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Unification And Validation Of Hplc Method For The Quantitative Determination Of Fensulkal In A Substance And Combined Medicinal Mixtures

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ABSTRACT

Research objective: to carry out studies on the optimization of the analysis conditions of HPLC and to create highly effective and theoretically substantiated methods for the qualitative and quantitative determination of dosage forms of complex compositions containing fensulkal.

Material and methods. During the research,HPLC method was applied, with determination of the main chromatographic parameters of fensulkal: retention time, retention factor, spectral ratios, concentration of the organic component in the mobile phase. For the studies we used standard and test sample solutions of fensulkal and cetirizine (in combined model mixtures).

Findings.HPLC methods for the analysis of fensulkal in the substance and model mixturecombined with cetirizine were developed and unified as end-to-end methods.Besides,the optimal conditions for the analysis were selected.The methods were certified and validated according to the criteria: "Linearity", "Convergence", "Trueness" and can be included in the normative document for fensulkal and cetirizine substances,as well as other combined medicinal remedies for quality control according to indicators "Identification" and "Quantitative determination".

Conclusion.On the basis of the conducted studies, the unified HPLC method was developed as end-to-end method for the analysis and standardization of drugs containing fensulkal in the model mixtures and dosage forms. The developed unified method is validated according to the indicators "Linearity", "Trueness", and "Convergence", and can be included in the ND for standardization and quality control of drugs containing fensulkal according to the above indicators.

Key-words:fensulkal, cetirizine, HPLC, substance, validation, method.

INTRODUCTION

The vector of development of the pharmaceutical industry in the period ofindependence is aimed at the creation and implementation of import-substituting drugs produced in our country. In the republic, special attention is paid to the development of the pharmaceutical industry and the provision of the population with domestically produced drugs. In this regard, the expansion of the antimicrobial and anti-inflammatory drugs production using domestic resources is a prior task in the development of science and technology,as well in the sphere of production and technologies modernization in order to

introduce workings of domestic drugs. Domestic drug manufacturers give preference to the workings from raw materials both of plant and synthetic origin.An important role in providing the population with effective and safe medicines is given to the standardization and quality control of drugs in accordance with modern requirements of good manufacturing practice.

The development and implementation of new highly sensitive drug quality control methods is one of the main tasks of pharmaceutical science. The efficiency of drug analysis is directly related to the use of physicochemical research methods. An increasing practical importance is given to the chromatographic methods, including high-performance liquid chromatography (HPLC), which provide high sensitivity, specificity, and rapidity of analysis.This method is included in the regulatory documents of the Russian Federation, CIS countries, and is also applied in the US, British, German and European Pharmacopoeias. In modern medical practice, a wide range of final dosage forms of anti-inflammatory action is used. Most of them are multicomponent drugs, which include substances with different classes of chemical compounds - antihistamines, analgesics, etc. The development of methods for assessing the drug data quality is a complex task that can be resolvedby the HPLC method, which allows to combine separation stages, identification and quantitative determination[1].

Fensulkal isa phenylglyoxylic acid derivative (its bisulfite derivative), has antimicrobial and anti-inflammatory effect and is referred to the NSAID group [2,3]. Fensulkal substance is to be included in a multicomponent transdermal dosage form. The ND proposes a spectrophotometric method or an alternative HPLC method for assessing the quality of fensulkal substance [4, 5]. The alternative method ofHPLC quantitative determination is considered imperfect, as does not allow to obtain reliable reproducible results and objectively assess the drug quality. The chromatographic conditions were selected empirically and have no theoretical justification.

So, taking into consideration the above, it seems relevant to conduct theoretical studies on the selection of optimal conditions for the analysis of fensulkalin the form of substance and multicomponent transdermal drug using HPLC. Based on the obtained theoretical data, it will be possible to use a scientific approach to develop a unified method for assessing the quality of complex dosage forms containing fensulkal.

RESEARCH OBJECTIVE

The aim of this research is to carry out studies on the optimizationof the analysis conditions of HPLC and to create highly effective and theoretically substantiated methods for the qualitative and quantitative determination of dosage forms of complex compositions containing fensulkal.

MATERIAL AND METHODS

The following chromatographic conditions were selected experimentally:

- analytical column dimensions: 150x3.0 mm, filled with ZorbaxEclipse XDB C8 sorbent, pore size 3.5 μm.

- column temperature - 40 °C;

- detection - 248nm;

-mobile phase: 0.05 M the solution of potassium dihydrogen phosphate (pH = 3.0 adjusted with phosphoric acid) and degassing methanol (35:65);

- the internal volume of the injector - 10 µl;

- the mobile phase speed - 1.0 ml / min.

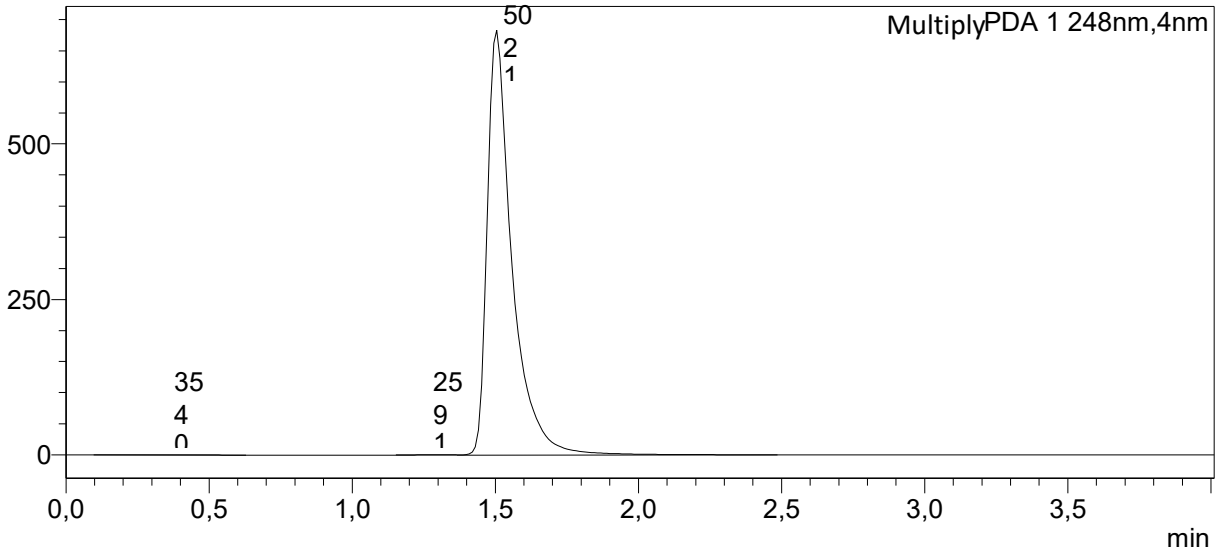
RESULTS

Preparation of a standard sample solution: 25 mg (so-called) of fensukalstandard sample was dissolved in the mobile phase in a 25 ml volumetric flask, and brought to the mark.

Preparation of a test sample solution: 25 mg (so-called) fensulkal substance in a 25 ml volumetric flask, was dissolved in the mobile phase and brought up to the mark with the same solvent.

System suitability test: The fensulkal peak in the chromatogram had a symmetry of 0.8 to 2.0, which proved the system suitability.

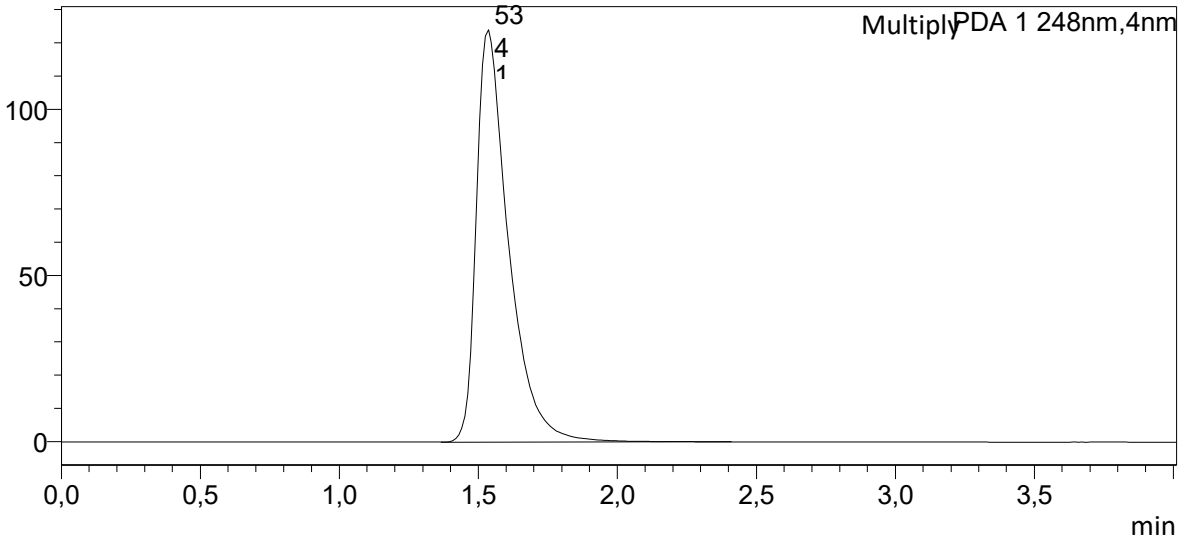
Chromatography was carried out on 5 samples of the standard and test solutions. Chromatograms of the retention time of the standard and test solutions are shown in Fig. 1.2.



Peak No.	Retention Time	Area	Height	Concent.	Meas.Unit	Mark	name
1	0,354	4087	298	0,000			
2	1,259	1352	190	0,000			
3	1,502	4124383	683107	0,000		V	
Total			683595				

		4129823					

Fig. 1. Chromatogram of thefensulkalstandard sample



Peak No.	Retention Time	Area	Height	Concetr.	Meas.Unit	Mark	name
1	1,534	1004980	124101	0,000			
Total		1004980	124101				

Fig. 2. Chromatogram of the fensulkaltest sample

Fig. 1 and 2 show, that the retention time of fensulkal of the standard sample (1, 502) and the test sample (1,534) are almost the same, which indicates the possibility of using a unified HPLC for quality control of fensulkal both in the substance and in the combined mixture in terms of "Identification".

The quantitative content of fensulkal in the substance is calculated by the formula:

$$X = \frac{S_1 \cdot a_0 \cdot P}{S_0 \cdot a_1}$$

where:

X is the amount of fensulkal in the substance,%;

S_1 is the area of the fensulkal peak on the chromatogram in the test solution;

S_0 is the area of the fensulkal peak in the chromatogram in the standard solution;

P – the amount of fensukal in the standard sample,%.

The method for the quantitative determination of fensulkal in the substance by HPLC was validated in terms of linearity, convergence, and trueness.

For determination oflinearity at the concentration 40 µg/ml to 140 µg/ml, solutions were prepared, which further were chromatographed. The linear relationship between the analytical response and the substanceconcentration is shown as a graph. (Fig. 3)

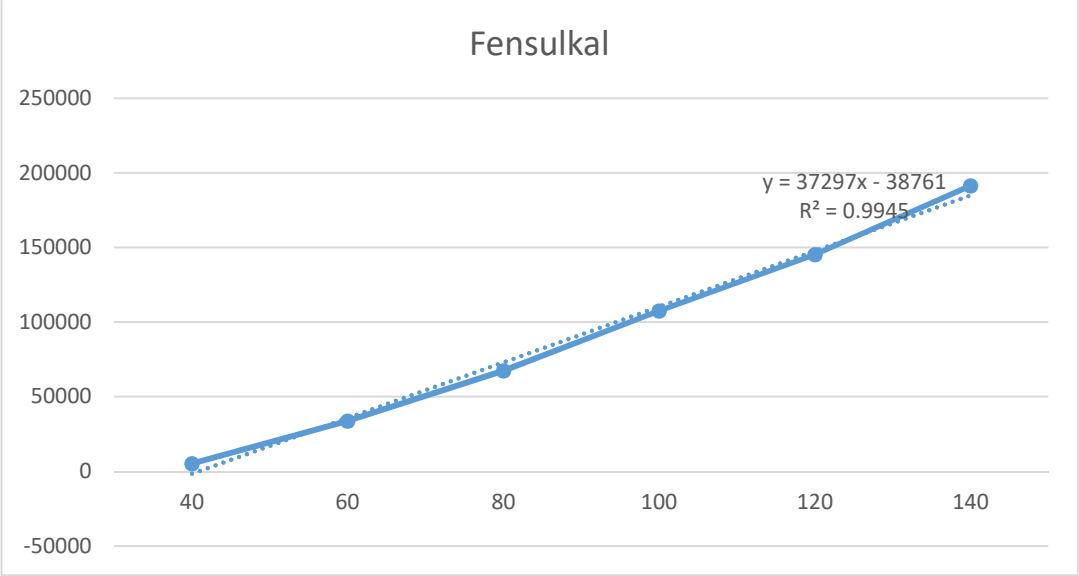


Fig. 3 Dependence of fensulkal concentration on peak height

Fig. 3 shows that the correlation coefficient is 0.9945 (R^2=0.9945), which proves the linearity of the developed method.

The concentration dependence on the peak height is shown in the Table 1.

Table 1

Dependence of concentration on the peakheight of fensulkal

№	Concentration, µg / ml	Peak height, [mAU]
1	40	5207
2	60	33815
3	80	67381
4	100	107635
5	120	145267
6	140	191362

As the Table 1 shows, with the increase offensulkal concentration in the analyzed sample, the peak height also grows in direct proportion.This factor may explain the concentration choose in the range 40 to 140 µg/ml in order to apply the method for fensulkal quality control both in the substance and mixtures according to the indicator "Quantitative determination".

The convergenceof method was determined by comparing the results of the analysis of 5 samples of fensulkal in the substance. The results are shown in the Table 2.

Table 2

Results of determining the fensulkal analyzes convergence in the studied samples

№	Weighted substance, mg	Peak height	Fensulkalamount in the substance,%.	Metrological characteristics
1	24,9631	835035	98,96	$\bar{X}$=100,2 % S^2 = 1,9180 S = 1,3849 ΔX = 3,8501 $\Delta\bar{X}$ = 1,7218 E= 3,84 % $\bar{E}$= 1,72 %
2	25,8310	843968	99,35	
3	25,3898	857845	100,83	
4	25,7504	840819	99,54	
5	26,0092	838990	102,34	

The Table 2 shows, that the results of the analyzes are close in values and correspondto the confidence interval of each individual analysis. The mean relative error of the method is 1.72%, which fits within the limits of the method.

The trueness of the method.We used the standard addition method. For this purpose, to 12 identical amounts of weighted fensulkal substanceswe added standard sample solutions and the results of analyzes were compared with theoretical values (Table 3).

Table 3

Results of determining of the unified HPLC method trueness for fensulkal in the analyzed samples (standard addition method)

№	Fensulkal content, mg	Added amount of fensulkal to the standard sample solution, mg	Theoreticalamount, mg	Determined amount, mg	Response, $\bar{X}$%
1	9,9500	1,5100	11,4600	11,7247	102,31
2	9,9500	1,5100	11,4600	11,3511	99,05
3	9,9500	1,5100	11,4600	11,3019	98,62
4	9,9500	3,0800	13,0300	12,9701	99,54
5	9,9500	3,0800	13,0300	13,1720	101,09
6	9,9500	3,0800	13,0300	12,8150	98,35
7	9,9500	4,5050	14,4550	14,7152	101,80
8	9,9500	4,5050	14,4550	14,1442	97,85
9	9,9500	4,5050	14,4550	14,2223	98,39
10	9,9500	6,0100	15,9600	16,0398	100,50
11	9,9500	6,0100	15,9600	15,6264	97,91

12	9,9500	6,0100	15,9600	15,7429	98,64

The Table 3 shows that the theoretical amountisclose by values to the determinedone, indicating the trueness of the method. The mean response value was 99.50%.

On the next stage of study,we developed HPLC method for quality control, standardization and validation of fensulkal in combined model mixtures (with cetirizine) for further implementation as end-to-end method in dosage form and inclusion in a regulatory documentin the future.

For the analysis of fensulcal in the model mixture, the following conditions were selected: the analytical column dimensions - 150x3.0 mm, filled with the Zorbax Eclipse XDB C8 sorbent,pore size of 3.5 μm; column temperature - 40 °C; detection - 230 nm; mobile phase - 0.05 M potassium dihydrogen phosphate solution (pH=3.0 adjusted with phosphoric acid) and degassing methanol (35:65); internal volume of the injector - 10 μl; the mobile phase speed - 1.0 ml / min.

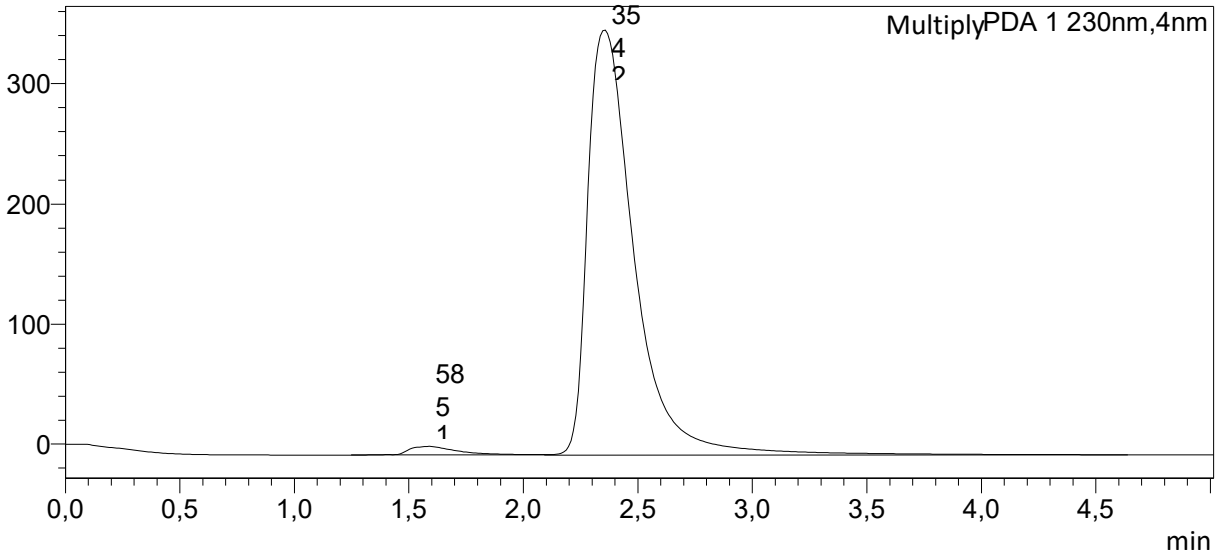
Preparation of Cetirizine Standard Solution: 20 mg (so-called) of thecetirizinestandard sample was dissolved in the mobile phase in a 100 ml volumetric flask, and the volume was brought to the mark with the same solvent.

Preparation of a mixture of standard samplesolutions: 5 ml of cetirizine and fensulkalstandard solutions in a 50 ml volumetric flask, were dissolved in the mobile phase, and brought to the mark.

Preparation of the test solution: 30 mg (so-called) of the model mixture in a 25 ml volumetric flask was dissolved in the mobile phase, and adjusted to the mark with the same solvent. 5 ml of the prepared solution was placed in a 50 ml flask, diluted with the mobile phase and brought to the mark.

*System suitability test:*Apeak symmetry was noted on the fensulkalchromatogram (0.8-2.0), indicating the method suitability for the quantitative determination of fensulkal.

Standard and test solutions were chromatographed in a fivefold mode.



Peak No.	Retention Time	Area	Height	Concentr.	Meas. Unit	Mark	Name
1	1,585	236494	15869	0,000			
2	2,354	8489394	600113	0,000		V	
Total		8725888	615982				

Fig. 4 Chromatogram of fensulkal and cetirizinemixture

From the Fig. 4 it can be seen that the unified HPLC method can be applied to determine the identification and quantitative content of fensulkal and cetirizine simultaneously without separating the mixture, which increases its advantages.

The quantitative content of fensulkal (cetirizine) was calculated using the formula:

$$X = \frac{S_1 \cdot a_0 \cdot P}{S_0 \cdot a_1}$$

where:

X -the quantitative content of fensulkal (cetirizine) in the mixture,%;

S_1 - peak area of fensulkal (cetirizine) in the chromatogram;

S_0- peak area of fensulkal (cetirizine)standard sample in the chromatogram;

P -quantitative content of fensulkal (cetirizine) in the standard sample,%.

The HPLC method for the quantitative determination of fensulkal and cetirizine in the model mixture was validated in terms of linearity, convergence, and trueness.

Thelinearity of the method: Thelinearityof the method wasdeterminedby solutions prepared and chromatographed within the concentration range 40 μg/ml to 140 μg/ml. The

linear dependency of the substance concentration from the peak height is shown in the Fig. 5.

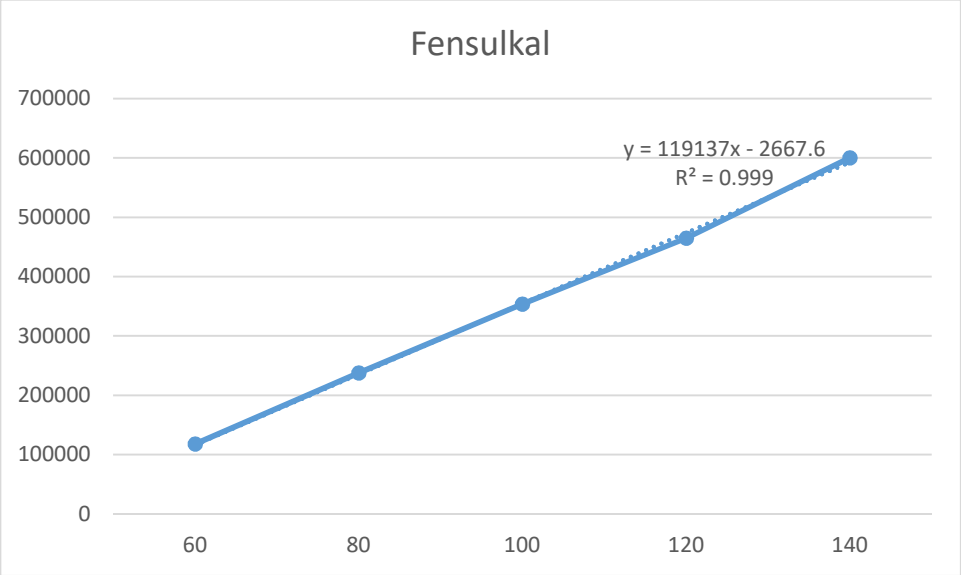


Fig. 5. Dependence of the peak height on the fensulkal concentration in the mixture

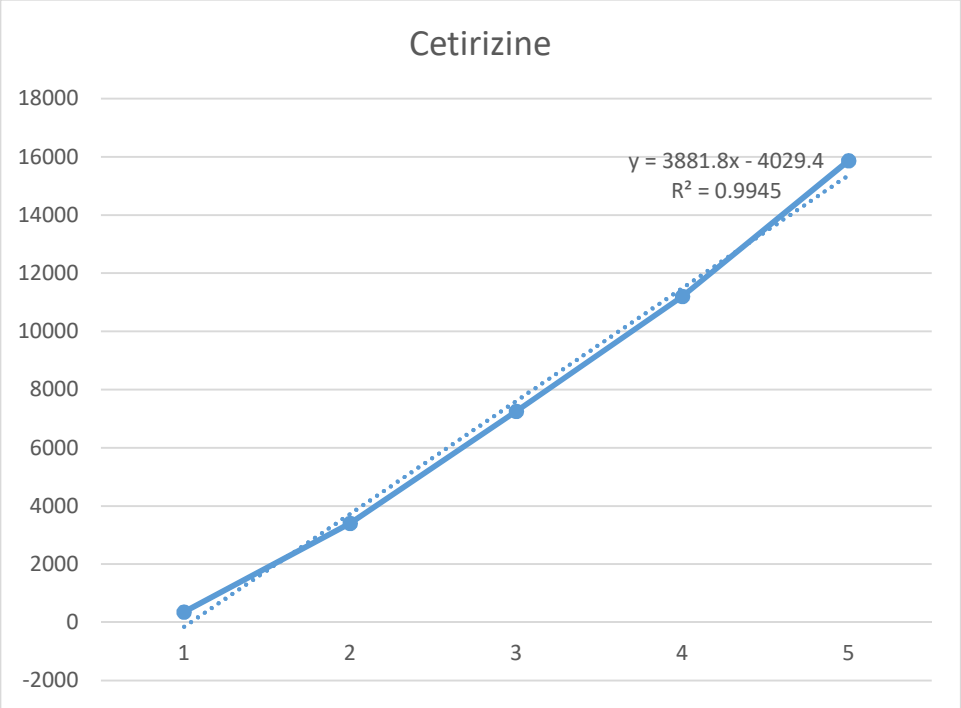


Fig. 6. The dependency of cetirizine peak height on concentration

Figures 5 and 6 show that the correlation coefficient is 0.999 (R^2=0.9990) for fensulkal and 0.9945 (R^2=0.9945) for cetirizine, which proves the linearity of the developed method for the simultaneous determination of fensulkal and cetirizine in the

mixture without separation by HPLC. The dependency of the peak height on fensulkal and cetirizine concentration are presented in the Table 4.

Table 4

Dependence of the peak height on fensulkal and cetirizine concentration in the mixture

№	Fensulkal		Cetirizine	
	Concentration, μg / ml (relative to the mixture)	Peak height, [mAU]	Concentration, μg / ml (relative to the mixture)	Peak height, [mAU]
1	200	117907	200	358
2	400	237575	400	3404
3	600	353590	600	7249
4	800	464535	800	11200
5	1000	600113	1000	15869

As the Table 7 shows, the HPLC method for the determination of fensulkal mixed with cetirizine is linear within the concentration range 200 to 1000 μg/ml.

Determination of the HPLC method convergence for the mixture of fensulkal and cetirizine. For this purpose, the mixture was analyzed 5 times, and the results obtained were compared, presented in theTable 5.

Table 5

№	Weighed sample of the drug, mg	Fensulkalpeak height	Cetirizine peak height	The quantitative content of fensulkal in the substance,%.	The quantitative content of cetirizine in the substance,%.
1	30.0583	470767	18703	82.81	16.42
2	30.0317	465104	19567	83.17	17.08
3	29.9924	468641	18525	83.59	16.76
4	30.0027	463406	19622	82.98	16.57
5	29.9897	464741	19072	83.27	16.61

From the data obtained, it can be seen that the results are close in value, and fit within the confidence interval. The relative error of the method is 1.72%, which is also within the permissible limit for this method.

*The truenessof the method:*The trueness of the method was determined using the standard addition method. 12 samples of the substance of same amount wereanalyzed, with addition of definitely precise amount of fensulkal. The data analyzed are shown in the Tables 6a and 6b.

Table 6a

Analysis data for determination of the HPLC method trueness in fensulkal and cetirizine mixture (the method of adding fensulkal standard sample)

№	The amount of fensulkal, mg	The added amount of fensulkal standard sample, mg	Theoretical amount, mg	Determined amount, mg	Responsedj $\bar{X}$%
1	10,0000	1,5000	11,5000	11,2332	97,68
2	10,0000	1,5000	11,5000	11,3769	98,93
3	10,0000	1,5000	11,5000	11,5196	100,17
4	10,0000	3,1000	13,1000	12,8393	98,01
5	10,0000	3,1000	13,1000	13,0358	99,51
6	10,0000	3,1000	13,1000	12,9900	99,16
7	10,0000	4,4800	14,4800	14,3613	99,18
8	10,0000	4,4800	14,4800	14,6624	101,26
9	10,0000	4,4800	14,4800	14,5423	100,43
10	10,0000	5,9600	15,9600	15,7717	98,82
11	10,0000	5,9600	15,9600	15,5387	97,36
12	10,0000	5,9600	15,9600	15,8786	99,49

Table 6b

Analysis data for determination of the HPLC method trueness in fensulkal and cetirizine mixture (method of adding cetirizine standard sample)

№	The amount of cetirizine, mg	The added amount of cetirizinestandard sample, mg	Theoretical amount, mg	Determined amount, mg	Response $\bar{X}$%
1	1,9880	0,1500	2,1480	2,1570	100,42
2	1,9880	0,1500	2,1480	2,1297	99,15
3	1,9880	0,1500	2,1480	2,1699	101,02
4	1,9880	0,3000	2,2980	2,3205	100,98
5	1,9880	0,3000	2,2980	2,2925	99,76
6	1,9880	0,3000	2,2980	2,3217	101,03
7	1,9880	0,4500	2,4480	2,4624	100,59
8	1,9880	0,4500	2,4480	2,4695	100,88
9	1,9880	0,4500	2,4480	2,4948	101,91
10	1,9880	0,6000	2,5980	2,5759	99,15
11	1,9880	0,6000	2,5980	2,6458	101,84
12	1,9880	0,6000	2,5980	2,5762	99,16

The Tables 6a and 6b show that the theoretical values are close to the determined amounts of fensulkal and cetirizine.The mean response value was 99.50%, which indicated the trueness of the method.

CONCLUSION

Thus, during the research we determined the main chromatographic parameters of fensulkal:retention time, retention factor, spectral ratios;as well, the identification conditions were optimized, based on the retention value direct dependency on the pH value and organic component concentration in the mobile phase.

Considering the chromatographic behavior of fensulkal, the optimal conditions for its separation were determined, and the unified HPLC method was developed suitable for assessing the drug quality according to indicators "Identification", "Dissolution", "Quantitative determination". On the basis of the conducted studies, the unified HPLC method was developed as the end-to-end method for the analysis and standardization of drugs containing fensulkal in their model mixtures and dosage forms. The developed unified method is validated according to the indicators "Linearity", "Trueness", and "Convergence", and can be included in the ND for standardization and quality control of drugs containing fensulkal according to the above indicators.

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