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VALIDATION OF THE HPLC METHOD FOR QUALITY CONTROL OF THE SUBSTANCE BENZKETOZONE.

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ABSTRACT

Annotation: A new improved method for the separation, detection and determination of the quantitative content of Benzketozone in a substance by HPLC has been developed. The developed methodology was validated. Parameters such as sensitivity, linearity, repeatability and accuracy are established. Also A comparative analysis of Benzketozone in the substance was carried out using UV spectrophotometric and HPLC analysis methods. It was shown that the relative error for the UV spectrophotometric method was 0.9%, and for HPLC - 1.09%. A one-way analysis of variance was carried out on the developed methods for the quantitative determination of the substance Benzketozone. Results of comparison of the calculated value of F with the table value: $F_{calc.} < F_{tables}$ show that the developed methods are comparable to each other. Therefore, for further qualitative and quantitative analysis of Benzketozone in soft dosage forms, it is advisable to use the developed methods of UV spectrophotometry and HPLC.

KEYWORDS: Benzketozone, HPLC, spectrophotometric method, validation assessment.

INTRODUCTION

The introduction of domestic developments of drugs and medical devices is a priority in the development of science and technology in the direction of modernizing production technology in the pharmaceutical industry.[1,2]

Biologically active compounds obtained on the basis of phenylglyoxylic acid have a wide range of pharmacological activity, and in particular anti-inflammatory, without a number of side effects actions. As a result, their use in practical medicine as locally developed drugs is of particular importance.[3,4.5,6]

Along with the search for new treatment agents, it is necessary to resolve the issues of developing methods for their analysis using end-to-end system methods. The task of our research includes the development and improvement of methods qualitative and quantitative analysis of benzketozone in the substance. Benzketozone in a substance is determined qualitatively and quantitatively by several methods, such as thin layer chromatography, chromatospectrophotometry, spectrophotometry, and HPLC.[7,8,9] The issues of developing an improved method for the identification and quantification of benzketozone and conducting a validation assessment in order to implement methods for the first time produced drugs containing this pharmaceutically active ingredient (PAI) are relevant.

Purpose of the study: Development and validation of an improved HPLC technique for quality control of Benzketozone.

OBJECTS AND RESEARCH METHODS

Benzketozone substance (FS 42 Uz-0850-2020). HPLC analysis was carried out on a liquid chromatograph from Shimadzu LC 2030 C Plus ", Japan. Data processing was carried out using the “Lab” program Solution ".

RESULTS AND DISCUSSION

Development and validation of an improved HPLC procedure for quality control of Benzketozone. To develop conditions for the separation and detection of Benzketozone by HPLC, solutions of a standard sample and the test substance, as well as the corresponding mobile phases, were prepared.
Shim - Pack - XR - ODSIII sorbent with a particle size of 2 μm, equipped with a DMD detector with variable wavelength (230 nm) and an isocratic pump. The relative standard deviation of the peak areas of the obtained

chromatograms should be no more than 2.0%. The benzketozone content in % was calculated using the formula:

$$X\ (\%) = \frac{S_1 * a_0 * 2 * P}{S_0 * a_1}$$

where S_1 is the peak area of Benzketozone on the chromatogram of the test solution; S_0 - peak area of Benzketozone on the chromatogram of the PCO solution; a_0 - weighed portion of RSO Benzketozone, in g; P – content of benzketozone in RSO, in%.

Preparation of a standard sample solution. 50 mg (t.n.) of a standard sample of Benzketozone is dissolved in the mobile phase in a 100 ml volumetric flask, the solution is adjusted to the mark.

Preparation of a solution of the test sample. 50 mg (t.n.) of the substance Benzketozone was placed in a 100 ml volumetric flask, dissolved in the mobile phase and brought to the mark with the same solvent.

Checking the suitability of the system. The Benzketozone peak in the chromatogram has a symmetry of 0.8 to 2.0, which proves the suitability of the system.

Chromatography was carried out on 5 samples of standard and test solutions. The quantitative content of benzketozone in the substance was determined by the formula:

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

g de , X– content of Benzketozone in the substance, %;

S_1benzketozone peak area on the chromatogram in the test solution;

S_0benzketozone peak area on the chromatogram in a standard solution;

P– content of benzketozone in the standard sample, %.

Validation characteristics were accuracy, repeatability, accuracy, specificity, sensitivity and linearity.

The linear dependence diagram is presented in Figure 1.

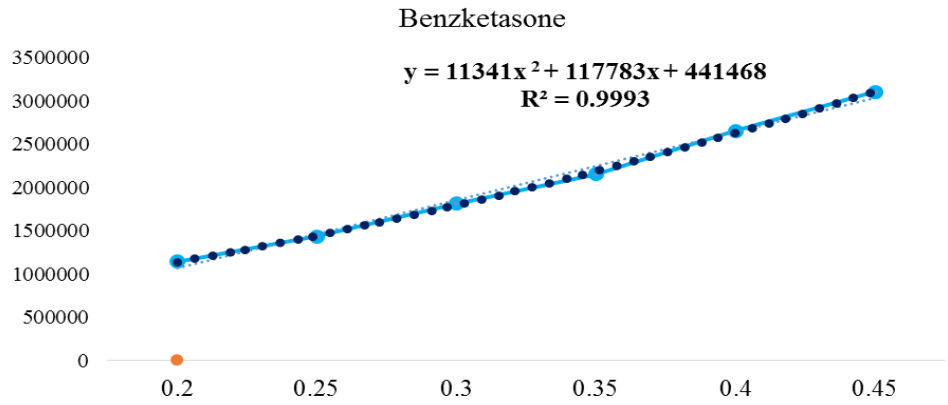
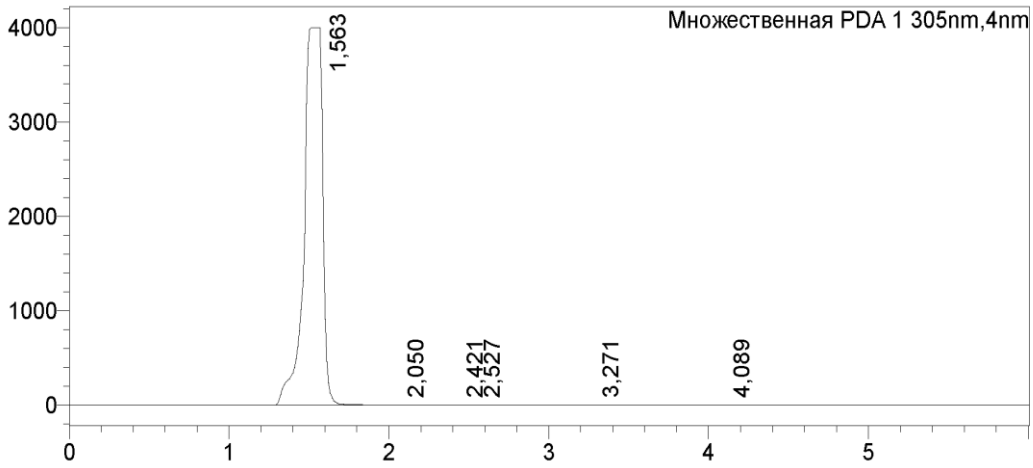


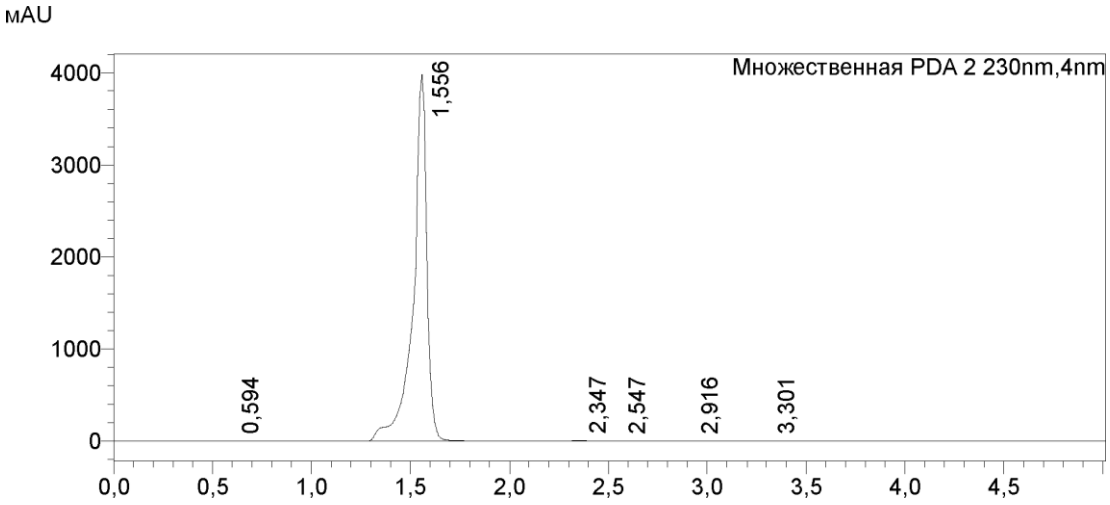
Figure 1: Linear relationship between the concentration of substances.

Determination of linearity of the method. For this purpose, special solutions were prepared for substance concentrations in the range from 40 µg/ml to 140 µg/ml, followed by chromatography of the samples (Fig. 2).



Peak No.	Vr. hold	Square	Height	Conc.	Unit change	Label	Name
1	1,563	30456728	3893564	0.000		S	
Sum		32156001	4004835				

PDA Ch2 230nm



<Peak table>
PDA Ch2 230nm

Peak No.	Hold time	Square	Height	Conc.	Unit change	Label	Name
1	0.594	5392	674	0.000			
2	1.556	20173524	3561252	0.000		S	
Sum		18912622	3989787				

Figure 2: Benzketozone RSO chromatogram.

From the above chromatograms (Figure 2) it is clear that at a column temperature of 20 ^{0} C, the retention time of Benzketozone is 1.248 minutes. The analytical signal for a given range and the linear relationship between the concentration of the substance is shown in Table 1.

Table 1: Relationship between concentration and sediment height.

	Concentration, mg/ml	Sediment height, [mAU]
1	0.10 _	572052.5
2	0.125 _	717264.5
3	0.15	907665.5
4	0.175	1077952
5	0.20	1327068.5
6	0.225	1552335.5

The graph shows that the correlation coefficient was 0.99 38 .

Repeatability of the method. For this purpose, the determination of the substance was repeated 5 times. From the data presented in Table 2 it is clear that the indicators are close in value and are in the confidence interval.

Table 2: Results of the method repeatability assessment.

No.	Drug weight, mg	Draft height	Volume of Benzketozone in the substance, %	Metrological Indicators
1	1 5, 0	2383185	9 8 , 77	$\bar{X}$= 99.51 % _
2	1 5.0	2380474	99.21	S^2= 0.8 901
3	1 5.1	2382526	99.03 _	S= 0.9 604
4	1 5.3	2386279	100 , 28	ΔX= 2.5106 _ _
5	1 5.2	2384157	100 , 25	$\Delta\bar{X}$= 1.2154 _ _
				E= 2.34 % _
				$\bar{E}$= 1.09 % _

It was determined that the relative error of this study did not exceed 1.09%.

The correctness of the method was determined based on the results of determining 12 identical masses of substances of the test substance. Table 3 presents a comparison of the obtained results with the results of theoretical calculations.

Table 3: Results of assessing the correctness of the method.

No.	Weight of Benzketozone, mg	Standard sample Benzketozone, mg	Calculated content, mg	Determined content, mg	$\bar{X}$%
1	10.1	1.5	11.6	11.5478	99.55
2	10.1	1.5	11.6	11.6568	100.49
3	10.1	1.5	11.6	11.7288	101.11
4	10.1	3.1	13.2	13.1802	99.85
5	10.1	3.1	13.2	13.3544	101.17
6	10.1	3.1	13.2	13.5234	102.45
7	10.1	3.9	14.0	14.0770	100.55
8	10.1	3.9	14.0	13.7102	97.93
9	10.1	3.9	14.0	13.8124	98.66
10	10.1	6.0	16.1	16.1113	100.07
eleven	10.1	6.0	16.1	15.8311	98.33
12	10.1	6.0	16.1	16.0292	99.56

The average efficiency rate was 9 9.5%.

Thus, we have developed a new method for the separation, detection and determination of the quantitative content of the substance Benzketozone using HPLC, and also validated this method. In this case, parameters such as sensitivity, linearity, repeatability, and accuracy are established.

A one-way analysis of variance was carried out on the developed methods for the quantitative determination of the substance Benzketozone.

The methods we developed for the quantitative determination of the substance Benzketozone served as the basis for the selection of methods for use in quality control, as well as biopharmaceutical and pharmacokinetic studies of gels and suppositories, both single-component and combined dosage forms.

To select the most optimal method, we carried out a comparative analysis of the developed methods of UV spectrophotometry and HPLC. A comparison of the results of determining Benzketozone using the proposed methods was carried out using mathematical modeling (Table 4). To do this, we compared the relative error of the average result of each analysis method $\varepsilon_{avg.}$ The obtained relative errors of the average results of the analysis methods are insignificant and meet the requirements of the State Fund XI.

Table 4: Comparison of the results of determination of Benzketozone using the proposed methods.

Medicinal substance	Relative error of the average result of the analysis method ε_{avg}	
	UV spectrophotometry	HPLC
Benketozone	0.88 % _	1.09 % .

Next, based on the results of the quantitative determination of Benzketozone using UV spectrophotometry and HPLC[10], a one-way analysis of variance was performed. When comparing inter- and intra-method variances, Fisher's test (F) was calculated and compared with the tabulated F value. The results of the analysis are presented in Table 5.

Table 5: Results of one-way analysis of variance.

	Sum of squares	F	Middle square	$F_{calc} S^2 / S$	$F_{table}(0.05;4;10)$
Between methods	S_{sp} =0.466	5-1=4	S^2 =0.3086	0.75	3.92
Inside Methods	S_{osh} =4.192	15-5=10	S =0.415		
total amount	S = 4.658	15-1=14	-		

Results of comparison of the calculated value of F with the table value: $F_{calc.} < F_{tables}$ show that the developed methods are comparable to each other. Therefore, for further qualitative and quantitative analysis of Benzketozone in soft dosage forms, it is advisable to use the developed methods of UV spectrophotometry and HPLC.

Thus, a comparative analysis of Benzketozone in the substance using UV spectrophotometric and HPLC analysis methods showed that the relative error for the UV spectrophotometric method was 0.9%, while for HPLC it was 1.09 %. The developed methods are included in the pharmacopoeial article of Benzketozone as alternative methods, and can be used for quality control not only of Benzketozone in FAI, but also in dosage forms containing it.

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