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Validation Of HPLC Methods For The Quantitative Estimation Of Benzketozone In Substances And Gels

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ABSTRACT

The article presents the results of the developed methods of identification and quantitative estimation of benzketozone in the substance and in 3% gels. Identification was carried out using chromatographic method in thin layers of sorbent (TLC) Benzketozone, the high-performance liquid chromatography (HPLC) method was used. The developed methods were validated according to the following parameters: accuracy, linearity, sensitivity and repeatability. Based on the results, it can be concluded that closely developed and tested methods flow between them. We find it appropriate that for further quality control of Benzketozone in soft dosage forms, we developed through TLC and HPLC methods. Methods have been developed for identifying and determining the quantitative content of benzethone in substances and gels by TLC and HPLC methods.The developed methods have been validated and certified in the laboratory

INTRODUCTION

Expanding the range of anti-inflammatory drugs using domestic developments is becoming a priority. Currently, the world is paying great attention to the development of effective, high-quality and safe non-steroidal anti-inflammatory drugs obtained by chemical synthesis. In the production of domestic pharmaceutical products (PP), preference is given equally to pharmaceutical products developed from raw materials of plant and synthetic origin. Based on aromatic α-keto acids, many works are known on the synthesis of biologically active compounds and the study of their pharmacological activity. Due to their high reactivity, α-keto acids are starting compounds in the synthesis of many biologically active compounds. Based on this acid, its ethyl ester and thiosemicarbozone, domestic scientists have developed a technology for the production of a number of drugs (1,2,3). One of which is benzketozone, which is a derivative of phenylglyoxylic acid (4). The development of quality control methods is an integral part in the search and implementation of new drugs using end-to-end systems.Further research is aimed at developing and improving methods for identifying and quantifying benzketozone in the substance and in gels. Also relevant are the issues of conducting a validation assessment of developed improved methods for quality control of benzketozone in order to introduce methods for new drugs containing this pharmaceutically active ingredient. (PAI) are relevant.

Today, the requirements for the production of pharmaceutical products are carried out in accordance with the GMP standard. Where the use of validated analytical techniques for standardization and subsequent quality control of drugs is prescribed. The article presents data obtained as a result of analysis of HPLC technique for determination of vitamin K1, validation was carried out and conclusions were drawn of the developed new gel from local plant raw materials (5-8).

HPLC methods used to determine the quality of active substances, despite their uniformity and accuracy, must be certified and validated according to the parameters (validation characteristies): accuracy (Accuracy), linearity (Linearity), specificity (Specificity) and precision (Precision) (5-9).

Purpose of the study: to develop and improve optimal end-to-end methodologies for the analysis of Benzketozone in substance and in gels. To assess the validation characteristics. When developing a TLC technique, chromatography using current methods on Silufol UV 254 plates (Merck) (Rf 0.66); “Sorbfil UV” Russia (RUB 0.50); “Silufol UV 254” (Czech Republic) (Rf 0.60); Silica gel KSK (Rf 0.45) with the addition of a luminescent indicator.

HPLC chromatography conditions: Shimadzu LC 2030 CPlus equipment; column length 50 mm; diameter 2.0 mm; sorbent Shim-Pack-XR-ODSIII.

Validation of analytical procedures in accordance with ICH (International Council for Harmonization) recommendations Topic Q 2 (R1) "Validation of text and methodology of analytical procedures"

Findings of the study: Benzketosone in the substance is qualitatively and quantitatively determined by several methods such as TLS, chromatospectrophotometry, spectrophotometry. (4,10).

Organic solvents were used as the mobile phase (MP) for the TLC chromatospectrophotometric method: chloroform, acetone, ethyl acetate, hexane, etc. in various variations and ratios (4).

The double extraction method was used to extract benzketozone from gels. A working standard sample of benzketozone (WSS) was prepared by dilution: solution A- with a concentration of 0.1%; solution B - 0.05%; solution C - 0.02%, respectively. On the starting line, 10 µl of the test solution of benzketozone, equivalent to 10, 5 and 2 µg of PSO solutions A, B and C, were applied successively after 15 mm. The chromatograms were opened when viewed in UV light, at 254 nm (wavelength). Then the Rf values of PCO were compared with the studied samples. The suitability of the chromatographic system was assessed by the chromatogram of benzketozone solution C, where the spot is clearly visible. Differences were assessed using Student's t-test at a significance level of $p < 0.05$ (4). Considering the poor solubility of Benzketozone in water and easy solubility in hot, and in non-polar and universal solvents (chloroform, acetonitrile, 96% alcohol). has weak hydrophilic properties. The chromatographic mobility of the substance benzketozone was studied to select the optimal composition of the mobile phase (MP). For this purpose, combined and individual solvents of different polarities were used. Modifier systems with different alkali and acid contents (ammonia solution (25%) and acetic acid) were also studied.

1.Preparation of the reference solution: 5.0 mg of benzketozone (standard sample of the company) was placed in a measuring flask with a capacity of 10 ml, 9 ml of methylene chloride P, one drop of triethypamine P were added, mixed until dissolved, the volume of the solution was brought to the mark with methyl chloride P and mixed (0.5 mg / ml of benzketozone).

One main spot should be found on the chromatogram of the test solution, corresponding in R_f value (about 0.60) to the benzketozone spot on the chromatogram of the reference solution.

2.Preparation of a standard solution of benzketozone: 5.0 mg of benzketozone substance was placed in a 10 ml volumetric flask, and 9 ml of methylene chloride P, one drop of triethypamide P was added, stirred into solution, the volume of the solution was brought to the mark with methylene chloride P and stirred (0.5 mg / ml of benzketozone).

15 µl of the test solution and the reference solution were applied twice to the start line of TLS plate with a layer of silica gel F 254 P with a solution of 10 x 20 cm with a layer thickness of 0.25 mm. The plate, saturated for 10 minutes, was placed in a chamber with a solvent system and chromatographed. Then it was viewed in UV light at a wavelength of 254 nm. A main spot was found on the chromatogram of the test solution, corresponding in R_f value (about 0.60) to the benzketozone spot on the chromatogram of the reference solution.

The definition of the TLC validation procedure according to the sensitivity parameter was as follows: preparation of aqueous solutions of benzketozone in various concentrations; preparation of the system benzene: hexane: ethanol: acetic acid: chromatography. Data on the study of the detection limit of benzketzone using the sensitivity method are presented in table. 1.

Table 1

Determination of the sensitivity and detection limit of benzketozone by various detection reagents

Amount of substance taken for analysis, mcg	UV-254	Iodine vapor	$CuSO_4$	$FeCL_3$
100	+	+	+	+
50	+	+	+	+
10	+	+	+	–
5	+	+	–	–
1	–	+	–	–
0,1	–	–	–	–

Based on the results of the table data, it can be concluded that the detection limit of benzketozone varies in the range from 100 to 5 µg; for development, a camera with UV light and iodine vapor is most suitable.

Further, validation parameters and characteristics such as specificity, selectivity, sensitivity and reproducibility of the technique were determined (10).

A series of chromatographic determinations (working and standard samples of benzketozone) were carried out to study the specificity of the developed TLC technique.

To prove the reproducibility of the TLC technique, various chromatographic plates were used: "Silufol UV254" (Merck) -R_f 0.66; Silica gel KSK -R_f 0.45; "Silufol UV 254" (Czech Republic) -R_f 0.60; "Sorbfil UV" (Russia) -R_f 0.50.

It should be noted the importance of the results of validation of chromatographic techniques, including TLC for the analysis of the finished dosage form. The sensitivity, selectivity, and reproducibility of benzketozone were studied using the above method. According to the results of chromatography "System", "Phase mobility" of benzketozone in individual solvents in different ratios, it was found that the benzene – hexane – ethanol – acetic acid system in a ratio of 5:1:1:0.5 is more suitable and provides optimal mobility, where R_f is 0.60.

Based on the value of dielectric constant, we studied the polarity of eluents in combination and individually the proportion of solvents included in the PF.

It was noted that the chromatographic mobility of benzketozone increased in the presence of an ethanol solution. The introduction of acetic acid into the PF composition leads to its decrease.

The influence of systems with different contents of acidic and alkaline reagents on chromatography was studied. The assessment was carried out as the average of five parallel determinations.

In the presence of an acid modifier, the mobility of benzketozone was also studied during chromatography using Silufol UV 254 plates (Merck) and a combination of eluents.

It is known that benzketozone has two main radicals in its structure, and therefore belongs to weak acids (1,2).
The presence of alkaline centers in the molecule determines the ability to ionize in both acidic and alkaline environments. When selecting a PF based on efficiency parameters, we settled on the system chloroform R-ethanol-R – acetic acid (5:1:0.5). In this case, the mobility coefficient of the benzketozone substance was Rf-0.60 (±0.02), and the sensitivity was 2 μg.
The results of the studies substantiate the choice of solvents, the composition of extractants and the frequency of extraction of active substances from the dosage form, sample volume, type of plate, as well as elution conditions for identifying benzketozone by TLC.
The results indicate that the developed TLC method based on Rf indicators confirms reproducibility and meets the requirements of the State Fund. Moreover, it is important to note that today "Silufol UV 254" plates are the most affordable and there is no need for special preparation.
Thus, the developed TLC procedure was validated in terms of sensitivity, selectivity and reproducibility, which are very important necessary (according to the requirements of the international harmonized quality control standards ICH) for quality control of the finished Benzketozone dosage form.

Development and validation of an improved HPLC technique for quality control of Benzketozone

To develop quality control methods for the quantitative determination, separation and detection of Benzketozone by HPLC, a necessary condition is: preparation of standard and test solutions of the test substance; appropriate mobile phases.
Conditions: liquid chromatograph from ShimadzuLC, 2030CPlus; column 50 mm long, 2.0 mm in diameter, filled with Shim-Pack-XR-ODSIII sorbent; with a particle size of 2 microns; DMD detector - variable wavelength (230 nm); isocratic pump. LabSolution programs were used to process the data. The data was processed using the "LabSolution" program. 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:

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

where, S1 is the peak area of Benzketozone on the chromatogram of the test solution;
S0 is the peak area of the Benzketozone on the chromatogram of the WSS solution; a0 is the weighed sample of the WSS of Benzketozone, in g; P is the content of Benzketozone in the WSS, in %.
The standard solution was prepared as follows: 50 mg (t.n.) of a standard sample of benzketozone -50 mg (t.n.) was dissolved in PF in a 100 ml volumetric flask, the solution was adjusted to the mark.
Test sample solution. To prepare the benzketozone substance, 50 mg (so) was placed in a 100 ml volumetric flask, dissolved in the mobile phase and diluted 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.
For chromatography, 5 samples of standard and test solutions were used. The quantitative content of benzketozone in the substance was determined by the formula:

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

where, X - the content of Benzoketozone in the substance, %;
S_1 - the peak area of the Benzocetozone on the chromatogram in the test solution;
S_0 - the peak area of the Benzoketozone on the chromatogram in standard solution;
P - the content of Benzoketozone in reference standard, %.
To validate analytical methods, the following parameters were studied: accuracy, repeatability, accuracy, specificity, sensitivity and linearity.
The linear dependence diagram is shown in Figure 1

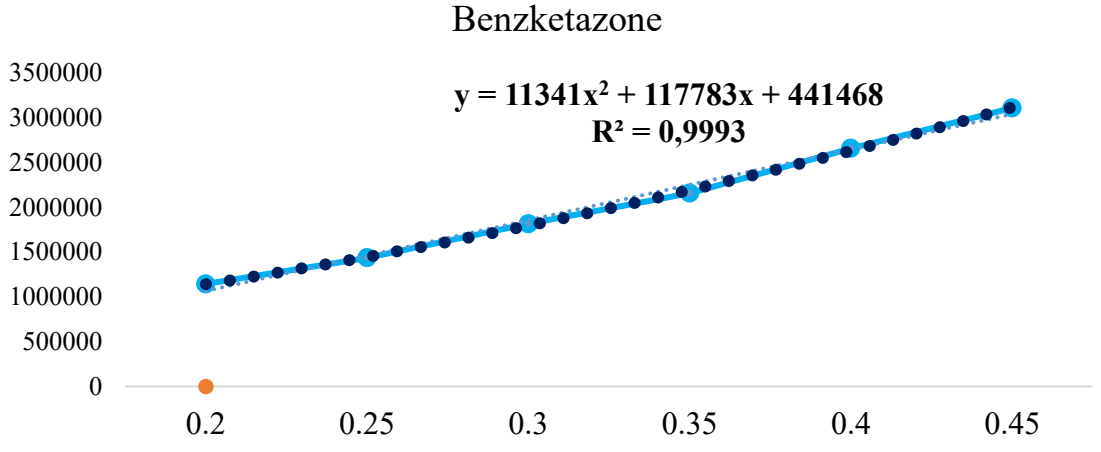

Figure 1. Interrelation of linear dependence on the concentration of substances.

Determination of the linearity of the method. For this purpose, special solutions were prepared for concentrations of the substance in the range from 40 micrograms/ml to 140 micrograms/ml with further chromatography of the samples (Fig.2a, b).

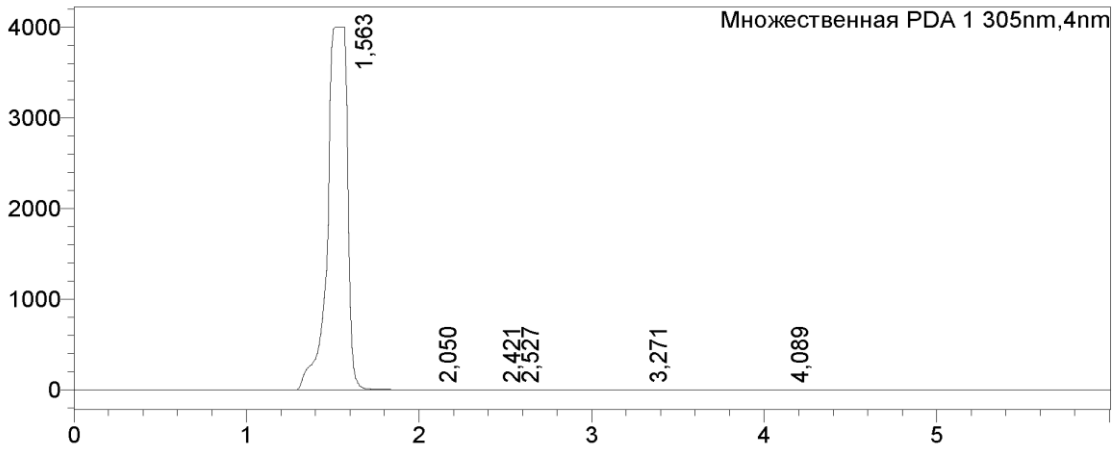

Figure 2.a Chromatogram of Benzketozone

Peak No.	Retention time	Area	Height	Concentration	Measurement unit	Mark	Name
1	1,563	30456728	3893564	0,000		S	
Sum		32156001	4004835				

PDA Ch2 230nm

мAU

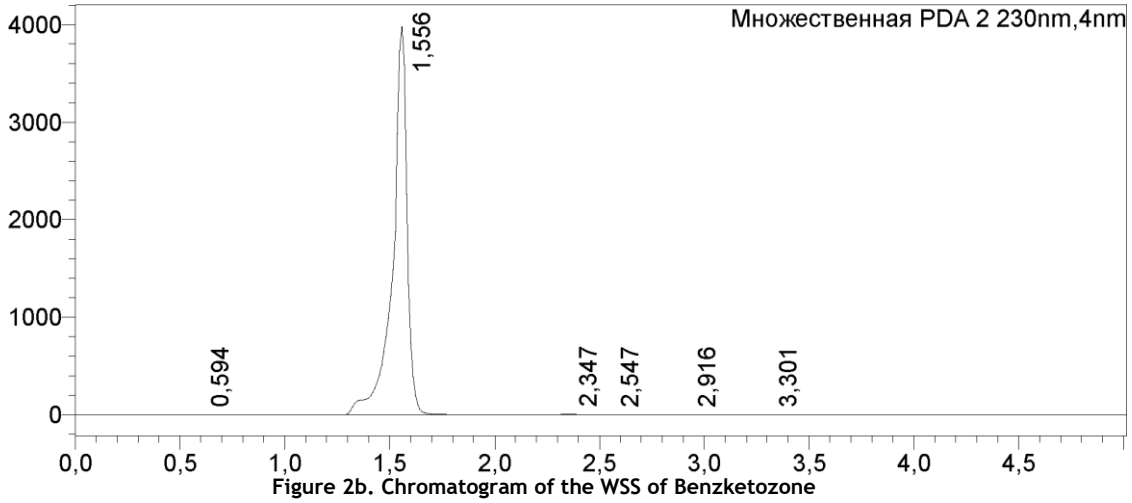

Figure 2b. Chromatogram of the WSS of Benzketozone

< Table of peaks >
PDACh2 230nm

Peak No.	Retention time	Area	Height	Concentration	Measurement unit	Mark	Name
1	0,594	5392	674	0,000			
2	1,556	20173524	3561252	0,000		S	
Sum		18912622	3989787				

From the above chromatograms (Figure 2) it follows that the retention time of Benzketozone is 1.248 minutes at a column temperature of 200C. The analytical signal for a given range and the linear relationship between the concentration of the substance is shown in Table 2.

Table 2

The 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 0.9938.
Method repeatability. For that purpose, the definition of the substance was repeated 5 times. From the data presented in Table 3, it can be seen that the indicators are close in value and are in the confidence interval.

Table 3.

Results of the repeatability assessment of the method

No.	Weigh of drug, mg	Sediment height	Volume of Benzketozone in the substance,%	Metrological parameters
1	15,0	2383185	98,77	$\bar{X}$=99,51 %
2	15,0	2380474	99,21	S^2 = 0,8901
3	15,1	2382526	99,03	S = 0,9604
4	15,3	2386279	100,28	ΔX = 2,5106
5	15,2	2384157	100,25	$\Delta\bar{X}$ = 1,2154 E= 2,34 % $\bar{E}$= 1,09 %

To determine the correctness of the method, we used the results of the test substance in 12 determinations of identical masses of substances. Below (Table 4) the results of comparing the obtained data with theoretical calculations are presented.

Table 4

Results of the method validity evaluation

No.	Benzketozone mass, mg	Benzketozone standard sample, mg	Calculated content, mg	Determined content, mg	$\overline{X}$ %
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
11	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 99.5%.
Thus, the results of the development of an improved end-to-end method for the separation, detection and determination of the quantitative content of the substance Benzketozone using HPLC are presented. The developed methodology was validated according to the following parameters: sensitivity, linearity, repeatability and accuracy.
The developed ones are an end-to-end methodology and served as the basis for their use in quality control, as well as biopharmaceutical research in the developed gels and suppositories, both single-component and combined dosage forms.Below are the results of one-way analysis of variance obtained based on the results of the quantitative determination of Benzketozone using previously developed methods of UV spectrophotometry (3) and HPLC (11,12). Tabular F values with inter-method and intra-method variances were compared and calculated using the Fisher test (F).The results of the analysis are presented in Table 4..

Table 4

Results of one-way analysis of variance

	Sum of squares	F	Average square	$F_{assessed}$ S^2/ S	$F_{tabular}$ (0,05;4;10)
Between methods	$S_{сп}$=0,466	5-1=4	S^2 =0,3086	0,75	3,92
Within methods.	$S_{ош}$=4,192	15-5=10	S =0,415		
Total sum	S = 4,658	15-1=14	-		

The results of the tabular data indicate that the calculated values of F and the tabulated Fcalc <Ftab are comparable to each other. For quantitative assessment in further studies in the analysis of Benzketozone, it is advisable to use the previously developed UV spectrophotometric technique and the new improved HPLC technique.

CONCLUSION

Thus, we have developed quality control methods for the identification and quantitative determination of Benzketozone in the substance and in 3% gels. Identification was carried out using the chromatographic TLC method. An HPLC method has been developed for the quantitative determination of the substance Benzketozone. To confirm the reliability of the developed methodologies, validation was carried out according to the following parameters: sensitivity, linearity, repeatability and accuracy.

According to the results of comparing the tabular value of the calculated F, Fcalculate<Ftable shows that the previously developed and improved new proven end-to-end methods are comparable to each other. Therefore, for the purpose of quality control in soft dosage forms of Benzketozone, it is advisable to use developed end-to-end advanced TLC and HPLC methods to determine qualitative and quantitative analysis. The developed methods have been validated and certified in laboratory conditions.

REFERENCES

- Leontieva L.I., Azizov U.M., Khadzhieva U.I. et al. Anti-inflammatory activity of derivatives of phenylglyoxylic acid // Chem.pharm.journal -Moscow, 1999.- No. 3-C.26-28.
- Zakirov E.U., Yuldoshev S.J., Karshiev D.N., Azizov U.M. Study of the pathogenetic action of phenylglyoxylic acid on various forms of inflammation. // Infection, immunity and pharmacology-Tashkent, 2004.- No.1 - P.176-179.
- Standard of Quality of Pharmaceutical Product. Pharmacopoeial monograph "Benzketozone" FS 42 Uz-0850 -2020.
- U.M. Tillaeva, D.B. Kasimova, D.T. Gaibnazarova, U.B. Yakhyaev. Validation of the metod of spectrophotometric quantitative determination of benzketozone in a pharmaceutical active ingredient.World Journal of Pharmaceutical and Life Sciences, India, January 2020,-P.24-26.
- ICH Guidelines "Validation of Analytical Methods. Content and methodology" Q2 (R1) // Pharmacia. -2008,- No.4.-C.3-10.
- **6.**Validation of analytical methodologies for drug manufacturers. Model manual of the enterprise for the production of pharmaceuticals. Edited by V.V. Beregovykh. Moscow: Litterra, 2008.-132 p.
- Aladysheva Zh.I., Belyaev V.V., Beregovykh V.V. Practical aspects of works on validation of analytical techniques // Pharmacia 2008. -No.7. - P.9-14.
- Mitkina L.I., Kovaleva E.L. Approaches to assessing the suitability of analytical techniques in the examination of the quality of pharmaceuticals //Bulletin of the Scientific center for the examination of medical products. -2012,-No.2.-P.6-9.
- GPM 1.1.0012.15 Validation of analytical methods. State Pharmacopoeia of the Russian Federation. XIII ed. - Vol. I, - M., -2015. - P.222 - 234.
- Tillayeva U.M. Quality specification for combined action suppositories contating Benzketozon // Turkish Journal of Physiotherapy and Rehabilitation. – 2021.- Volume 32, Issue 2. – P.4072-4078.
- **11.**Tillaeva U.M., Kasimova D.B., Tillaeva G.U., Gaibnazarova D.T., Yakhyaev U.B. Validation of the method of spectrophotometric quantitative determination of benzketazone in a pharmaceutical active ingredient. World journal of Pharmaceutical and Life Sciences. India. 2020;l6(5):24-26.
- **12.**Tillaeva U.M., Rakhmanova Z.A., Tillaeva G.U. Pharmaceutical analysis and pharmacological studies of Benzketozone. Farmatsiya,Scientific and practical journal. 2022.No.2. P.22-28.