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DEVELOPMENT AND VALIDATION OF HPLC METHODS FOR THE ANALYSIS OF AZITHROMYCIN IN A MODEL MIXTURE WITH CETIRIZINE

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ABSTRACT

A method for quality control of azithromycin in a model mixture with cetirizine using HPLC has been developed and validated. Chromatography conditions have been selected. The HPLC analysis method has been validated according to the following criteria: linearity, specificity, accuracy, sensitivity, accuracy, repeatability, reproducibility.

KEYWORDS: Azithromycin, cetirizine, model mixture, HPLC, validation.

INTRODUCTION

Antibacterial drugs are non-renewable resources; this is due to the continuous development of antibiotic resistance in microorganisms. The discovery and introduction into clinical practice of macrolide antibiotics has become one of the largest achievements in the history of antimicrobial therapy. This, in turn, places increased demands on the quality control of medicines and the improvement of methods for the quantitative determination of antibiotics, and in particular the group of macrolides.

Relevance: Currently, there is a relatively small number of works on the determination of azithromycin. An important problem remains the development of new, more sensitive and selective methods for their analysis. Determination of macroquantities of azithromycin is important for assessing the pharmacological action and effectiveness of antibacterial therapy, identifying active substances in dosage forms, as well as its metabolites in biological matrices.[1,2,3]

Goal: Development and validation of HPLC methods for the analysis of azithromycin and a model mixture with cetirizine.

MATERIALS AND METHODS

Azithromycin, high-performance liquid chromatography, cetirizine.

RESULTS AND DISCUSSION

A method for the quantitative determination of azithromycin in a model mixture has been developed. The following chromatographic conditions were selected.

X chromatography column: Column 150 mm long, 4.6 mm i.d., Waters X-Terra RP18, 5 µm, or an equivalent column may be used after appropriate validation.
Mobile phase: Ammonium phosphate buffer solution, pH 9.8: Acetonitrile (35: 65).
Flow rate: 1.0 ml/min.
.Detector: UV at 210 nm.
Temperature: 40 ^{o}C.
Injection volume: 10 µl
Analysis time: 10 min.
Working concentration: azithromycin – 500 µg/ml, cetirizine – 50 µg/ml.
Suitability of the chromatography system:
RSD: ≤ 2.0%
Resolution between azithromycin and cetirizine peaks: 1.0.
Asymmetry coefficient: 1.5.
Preparation Ammonium phosphate buffer solution, pH 9.8: dissolve 0.264 g of dibasic ammonium phosphate in 1000 ml of water (0.002
M) and adjust the pH to 9.8 by adding ammonia solution using a pH meter to control RA. Filter and degas under vacuum through a 0.45 µm membrane filter.
Solvent preparation: Mobile phase
Preparation of a standard solution of azithromycin: about 50 mg (t.n.) of azithromycin CO is placed in a 100 ml volumetric flask, dissolved in a solvent, the volume of the solvent solution is adjusted to the mark and mixed. (500 µg/ml).
Preparation of a standard solution of cetirizine: about 50 mg (ton) of cetirizine CO is placed in a 100 ml volumetric flask, dissolved in a solvent, the volume of

the solvent solution is adjusted to the mark and mixed. 10 ml of the resulting solution is placed in a 100 ml volumetric flask, the volume of the solvent solution is adjusted to the mark and mixed (50 µg/ml).
Preparation of the test solution: about 2.5 g (t.n.) of the gel is placed in a 100 ml volumetric flask, dissolved in the solvent, the volume of the solvent solution is adjusted to the mark and mixed. Filter through a 0.45 µm membrane filter.
10 µl of the test solution and standard solutions are alternately chromatographed on a liquid chromatograph, obtaining at least 5 chromatograms for each solution.

The content of azithromycin, in grams, per 100 g of gel, is calculated using the formula.

$$X = \frac{S_1 \cdot a_0 \cdot 100 \cdot P \cdot 100}{S_0 \cdot a_1 \cdot 100 \cdot 100} = \frac{S_1 \cdot a_0 \cdot P}{S_0 \cdot a_1} \quad (1)$$

Where:

S_1– the average value of the peak areas of azithromycin, calculated from the chromatograms of the test solution;

S_1– average value of azithromycin peak areas, calculated from chromatograms of azithromycin CO solution;

a_0– mass of a sample of azithromycin CO, in grams;

a_1– weight of the gel sample, in grams;

P– content of azithromycin in SOazithromycin, as a percentage.

The content of cetirizine, in grams, per 100 g of gel, is calculated using the formula.
Where:

$$X = \frac{S_1 \cdot a_0 \cdot 100 \cdot 10 \cdot P \cdot 100}{S_0 \cdot a_1 \cdot 100 \cdot 100 \cdot 100} = \frac{S_1 \cdot a_0 \cdot P \cdot 0{,}1}{S_0 \cdot a_1} \quad (2)$$

S_1 – average value of cetirizine peak areas, calculated from
chromatogram of the test solution;

S_1– average value of cetirizine peak areas, calculated from chromatograms of cetirizine CO solution;

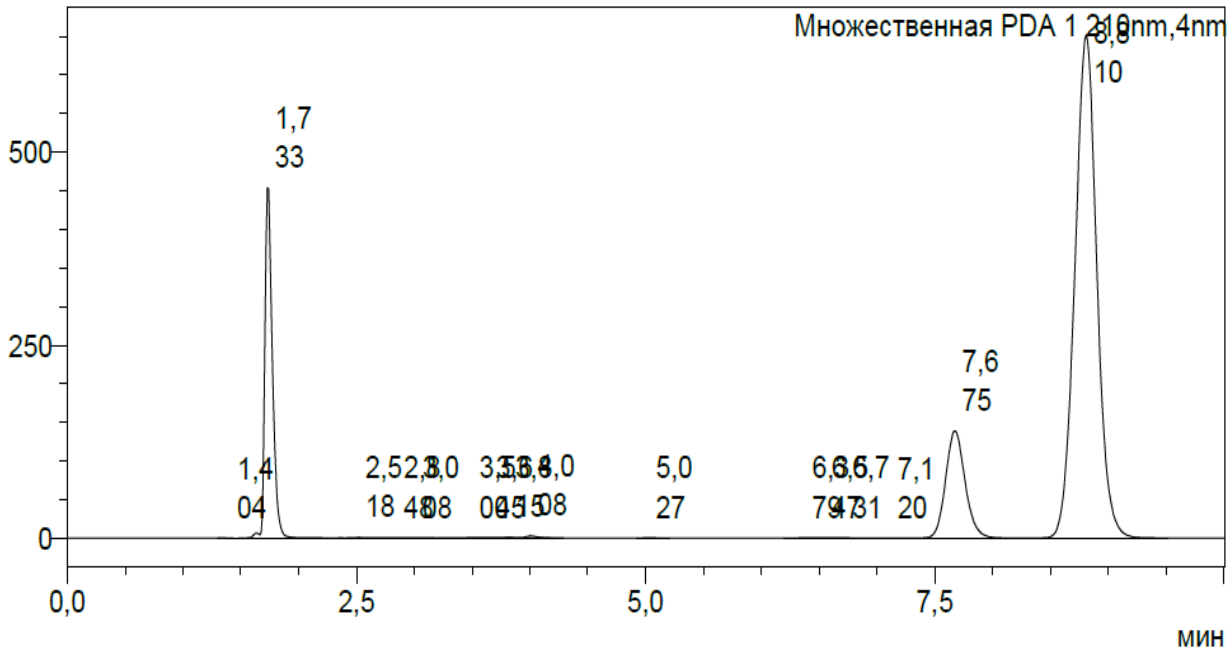
a_0– mass of a sample of cetirizine CO, in grams;

a_1– weight of the gel sample, in grams;

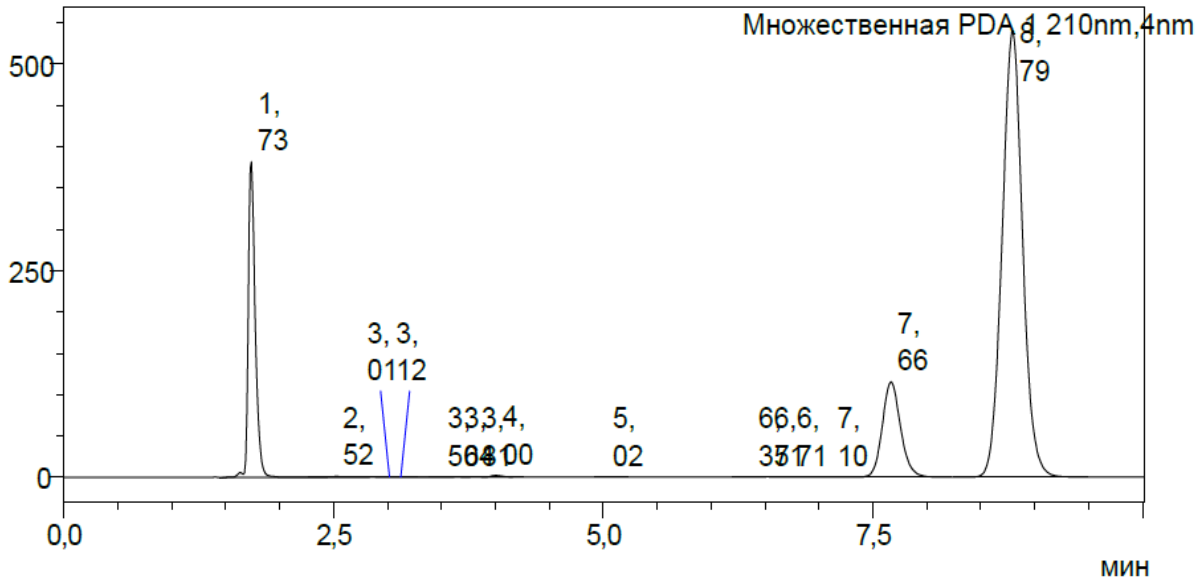
P– content of cetirizine in SOcetirizine, in percent.

Determining Method Specificity

The specificity of this analytical procedure was demonstrated by comparing the analyte and a standard sample.



Rice. 1: Chromatogram of a standard solution.



Rice. 2: Chromatogram of the test solution gel (azithromycin 500 µg/ml, cetirizine 50 µg/ml).

In the obtained chromatograms, the retention time of the main peak of azithromycin and cetirizin is observed; the chromatogram of the test solution corresponds to the retention time of the peak of azithromycin and cetirizine in the chromatogram of the standard solution.

Acceptance criterion: the retention time of azithromycin and cetirizine in the sample chromatogram must correspond to the retention time of azithromycin and cetirizine in the standard chromatogram.

Determination of method linearity

The test was performed by one analyst.

The linearity of the method was determined at five concentration levels: 80%, 90%, 100%, 110%, 120% of the working concentration of the analyte (in three parallels). The content of azithromycin 500 µg/ml and cetirizine 10 µg/ml was taken as 100% working concentration.

Table 1: Results of Determining The Linearity of The HPLC Method For Analyzing The Components Of A Model Mixture.

% of working concentration	No.	Content of cetirizine (azithromycin) in, mcg/ml	Peak height, mAU	Linear equation, coefficient of determination and correlation coefficient
Cetirizine				
80	1	8	266351	y = 46050 x + 223142 R² = 0.9989 (coefficient of determination) R = 0.9994 (correlation coefficient)
	2		268955	
	3		265074	
90	1	9	316547	
	2		320509	
	3		317222	
100	1	10	365507	
	2		360950	
	3		362881	
110	1	eleven	402577	
	2		405771	
	3		405905	
120	1	12	454477	
	2		452991	
	3		453682	
Azithromycin				
80	1	400	379624	y = 67509 x + 317222 R² = 0.9987 (coefficient of determination) R = 0.9993 (correlation coefficient)
	2		383592	
	3		380249	
90	1	450	456525	

	2		449678	
	3		452388	
100	1	500	526893	
	2		524955	
	3		525137	
110	1	550	589632	
	2		585607	
	3		588346	
120	1	600	651388	
	2		649865	
	3		652344	

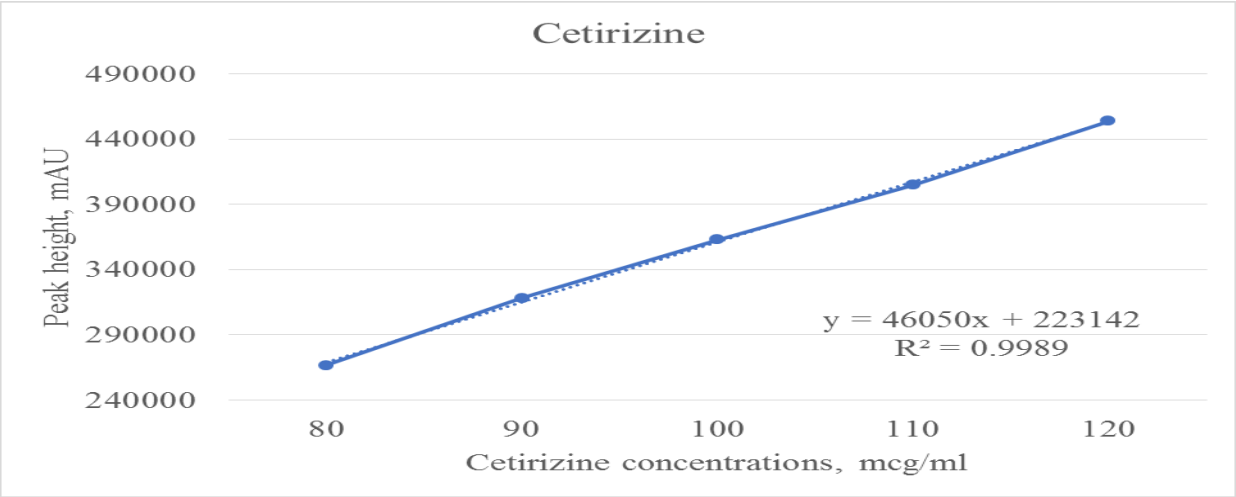


Fig.3. Linear graph of cetirizine.

The presented graph shows the presence of a well-defined linear relationship with a correlation coefficient of 0.9996.

Acceptance criterion: the correlation coefficient must be at least 0.990.

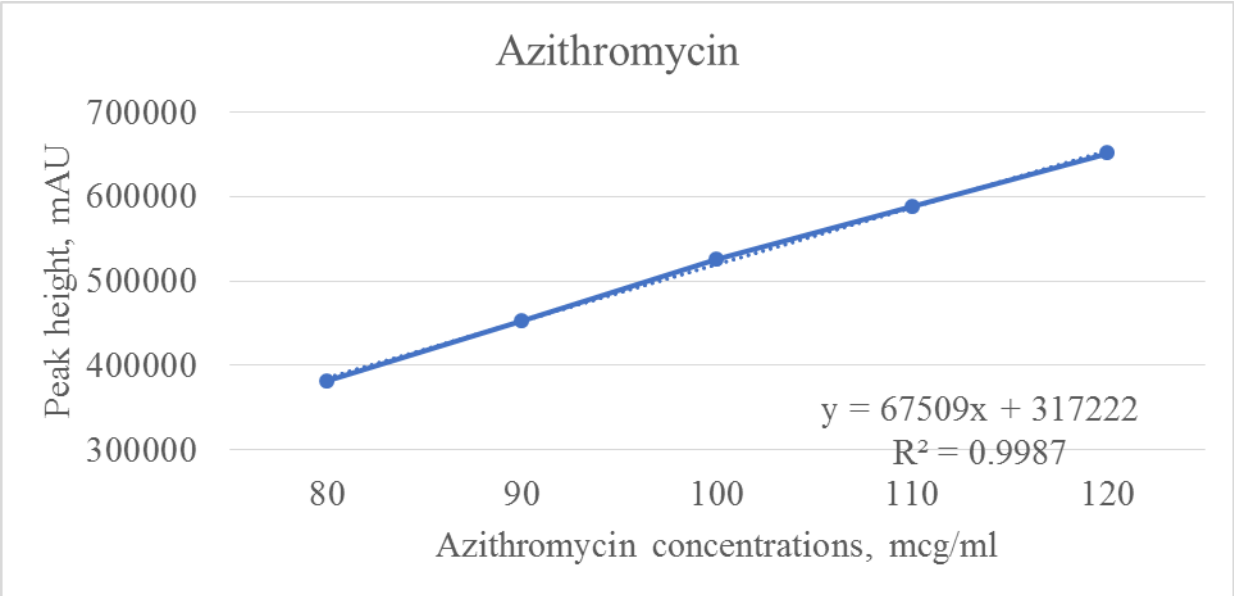


Fig 4: Linear dependence graph of azithromycin.

The presented graph shows the presence of a well-defined linear relationship with a correlation coefficient of 0.9993.

Acceptance criterion: the correlation coefficient must be at least 0.990.

Determining the correctness of the method

The test was performed by one analyst.

The accuracy of the analytical procedure was confirmed using nine prepared solutions of a standard sample in the concentration range from 80 to 120% of the working concentration of the analyte.

Calculation of the deviation of the obtained results using the formula:

$$\delta = \frac{(X_{cp} - X_P)}{X_P} \cdot 100 \qquad (3)$$

where: δ- relative value of systematic error, %;

X_{cp}- average concentration, in milligrams per milliliter;

X_P- calculated concentration from the target, in milligrams per milliliter.

Table 2: Results of determining the correctness of the HPLC method for analyzing the components of the model mixture.

% of working concentration	Cetirizine (azithromycin) taken, mcg/ml	Peak height, mAU.	Found cetirizine (azithromycin, mcg/ml	Yield value of cetirizine (azithromyci), %	Average, %	SD	RSD, %
Cetirizine							
80	8.01	266351	7.98	99.63	99.79	0.74	0.74
	8.01	268955	8.06	100.60			
	8.01	265074	7.94	99.15			
100	10.04	365507	9.93	98.90	98.26	0.62	0.63
	10.04	360950	9.81	97.67			
	10.04	362881	9.86	98.19			
120	119.98	454477	12.01	10.01	9.99	0.02	0.16
	119.98	452991	11.97	9.98			
	119.98	453682	11.99	9.99			
Mean=99.37; SD=0.50; RSD =0.51%; -μ= 0.74; t_{calc} =0.57; tp,f =0.92,							
Azithromycin							
80	401.5	379624	400.09	99.65	100.05	0.56	0.56
	401.5	383592	404.27	100.69			
	401.5	380249	400.75	99.81			
100	500.5	526893	495.22	98.95	98.71	0.20	0.20
	500.5	524955	493.40	98.58			
	500.5	525137	493.57	98.62			
120	600.9	651388	598.60	99.62	99.59	0.19	0.19
	600.9	649865	597.20	99.38			
	600.9	652344	599.48	99.76			
Mean=99.45; SD =0.31; RSD =0.32%; -μ = 0.76; t_{calc} = 0 , 39 ; $t_{p,f}$ =0.54,							

The results obtained using this method are not burdened by systematic error ($t_{calculated} < t_{p,f}$), true confidence interval (for cetirizine 100 ± 0.74, for azithromycin 100 ± 0.02) and relative standard deviation (RSD) < 2.0 %, and the yield of the analytical procedure ranges from 90 to 110%, which corresponds to the accepted criterion. The chosen technique is characterized by good repeatability of results.

Acceptance criterion: the value of the relative systematic measurement error of 5.0% when analyzing solutions with active substance content in the range from 80% to 120% should not exceed 2%, confidence interval 0.95.

Determination of method repeatability

The test was performed by a single analyst by repeating it multiple times with the same homogeneous sample (9 repetitions). A rutin content of 0.05% was taken as 100% working concentration.

Calculation of coefficient of variation

$$V = RSD \cdot 100 \qquad (4)$$

where: V- coefficient of variation (dispersion) %;

RSD- relative standard deviation.

$$RSD = S/Xcp \qquad (5)$$

where: S- standard deviation;

Xcp- average concentration of all determinations.

$$S = \sum_{i=1}^{n} (X - X_{cp})^2 / (n-1) \qquad (6)$$

where: S- standard deviation;

X- concentration obtained as a result of the experiment,

%;

X_{cp}- average concentration of all determinations;

n- number of definitions.

Table 3: Results of determining the repeatability of the HPLC method for analyzing the components of a model mixture.

No.	Weight of the drug, g	Peak height of cetirizine, mAU	Azithromycin peak height, mAU	Cetirizine content, mg/0.255 g	Azithromycin content, mg/0.255 g
1	0.5101	526893	365507	249.80	10.10
2	0.5106	524955	360950	249.13	9.94
3	0.5098	525137	362881	248.82	9.99
4	0.5099	526643	362341	249.58	10.01
5	0.5101	525913	360955	249.34	9.96
b	0.5104	524773	360723	248.94	9.93
7	0.5097	529135	365991	250.67	10.16
8	0.51	526009	360457	249.33	9.94
9	0.5103	523995	361099	248.52	9.92
Average	0.5101	525939.2222	362322.6667	249.3476	9.9940
SD	0.0003	1515.5806	2097.6312	0.6295	0.0825
RSD, %.	0.0572	0.2882	0.5789	0.2525	0.8251

The closeness of the results of individual tests performed by the same chemist is confirmed. The coefficient of variation does not exceed 2%.

Acceptance criterion: coefficient of variation should not exceed 2%.

Determination of method reproducibility

The test was performed by two analysts under different conditions with two homogeneous samples. A solution with a concentration of 100% was taken as a standard sample.

Calculation of the coefficient of variation according to formulas.[4-6]

Table 4: Results of determining the reproducibility of the HPLC method for analyzing the components of a model mixture.

No.	Performer - 1	Performer - 2	Performer - 1	Performer – 2
	Azithromycin content, mg/0.255 g		Cetirizine content, mg/0.255 g	
1	249.80	250.1	10.1	9.92
2	249.13	250.6	9.94	9.96
3	248.82	249.6	9.99	10.12
4	249.58	249.7	10.01	10.09
5	249.34	248.3	9.96	10.07
6	248.94	249.5	9.93	9.99
7	250.67	250.5	10.16	9.86
8	249.33	249.1	9.94	9.95
9	248.52	248.6	9.92	10.09
Average value	249.3478	249.5556	9.9944	10.0056
SD	0.6309	0.7907	0.0834	0.0904
RSD, %	0.9000	0.3169	0.8343	0.9038
Dispersion	0.00001864	9.182E-05	0.001241962	0.000836296
Confidence interval	9.75114E-06		0.000240214	
Overall average	249.4516667		10	
Total SD	0.702141682		0.084575062	
Total RSD, %	0.281474039		0.845750624	
Total variance	0.00014408		2E-07	

The degree of consistency of results obtained when analyzing the same homogeneous samples by different chemists is confirmed. Acceptance criterion: coefficient of variation does not exceed 2%.

CONCLUSION

A method for quality control of azithromycin in a model mixture with cetirizine using HPLC has been developed.

Chromatography conditions have been selected. The HPLC analysis method has been validated according to the following criteria: linearity, specificity, accuracy, sensitivity, accuracy, repeatability, reproducibility.

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