QUALITY SPECIFICATION FOR COMBINED ACTION SUPPOSITORIES CONTAINING BENZKETAZON

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ANNOTATION

In the Republic of Uzbekistan, special attention paid to the development of the pharmaceutical industry and the provision of domestic medicinal products to the population. In this regard, expanding the range of antimicrobial and anti-inflammatory drugs using domestic resources is a priority in developing science and technology in the direction of modernization of production and technologies to introduce domestic developments of medicinal products (MP) and treatments.

The scientific staff of the Uzbek Scientific Research Chemical-Pharmaceutical Institute A. Sultanov carried out several works on synthesizing new biologically active compounds based on aromatic α-keto acids and study their pharmacological activity [1, 2]. Biologically active compounds obtained based on phenylglyoxyl acid have an extensive range of pharmacological activity, particularly anti-inflammatory, without several side effects, which makes it especially important to use them in domestic medicine as drugs of local development.

In its activity, Benzketozon (47.7%) surpasses known drugs: Butadione (26.7%), Voltaren (42.2%), with low toxic compounds (LD₅₀ 2394 mg/kg) [3]. Together with the Tashkent Pharmaceutical Institute, anti-inflammatory drugs in soft medical forms (MF) of combined action are being carried out.

I. INTRODUCTION

The study on effective MF with already known medicinal products proved well in treating a particular disease and received overall development. Moreover, a specific interest in this direction is represented by combined MF when it already has complete characteristics of the dependence of effects and a wide range of doses for each drug [4]. The specific activity of suppositories compared with a one-component due to the potential action of acting substances is proved when reducing dosage and corresponding side effects [5]. The combined action MP application increasingly used, and industrial production of many products is established and has a significant percentage of the segment of the registered MP [6].

Drugs containing antibacterial, steroid and non-steroid drugs considered among the applicable combinations [7, 8, 9]. Pharmacological interaction is connected with the fact that one substance alters the pharmacokinetics or pharmacodynamics of another component of the mixture. The pharmacokinetic type of interaction may involve intake, biotransformation, transport and injection of one of the substances. Pharmaceutical type of interaction results from direct or indirect interaction of substances at the level of receptors, cells, enzymes, organs or physiological systems. In modern medical practice, combined MPs are increasingly crucial for treating complex pathologies associated with enzyme deficiency, reduced body resistance, and in medicinal mixtures, they most optimally display their pharmacological properties [10].

Modern medicine increasingly uses protein-nature drugs as promising combinations in one MP due to high activity and specificity [11]. We conducted a content analysis using a pharmacized method of studying textual, graphic information in quantitative indicators and its statistical processing [12, 13, 14]

Papaya (melon tree) is cultivated in Uzbekistan, has a proteolytic effect, and can split proteins into polypeptides and amino acids [15, 16]. It is an indispensable means for healing wounds, as detected by a mine. The NSAID with antibacterial agents and enzymes Senergically must contribute to the strengthening of their positive action. In order to obtain a purposeful combination of LV, we have developed the suppositories containing Benzketazon and papaya (exterior part) as active substances.
It is known that NSAID considered to be long-term drugs and have unwanted side effects and negatively affect the gastrointestinal tract. For this purpose, rational use of rectal PF.

The aim of the research

Conduct quality control methods and standardization of combined-action suppositories containing Benzketazon and Papaya extract following modern requirements of good production practice.

II. MATERIALS AND METHODS

In work used devices: SF 2000 spectrophotometer, T-A-13 analytical scales. Standard sample solutions (SS) of Benzketozon (FS 42-0849-10) prepared at 0.05 mg/ml (for UV spectrophotometry). For the determination of Benzketozon by UV spectrophotometry, we used the technique developed earlier [3]: About 0.1 g of the drug (so-called) dried to a constant mass of Benzketozon transported to a measured bulb with a capacity of 200 ml, dissolved in 50 ml of purified water, heated on the water bath to the full dissolved, cooled, marked with the same solvent and mixed (solution A). 1 ml of solution A carried to a 100 ml dimension bulb, brought to a mark by the same solvent and mixed (solution B). The optical density of solution B obtained measured at 305 nm in the cuvette with a layer thickness of 10 mm. Clean water used as a comparison solution.

In parallel, a solution of the working standard sample of Benzketozon prepared. For which about 0.1g of the drug (so-called) dried to a constant mass of Benzketozone carried to a measured bulb with a capacity of 200 ml, dissolved in 50 ml of purified water, heated on the water bath to the full dissolved, cooled, marked with the same solvent and mixed (solution A). 1 ml of solution A carried to a 100 ml dimension bulb, marketed by the same solvent and mixed (solution B).

The formula calculated quantitative Benzketozon content in % (X):

\[
X = \frac{A_x \cdot a_{cm} \cdot 100,00 \cdot 25,00 \cdot 5,00 \cdot P}{A_{cm} \cdot a_x \cdot 5,00 \cdot 25,00 \cdot 100,00} = \frac{A_x \cdot a_{cm} \cdot P}{A_{cm} \cdot a_x}
\]

Where, \( A_x, A_{cm} \) - optical density of the analyzed solution and the solution of petrol-ozone CO respectively;

\( a_x, a_{cm} \) - of the analyzed solution and Benzketozon SS, respectively, g;

The content of Benzketozon in the drug must be at least 97.5%.

III. RESULTS AND DISCUSSION

For the analysis, the suppositories prepared by the traditional method of extracting consist of a suppository base and an MD. The replacement factor calculated using a known technique (Strakova I.M.) The lubricant was a mixture of soft soap, glycerin and ethanol at 1:1:5. According to the type of suspension, a weighted quantity of pre-shredded MD (degree of grinding to the state of "the tiniest "SP x.s. 857), put the mixture into the molten base and shaken to complete homogenization. Then they filled the form cell up to the edges of 2.6 g and put them in the refrigerator. According to the specified method, several suppositories received were subject to quality control.

Physical-chemical characteristics and indicators met the requirements of SP XI (paragraph 2). The average mass of suppositorie s 2.6 (from 2.4 to 2.8). The melting temperature was not higher than 37°, the time of total deformation not more than 15min at temperature (37±1°), hardness on Kaminisk [17]

Study of the impact of the interaction of the suppositories base (SB) with the active substances (Benzketazon and Papaya) in the storage process. Given that SB's interaction with the drugs included in the composition of the suppositories and SB could influence the properties of the MP, we determined the basic parameters of the prepared suppositories: melting temperature, hardening temperature, acidic number, viscosity and TDT (Total Deformation Time). As a control, placebo suppositories used. The indicators were determined after the melting of the suppositories and extracting the Benzketazon and Papaya hot filtered. Before filtering, the bases cooled to solidification (on the SB filter), and the filter used for further research. At the time of preparation, the active substances had practically no influence on the fundamental Physico-Chemical and Structural-Mechanical indicators of the examined supposers and vice versa.
Storage research: For this purpose, the prepared suppositories divided into two series after packing into boxes. One series kept in the refrigerator at a temperature of 3-5°C, the other - at a room temperature of 20±2°C. During the storage process (24 months), every three months, the following values determined: melting temperature, acidic amount, iodine amount, and TDT. Table 1 showed the results of the definitions of the above parameters in the storage process.

<table>
<thead>
<tr>
<th>Medicinal Forms</th>
<th>Indicators</th>
<th>Storage period in months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Suppositories «Benzpap»</td>
<td>Iodine amount</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Acidic amount</td>
<td>0,25</td>
</tr>
<tr>
<td></td>
<td>TDT, min</td>
<td>5'22&quot;</td>
</tr>
<tr>
<td></td>
<td>Melting temperature, °C</td>
<td>37,0</td>
</tr>
<tr>
<td>Suppositories «Benzpap 10»</td>
<td>Iodine amount</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Acidic amount</td>
<td>0,58</td>
</tr>
<tr>
<td></td>
<td>TDT, min</td>
<td>5'15&quot;</td>
</tr>
<tr>
<td></td>
<td>Melting temperature, °C</td>
<td>36,8</td>
</tr>
</tbody>
</table>

Note: The upper digit indicated the value of the indicators at storage temperature +20±20°C. Lower digit - at +3±50°C.

Table 1 showed that in the two-year storage of suppositories at different temperature conditions, melting temperature, suppositories practically did not change. The iodine and acidic amount of suppositories at reduced storage temperature remained practically unchanged. A linear relationship between the time of total deformation (TDT) and melting point also observed. There was some increase in the TDT in all suppositories at the temperature of 3-5°C and at 20±2°C. However, the fluctuation in the values of all indicators did not exceed the permissible limits.

All indicators determined following the requirements of the general article "Suppositories" of SP XI.

The studies to determine the shelf life of the studied supposers based on the method of "Accelerated ageing" at elevated temperature carried out under the instructions I-42-2-82. The method of "Accelerated ageing" was to withstand the test MP at temperatures above its melting point and allows to establish the MD stability in the rectal MF (RMF) for a relatively short period.

It is known that the quantitative content of active substances in the MF storage process was one of the main factors characterizing stability.
In order to determine the quantitative content of the active substances in the process of storage, we used the methods of TLC (Thin Layer Chromatography) and SP (Spectrophotometry).

In the course of the "Accelerated ageing" study of samples of the studied suppositories stored at a temperature of 30°C (the temperature recommended by the instructions I-42-2-82 for suppositories), within three months, we found minor losses of active substances in the suppositories also traced of their decomposition products.

Only a tiny amount of material was found in the refrigerated suppositories. In this case, we also used the TLC method, which was essentially a semi-quantitative method and allowed for the intensity of stain colouring compared to SS to judge the substance contained in the test solution.

It should be noted that to extend or increase the stability of most suppositories and optimal storage conditions stored at a reduced temperature. The form of the base and storage conditions (oxygen presence, storage temperature, illumination) had a significant impact on the stability of the supposers.

As already noted, active MP could interact with SS to store suppositories, resulting in reduced content or reduced effectiveness of the drug. As it is known, the main requirement for quality control of finished MF was the qualitative and quantitative determination of acting substances in the drug.

According to the previously developed and validated SP method, the control of the quantitative content of Benzketazon acting in the developed suppositories carried out. [18]. As the results of Table 1 in the refrigeration chamber conditions showed, the suppositories under investigation practically changed the quality indicators during the 24 months of storage.

Separation of the active substances from the forming components of the RMF carried out by extraction. In choosing an organic solvent, the solubility of the active substances took into account. The investigated substances extracted from 5 suppositories in 20-25ml of water purified by heating on the water bath. The extraction was filtered, the filter divided into 5 parts and carried out identification reactions.

The methods of quality control and standardization, developed using modern Physico-Chemical methods, were subject to certification, which required to confirm the suitability of this technique for objective evaluation of the quantity content of such substances both in the active pharmaceutical ingredient (API). Moreover, later in the various drugs derived from the experiments and as the justification of the parameters for submission of validation analytical methods, part of a registration application submitted to the EU, Japan, Uetcetc. [19,20].

The further aim of the work was to assess the adequacy of the analytical methodology proposed for the quantitative determination of Benzketazon in FAI and from suppositories. The following resulted from validating the developed methods on parameters: specificity, linearity, correctness, and repeatability.

Before the statistical processing of the data, the homogeneity of the samples checked, and it determined that all of them did not contain a gross error. \( Q_1 < Q \) \( (n=5, P=95\%) \), i.e. \( Q_1 < 0,64 \). The methods developed implemented according to the Federal Reserve Board 42-0113-09 "Validation of analytical methods".

The analytical area of the technique was within linear dependence and is 42-58 \( \mu g/ml \) of Benzketazon and described by equation \( y=128.7x-0.101 \) with correlation coefficient \( r=0.995 \), as well as performed linear dependency condition \( r^2 \geq 0.99 \). The accuracy of the proposed methodology determined on 6 samples of solutions of model mixtures of Benzketazon (Table 2).

<table>
<thead>
<tr>
<th>Method</th>
<th>( \mu, \ g )</th>
<th>( x, % )</th>
<th>( R, % )</th>
<th>Metrological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV spectrophotometry</td>
<td>0,1107</td>
<td>0,1098</td>
<td>99,57</td>
<td>( R=99,15 )</td>
</tr>
<tr>
<td></td>
<td>0,1093</td>
<td>0,1021</td>
<td>96,56</td>
<td>( s^2=4,91 )</td>
</tr>
</tbody>
</table>
The methodology respected the inequality $t_{\text{calc}} < t_{\text{table}} (P, f)$, so the results presented did not aggravate by a systematic error and were correct.

In order to verify the repeatability of the techniques, a three-level experiment conducted on 3 experiments at each level. The measurement range selected based on the variation in the amount of Benzketozon in the FAI ($\pm 20\%$). Thus, the upper level corresponds to the attachment 0.18 g, the average - 0.20 g, the lower - 0.22 g.

In order to obtain metrological characteristics of the methods, statistical processing of results of quantitative determination of Benzketozon in FAI and from suppositories carried out by UV spectrophotometry (Table 3).

Table 3

Results of determination of repeatability (precision) of the method of determination of Benzketozon method of UV spectrophotometry

<table>
<thead>
<tr>
<th>Method</th>
<th>Level</th>
<th>Benzoketozon, %</th>
<th>$R$,</th>
<th>$f$</th>
<th>$x$</th>
<th>$s^2$</th>
<th>$s$</th>
<th>$P$</th>
<th>$t_{\text{calc}}$</th>
<th>$t_{\text{table}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>taken</td>
<td>found</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV spectrophotometry</td>
<td>I</td>
<td>0,181, 0,178</td>
<td>3</td>
<td>8</td>
<td>99</td>
<td>1,5</td>
<td>1,2</td>
<td>95</td>
<td>0,9</td>
<td>0,9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0,180, 0,179</td>
<td>7</td>
<td>3</td>
<td>99</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0,9</td>
<td>0,9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0,182, 0,178</td>
<td>4</td>
<td>5</td>
<td>97</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0,9</td>
<td>0,9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0,210, 0,209</td>
<td>7</td>
<td>8</td>
<td>99</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>0,9</td>
<td>0,9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0,208, 0,209</td>
<td>0</td>
<td>8</td>
<td>100</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>0,9</td>
<td>0,9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0,207, 0,205</td>
<td>5</td>
<td>4</td>
<td>98</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>0,9</td>
<td>0,9</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0,221, 0,219</td>
<td>5</td>
<td>5</td>
<td>99</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0,9</td>
<td>0,9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0,218, 0,212</td>
<td>8</td>
<td>7</td>
<td>97</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>0,9</td>
<td>0,9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0,221, 0,224</td>
<td>0</td>
<td>1</td>
<td>101</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0,9</td>
<td>0,9</td>
</tr>
</tbody>
</table>

According to the data of table 3 $t_{\text{calc}}(1,2)<t_{\text{table}}$, which allowed considering the results of the sample of the technique free from systematic error.

Papaya (a dry extract of the above-ground part) was determined from the same filter and carried out reactions on amino acid composition to identify TLC and paper chromatography indicators. Also, the identification of TLC carried out on alkaloids, flavonoids and ascorbic acid. Quantitative content on the analyzer "MIKROTECHNA - ANALIZER T-339" (Prague-Czechoslovakia), on the output of the order of peaks, therefore: asparagine acid,
threonine, glutamine acid, glycine, alanine, valine, isolectin, leucine, phenylalanine and lezins -serin, glycinn, tyrosine, amino acids.

Ascorbic acid content determined by TLC method: Silufol and Merck 200 x 150 ml, 5% ascorbic acid solution, 80:20 - ethyl acetate - icy acetic acid. The time in the endurance was 20 minutes. Drying: In the air, the developer - 0.04% 2.6-dichlorophenolindophenolate sodium solution (0.001 mol/l). Rf - 0.42. The data corresponded to the previously developed methodology [21]. They also determined the proteolytic activity of enzymes using the modified Anson method based on hydrolysis. Optical density determined by the photo-electrocolorimetric method. The Cuvette was 10 mm thick, and the wavelength is 630-670 nm; the optical density in the disposition is 0,2-0,60. The activity of proteolytic calculated according to the schedule tyrosine equivalent (TE). 1 ml standard solution was 1 mk tyrosine [22].

IV. CONCLUSION

1. The methods of natural and accelerated storage examined the factors influencing the stability of the suppositories in the storage process (type of basis used, temperature and time of storage). It was found that in the refrigerating chamber at temperature +3-5°C the tested suppositories practically did not change quality indicators during 24 months of storage. The basis slightly influenced the adjustment of the Physico-Chemical parameters of the suppositories "Benzpap" and "Benzpap 10".

2. For quality specification, the SP method is used; the lack of interaction of SS with the active substances of the examined suppositories proved.

3. With the help of validation assessment, it was established that the developed end-to-end method of quantitative determination of Benzketazon of suppositories using the Spectrophotometric method was correct, precise, reproducible, and linear in the analytical field.

4. The techniques developed adapted as cross-cutting to the qualitative assessment of the contents of active substances in the suppositories.

CONFLICT OF INTERESTS AND CONTRIBUTION OF AUTHORS

The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article and report on each author's contribution.

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