World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

VALIDATION OF THE METHOD OF SPECTROPHOTOMETRIC QUANTITATIVE DETERMINATION OF BENZKETOZONE IN A PHARMACEUTICAL ACTIVE INGREDIENT

U. M. Tillaeva*, D. B. Kasimova, G. U. Tillaeva, D. T. Gaibnazarova and U. B. Yakhyaev

Republic of Uzbekistan, Tashkent pharmaceutical Institute.

***Corresponding Author: Tillaeva U. M.**
Uzbekistan, Tashkent, Tashkent Pharmaceutical Institute.
Email Id: umida.tillaeva@gmail.com,

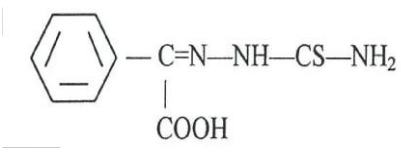
Article Received on 10/03/2020 **Article Revised on 30/03/2020** **Article Accepted on 21/04/2020**

ABSTRACT

In the Republic of Uzbekistan, special attention is paid to the development of the pharmaceutical industry and the provision of domestic products to the population. In this regard, the expansion of the range of antimicrobial and anti-inflammatory drugs, using domestic resources, is a priority in the development of science and technology in the direction of modernization of production and technology in order to introduce domestic developments of medicines and medical products.

Scientific staff of the Uzbek scientific research chemical and pharmaceutical Institute named after A. Sultanov conducted a number of works on the synthesis of new biologically active compounds based on aromatic a-ketoacids and the study of their pharmacological activity.[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, which makes it particularly important to use them in domestic medicine as locally developed drugs.

According to its activity, benzketozone [47.7%] exceeds the known preparations of butadion [26.7 %], voltaren (42.2%), while it belongs to the low-toxic compounds (LD 50 2394 mg/kg). Together with the Tashkent pharmaceutical Institute, the development of anti-inflammatory drugs in the form of soft dosage forms of combined action is being carried out. It is relevant to develop new and improved methods of quality control and standardization of both substances and dosage forms in accordance with modern requirements of good manufacturing practice. Thus, U. M. Azizov, L. I. Leontieva and others found that thiosemicarbazone of phenylglyoxylic acid (benzketosone) shows a pronounced anti-inflammatory effect.



Methods of quality control and standardization, developed using modern physical and chemical methods are subject to certification, which in turn is required in order to confirm the suitability of this method for objective evaluation of quantitative content such as a pharmaceutically active ingredient (PhAI) and in the future in various medicines obtained on their basis and justification of parameters to represent the validation of analytical methods is part of a registration application submitted in the EU, Japan USA and other.[3]

The purpose of the work is to assess the adequacy of the analytical methodology proposed for the quantitative determination of benzketozone in PhAI.The article presents the results of validation of the developed methods by parameters: specificity, linearity, correctness and repeatability.

MATERIALS AND METHODS

We used instruments: SPh 2000 spectrophotometer, analytical scales of the t-A-13 brand .Solutions of reference standards (RS) of benzketosone (PhA 42-0849-10) were prepared in a concentration of 0.05 mg/ml (for UV spectrophotometry) To determine benzketozone by UV-spectrophotometry, we used a technique developed earlier.[4] about 0.1 g of the drug (a. w.)the benzketozone dried to a constant mass was transferred to a measuring flask with a capacity of 200 ml, dissolved in 50 ml of purified water, heated in a water bath until completely dissolved, and cooled, brought to the mark with the same solvent and mixed (solution A). 1 ml of solution A was transferred to a measuring flask with a capacity of 100 ml, brought to the mark with the same solvent and mixed (solution B). The optical density of the resulting solution

B was measured at 305 nm in a cuvette with a layer thickness of 10 mm. Purified water was used as a comparison solution.

In parallel, a solution of a working standard sample of benzketozone was prepared. For this, about 0.1 g of the preparation (a.w.) dried to a constant weight of benzketozone was transferred into a volumetric flask with a capacity of 200 ml, dissolved in 50 ml of purified water, heated in a water bath until completely dissolved, cooled, adjusted to the mark with the same solvent and stirred (solution A). 1 ml of solution A was transferred into a 100 ml volumetric flask, made up to the mark with the same solvent and stirred (solution B).

The quantitative content of benzketozone in% (X) was calculated by the formula:

$$x = \frac{Dx * 200 * 100 * a_0 * 100}{D_0 * a_x * 1}$$

Where, Dx, D_0 are the optical density of the analyzed solution and the RS solution of benzketozone, respectively;
a_x, a_{RS} – accurate weight of the analyzed solution and RS of benzketozone, respectively, g;

The content of benzketozone in the drug should be at least 97.5%.

RESEARCH RESULTS AND DISCUSSION

Before statistical data processing, a homogeneity of the samples was verified and it was found that all of them did not contain a gross error, because Q1 <Q (n = 5, P = 95%), i.e. Q1 <0.64. Validation of the developed methods was carried out in accordance with the draft GPhA 42-0113-09 "Validation of analytical methods".[3,5]

The analytical region of the technique is within linear relationship and amounts to 42–58 μg / ml benzketozone and is described by the equation y = 128.7x-0.2380 with a correlation coefficient r = 0.995, and the necessary condition for the linear dependence $|r| \geq 0.99$ is also satisfied.

The correctness of the proposed methodology was determined on 6 samples of solutions of model mixtures of benzketozone in the PhAI (Table 1).

Table 1: Determining the correctness of the spectrophotometric method for determining benzketozone.

Method	taken, g	x,%	R, %	Metrological characteristics
UV spectrophotometry	0,1107	0,1008	100,09	R= 100,10
	0,1003	0,1002	100,09	s^2= 0,0086
	0,1003	0,1001	100,19	s= 0.0927
	0,1005	0, 1004	100,09	Δxmean = 0,2380
	0,1002	0,1001	100,09	ε, % = 0,24
	0,1001	0,1000	100,09	t_{tab}=2,57

In the technique, the inequality $t_{det} < t_{table}$ (P, f) is observed; therefore, the presented results are not burdened by a systematic error and they are correct.

In order to verify the repeatability of the methods, a three-level experiment of 3 experiments at each level was carried out. The measurement range was chosen based on the variation in the amount of benzketozone substance in the PhAI (± 20%). Thus, the upper level corresponds to a 0.22 g sample, the average - 0.20 g, and the lower - 0.18 g.

In order to obtain the metrological characteristics of the methods, statistical processing of the results of the quantitative determination of benzketozone in the PhAI was carried out by UV spectrophotometry.

Table 2: The results of determining the repeatability (precision) of the method for determining benzketozone by UV spectrophotometry.

Method	Level	Benzketozone, %		R , %	f	X_{mean}	s^2	S	P	Δx_{mean}	ε, %
		Taken	Found								
UV spectrophotometry	I	0,1810	0,1803	99, 61	9	99,45	0,8050	0,8972	95%	0,6758	1,53
		0,1807	0,1793	99,20							
		0,1824	0,1785	97,85							
	II	0,2107	0,2098	100, 42							
		0,2080	0,2078	100,09							
		0,2075	0,2054	98,98							
	III	0,2215	0,2205	99,54							
		0,2188	0,2177	99,49							
		0,2210	0,2208	99,90							

According to the data in Table 2, $t_{det} < t_{tab}$, which allows us to consider the results of the sample technique free from systematic error.

Note: t_{det-} -t determined
t_{tab} -t in table

CONCLUSIONS

Using a validation assessment, it was established that the developed method for quantitative determination using the spectrophotometric method in the PhAI is correct, precise, reproducible and linear in the analytical field.

REFERENCES

1. Azizov U.M., Leontyeva L.I., Zakirov U.B. Potassium salt of α-hydroxy, α-sodium sulfonate acetic acid, which has anti-inflammatory activity. Pat. RUz No. 4521, dated June 13, 1997.
2. Zokirov U.B., Maksudov A.A., Azizov U.M. The study of the effectiveness of benzketozone in the treatment of eye burns // Pharmaceutical journal. Tashkent, 2004; 1: 90-92.
3. Validation of analytical methods: text and methodology ICH Q2 (R1).
4. Temporary pharmacopoeial article of the benzketozone TPhA 42 Uz-0850-2010.
5. GPhA 42-0113-09 "Validation of analytical methods.