctness. The correctness of the method is characterized by the deviation of the average result of the determinations made with its use from the value taken as true.

To confirm the correctness of the method of quantitative analysis of ophthalmic solutions, the following approach was applied: 3 model solutions were prepared at concentrations of 80, 100 and 120%. Then analyzed by UV spectrophotometry, calculated the concentration, found concentration, average value, standard deviation (SD) and relative standard deviation (RSD). The experimental results are shown in Tables II and III.

TABLE I. THE RESULTS OF THE CONFIRMATION OF THE ANALYTICAL RANGE AND THE LINEARITY OF THE METHOD FOR THE QUANTITATIVE DETERMINATION OF FEXOFENADINE HYDROCHLORIDE AND CYANOCOBALAMINE IN A MODEL OPHTHALMIC SOLUTION

No	API content, % of LC	Fexofenadine hydrochloride		Cyanocobalamine	
		Optical density	Actual content, %	Optical density	Actual content, %
1	120	0.811	0.119	0.535	0.065
2	110	0.751	0.110	0.469	0.057
3	100	0.689	0.101	0.423	0.051
4	90	0.627	0.092	0.364	0.044
5	80	0.566	0.083	0.301	0.036

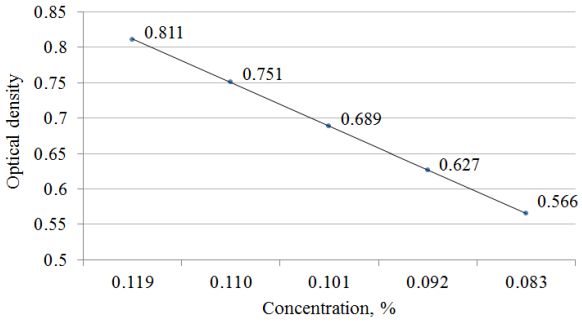

Fig. 3. A calibration graph of the linear dependence of the optical density on the concentration of Fexofenadine hydrochloride.

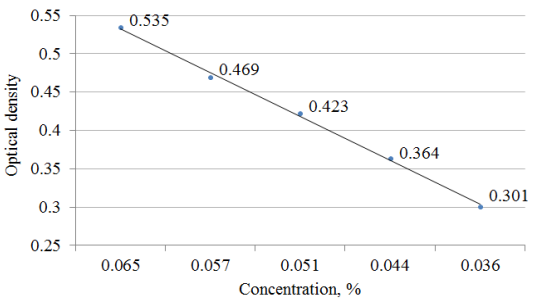

Fig. 4. A calibration graph of the linear dependence of the optical density on the concentration of Cyanocobalamine.

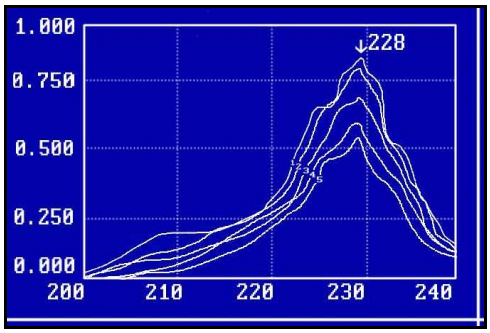

Fig. 5. Generalized dynamics of UV absorption by Fexofenadine hydrochloride solution.

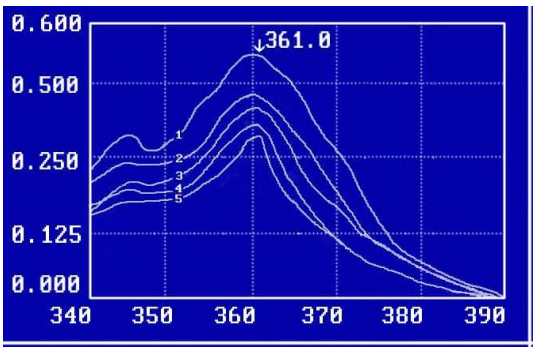

Fig. 6. Generalized dynamics of UV absorption by Cyanocobalamine solution.

From Tables II and III it can be seen that at all three concentration levels, the relative standard deviation is 1.26% for Fexofenadine hydrochloride and 2.71% for Cyanocobalamin, which indicates the correctness of the methods. For all test samples, the found value was approximately 100%.

According to monograph 1.1.0012.15 «Validation of analytical methods», the repeatability of the analytical method should be assessed by independent results obtained under the same regulated conditions in the same laboratory, of the same artist within a short period of time.

To confirm repeatability, 6 model solutions of each dosage form containing the regulated value of Fexofenadine hydrochloride and Cyanocobalamine were prepared and analyzed. The experimental results are shown in Tables IV and V.

From the data of tables 4 and 5 it follows that the results of the quantitative determination of fexofenadine hydrochloride are similar, as evidenced by the average value of the found content, standard deviation and relative standard deviation.

IV. Discussion

By the obtained experimental data it was established that the proposed assay methods for Fexofenadine hydrochloride and Cyanocobalamine ophthalmological solutions are reliable and valid.

TABLE II. The Results of Determining the Correctness of the Method for Quantitative Determination of Fexofenadine Hydrochloride at Three Concentration Levels

No	Optical density	Content,%	Found, %	C av., %	SD	RSD, %
1	0.809	0.118274854	98.56237817			
2	0.813	0.118859649	99.0497076			
3	0.804	0.11754386	97.95321637			
4	0.680	0.099415205	99.41520468			
5	0.671	0.098099415	98.0994152	99.29	1.2545	1.26
6	0.677	0.098976608	98.97660819			
7	0.547	0.07997076	99.96345029			
8	0.544	0.079532164	99.41520468			
9	0.559	0.081725146	102.1564327			

TABLE III. The Results of Determining the Correctness of the Method for Quantitative Determination of Cyanocobalamine at Three Concentration Levels

No	Optical density	Content,%	Found, %	C av., %	SD	RSD, %
1	0.543	0.06558	99.3632			
2	0.537	0.064855	98.26526			
3	0.549	0.066304	100.4611			
4	0.418	0.050483	100.9662			
5	0.407	0.049155	98.30918	100.58	2.7214	2.71
6	0.401	0.04843	96.8599			
7	0.313	0.037802	105.0054			
8	0.304	0.036715	101.986			
9	0.310	0.03744	103.9989			

TABLE IV. REPEATABILITY RESULTS OF QUANTITATIVE DETERMINATION OF FEXOFENADINE HYDROCHLORIDE AT THREE CONCENTRATION LEVELS

No	Optical density	Content,%	Found, %	C av., %	SD	RSD, %
1	0.683	0.100	99.8538			
2	0.671	0.098	98.09942			
3	0.698	0.102	102.0468	99.683	1.3381	1.34
4	0.680	0.099	99.4152			
5	0.676	0.099	98.83041			
6	0.683	0.100	99.8538			

TABLE V REPEATABILITY RESULTS OF QUANTITATIVE DETERMINATION OF CYANOCOBALAMINE AT THREE CONCENTRATION LEVELS

No	Optical density	Content,%	Found, %	C av., %	SD	RSD, %
1	0.683	0.100	99.8538			
2	0.671	0.098	98.09942			
3	0.698	0.102	102.0468	99.683	1.3381	1.34
4	0.680	0.099	99.4152			
5	0.676	0.099	98.83041			
6	0.683	0.100	99.8538			

V. CONCLUSION

The new methods for Fexofenadine hydrochloride and Cyanocobalamine ophthalmological solutions were proposed and successfully validated in from the perspective of specificity, linearity, analytical range, correctness and repeatability

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