Quantitative determination of the new substance 7-(4-fluorobenzyl)-3-thioxo-2,3-dihydro[1,2,4]triazolo[4,3-a]pyrazin-8(7H)-one as a potential pharmaceutical agent

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The article presents results of studies on the development of a quantification method for a substance 7-(4-fluorobenzyl)-3-thioxo-2,3-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-8(7H)-one using the method of non-aqueous potentiometric titration. Validation of the developed method has been carried out confirming the following characteristics: linearity, accuracy and precision that match the acceptance criteria for these measures.

Keywords: quantification, potentiometric titration, [1,2,4]triazolo[4,3-A]pyrazin-8-ones, analytical validation

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INTRODUCTION

In the last decade, the number of new antimicrobial drugs, which are being introduced into the world pharmaceutical market, has sharply decreased. This is due, first of all, to limited resources for conducting clinical trials of medicinal substances and the rapid development of microorganism resistance to antimicrobial agents that certainly serves as a deterrent to an uncontrolled arrival of new products onto the market.

Literature data indicate a high antimicrobial activity of the compounds containing the nucleus of [1,2,4]triazolo[4,3-a]pyrazine [1–3]. The synthetic scheme for synthesis of [1,2,3]triazolo[4,3-a] pyrazine derivatives based on substituted amides of oxalamic acids was developed. This scheme makes it possible to bring into the structure, of the desired products, various substituents in positions 3 and 7 of heterocycle. The proposed approach allows the synthesis of large arrays of target products for the screening of pharmacological studies [4].

The microbiological screening of the first synthesized derivatives of 3-thioxo-[1,2,4] triazolo[4,3-a]pyrazine, performed by us, made it possible to isolate substances with a high antimicrobial and antifungal activity among compounds of this series [5]. According to the results of the microbiological screening for an in-depth study, the substance 7-(4-fluorobenzyl)-3-thioxo-2,3-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-8(7H)-one has been selected (Fig. 1), the antimicrobial activity of which was the highest in relation to gram-negative bacteria (MICs 12.5 µg/ml, MBCs 25.0 µg/ml) [5].

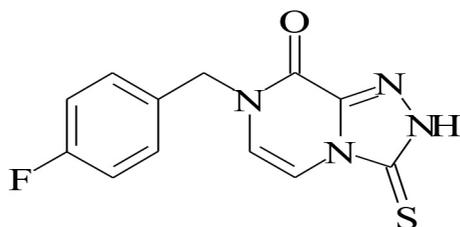The aim of the study is to develop a method for quantitative determination of the substance 7-(4-fluorobenzyl)-3-thioxo-2,3-dihydro-[1,2,4] triazolo[4,3-a]pyrazin-8(7H)-one with its subsequent validation for the introduction of the resulting substance into pharmaceutical practice. Article [8] describes a standardized procedure for validation of titrimetric methods, with its use for indicator titration, and the specifics of using this standardized procedure for the most common method of titrimetric analysis – potentiometric titration has not yet been experimentally described.

EXPERIMENTAL

The test substance is a crystalline powder of white or almost white colour with a grayish tinge and a slight specific odour. The solubility of the obtained substance was established experimentally. The procedure for studying the solubility was performed according to the requirements of SPhU. The substance of 7-(4-fluorobenzyl)-3-thioxo-2,3-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-8(7H)-one was found to be freely soluble in dimethylformamide, dimethylsulfoxide and triethylamine, slightly soluble in methanol and ethanol, and practically insoluble in water. An important step in the development of a drug is the development of accurate and reliable methods of its analysis. Due to the absence of a standard sample of the synthesized substance and on the basis of its physicochemical properties, a direct method of determination was chosen for quantitative determination – potentiometric titration in a non-aqueous medium. The structure of the 7-(4-fluorobenzyl)-3-thioxo-2,3-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-8(7H)-one molecule contains nitrogen-containing heterocycles and exhibits basic properties. In the course of the work the approach of European Pharmacopoeia [7] was used, according to which the greatest preference is given to direct methods of quantitative determination, which include potentiometric titration. Potentiometric titration is leading to determine the content of the active substance in pharmaceutical substances. Acidimetry in a non-aqueous medium allows the weak bases and their salts to be determined with the necessary accuracy.

It was established experimentally that the most suitable solvent is a mixture of acetic acid and acetic anhydride, and the titrant is a pharmacopoeial titrated solution of 0.1 M perchloric acid.

Fig. 1. The chemical structure of 7-(4-fluorobenzyl)-3-thioxo-2,3-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-8(7H)-one

Potentiometric titration was performed by using an automatic titrator 7025 M Titrino Metrohm (Switzerland) with a burette volume of 10 ml.

The amount of 7-(4-fluorobenzyl)-3-thioxo-2,3-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-8(7H)-one in the substance in % was calculated by the formula

$$X = \frac{V \cdot 0,02763 \cdot K \cdot 100 \cdot 100}{m \cdot (100 - W)},$$

where V is the volume of 0.1 M of perchloric acid solution, which was used for titration of the tested solution, ml; K is the correction factor to the molarity of titrant; 0.02763 is the number of grams of $C_{12}H_9FN_4OS$, which corresponds to 1 ml of 0.1 M of perchloric acid solution; m is the mass of a sample weight; W is the weight loss value on drying substance, %.

1 ml of 0.1 M of perchloric acid solution corresponds to 27.63 mg of $C_{12}H_9FN_4OS$.

RESULTS AND DISCUSSION

When using the method of acid-base titration, the uncertainty of the result of the final analytical operation is associated with taking a sample of the sample under investigation, determining the volume of the burette and determining the correction factor for the titrant solution [12]

$$\Delta_{X,r} = \sqrt{\left(\Delta_m\right)^2 + \left(\Delta_{Vt}\right)^2 + \left(\Delta_{C_M}\right)^2},$$

where Δ_m is the sample uncertainty; Δ_{Vt} is the titration burette volume uncertainty; Δ_{C_M} is the uncertainty of the molar concentration of the titrated 0.1 M perchloric acid solution.

To prevent errors during titration, the weighed sample of the substance to be analysed should be taken from the calculation so that 80 ± 10% of the volume of the burette is spent on titration [8].

As a result of experimental studies for the quantitative determination of 7-(4-fluorobenzyl)-3-

thioxo-2,3-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-8(7H)-one the following method was proposed: 0.250 g of the substance is dissolved in a mixture of 30 ml of *acetic acid anhydrous P* and 30 ml of *acetic anhydride P*, titrated with 0.1 M of perchloric acid solution potentiometrically till the first jump in potential on the titration curve. The quantitative content of the main active substance in the substance should be from 99.0 to 101.0% in terms of dry substance. Characteristics of the developed methods of acid-base titration are presented in Table 1.

The uncertainty of the result of the final analytical method for the quantitative determination of 7-(4-fluorobenzyl)-3-thioxo-2,3-dihydro-[1,2,4] triazolo[4,3-a]pyrazin-8(7H)-one is 0.22%, which does not exceed the maximum permissible uncertainty of the analytical method (max Δ_{as}, %): 0.22 ≤ 1.00.

Earlier it was reported about the development of techniques for controlling concomitant impurities in the substance 7-(4-fluorobenzyl)-3-thioxo-2,3-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-8(7H)-one [6]. The HPLC method was used to determine the main impurities. Impurity A (1-(4-fluorobenzyl)-3-hydrazino-pyrazin-2(1H)-one) was a semiproduct in the synthesis of the corresponding 3-thioxo-derivatives of [1,2,4]triazolo[4,3-a]pyrazine. Impurity B (7-(4-fluorobenzyl)-[1,2,4]triazolo[4,3-a]pyrazine-3,8(2H, 7H)-dione) was an impurity of the decomposition of the substance (oxidation under inappropriate storage conditions etc.) (Fig. 2). The total content of impurities in the substance should not exceed 0.5%. Thus, the accompanying impurities do not interfere with the quantitative determination of the active substance in the substance by potentiometric titration.

On the basis of the previously obtained experimental data, the loss in mass during the drying of the substance was 0.2–0.4%. On the basis of experimental data, the weight loss during the drying of a substance is normalized at a level not exceeding 0.5%.

Table 1. **Characteristics of the developed acid-base titration method**

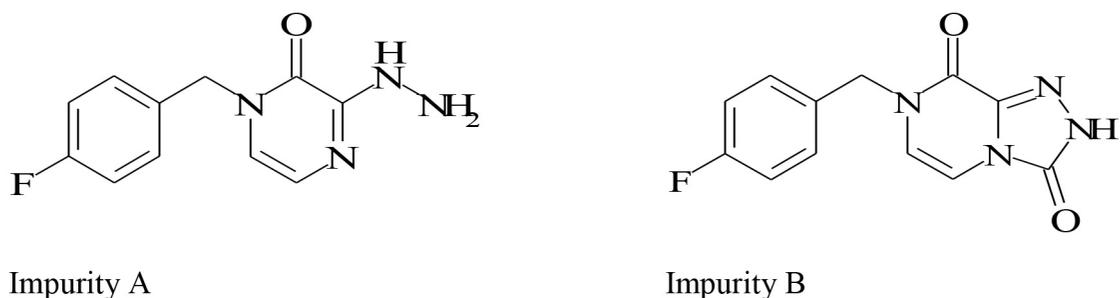| Content tolerances, %	Max Δ_{as}, %	Max Δ_s, %	Sample weight for analysis, m, g	Max Δ_m, %	Nominal titration volume, V_{nom}, ml	Δ_{Vt}, %	% of 10 ml burette volume	Δ_{C_M}, %
99.0–101.0	1.0	0.32	0.25	0.080	8.95	0.20	89.5	0.063

Impurity A   Impurity B

Fig. 2. The chemical structure of impurities A and B

For further use of the developed method, it was necessary to determine its validation parameters. Validation of the developed method was carried out according to the requirements of the State Pharmacopoeia of Ukraine (SPhU) by studying its linearity, accuracy and precision.

The establishment of a linearity was performed by titration of nine different concentration samples in a range of 80–120% of the nominal sample of the test substance described in the method. Accordingly, a series of working solutions with concentrations of 80, 85, 90, 95, 100, 105 110, 115 and 120% of 250 mg was prepared. To study the reproducibility of the results in the process of studying the linearity, we took 3 samples for each point in a range of 80–120%.

Using the rule of concurrent validation, accuracy and precision were determined using the data obtained in the determination of linearity. To calculate the normalized coordinates, we took the nominal sample weight and the nominal volume presented in Table 1. The results of studying the linearity of the entire sample of 27 points and a comparison with the criteria [8] are presented in Table 2.

The results of the study of linearity allow us to conclude that the criteria $|a|$ and $|1-b|$ do not satisfy the requirement of statistical insignificance, but satisfy the requirement of practical acceptability of a linearity. The results of determining the precision and accuracy of the developed methodology are presented in Table 3.

Table 2. **Characteristics of the linearity $Y = a + b \cdot X$ for the developed method**

Parameter	Value	Statistical insignificance criterion	Practical acceptability criteria	Conclusion
a	−0.92	$|a| \leq 0.131$		not correspond
b	1.0111			
$|1-b|$	0.0111	$|1-b| \leq 0.0013$		not correspond
s_0	0.255		≤0.39	correspond
r	0.99982		≥0.99959	correspond
r^2	0.99965		≥0.99917	correspond
$\delta_{RL,80}$	0.039		≤0.67	correspond
$\delta_{RL,120}$	0.34		≤0.67	correspond

Table 3. **Accuracy and precision of the developed analytical method**

X, %	Y, %	Z, %
80.08	80.22	100.18
80.24	80.45	100.26
79.92	79.78	99.82
85.12	85.47	100.42
85.24	85.81	100.67
84.88	84.47	99.52

Table 2. **(continued)**

X, %	Y, %	Z, %
89.84	89.50	99.62
90.08	89.94	99.85
90.32	90.28	99.95
94.88	95.20	100.33
94.68	94.53	99.84
95.12	95.42	100.31
100.24	100.45	100.21
100.00	100.11	100.11
99.96	99.89	99.93
104.72	104.80	100.08
105.12	105.25	100.12
105.04	105.03	99.99
109.92	110.17	100.23
110.08	110.73	100.59
110.24	110.84	100.54
114.96	115.31	100.30
114.68	114.97	100.25
114.92	115.53	100.53
120.26	120.45	100.24
119.92	120.56	100.53
120.20	120.45	100.21
$\bar{x}$		100.17
SD		0.29
ΔR		0.19
$\delta = |\bar{x} - 100|$		0,17
Statistical insignificance, $\delta \leq \Delta_R$ **0.17 ≤ 0.19**		performed
Practical insignificance, $\delta \leq 0.67$ **0.17 ≤ 0.67**		performed
Practical acceptability of precision (6 titrations), $\Delta_R \leq \frac{1}{3}\Delta_{as}$ **0.25 ≤ 0.33**		performed

The developed method meets the requirements of accuracy and precision.

The obtained experimental data and the calculations based on them indicate that all the validation parameters of the developed method correspond to the necessary acceptance criteria.

CONCLUSIONS

1. Based on the results of the study, a quantification technique for determination of the basic substance in the new pharmaceutical substance 7-(4-fluorobenzyl)-3-thioxo-2,3-dihydro-[1,2,4]triazolo[4,3-a]pyrazine-8(7*H*)-one was developed.

2. Validation of the proposed technique was performed, which confirms matching the following characteristics: specificity, linearity, correctness, precision, convergence (repeatability) and intermediate (intralaboratory) precision matching the acceptance criteria for these measures.

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NAUJOS MEDŽIAGOS 7-(4-FLUOROBENZIL)-3-TIOKSO-2,3-DIHIDRO[1,2,4]TRIAZOLO[4,3-A] PIRAZIN-8(7H)-ONO, KAIP POTENCIALAUS FARMACINIO AGENTO, KOKYBINIS NUSTATYMAS

S a n t r a u k a

Straipsnyje pateikiamas naujos medžiagos 7-(4-fluoro-benzil)-3-tiokso-2,3-dihidro[1, 2, 4]triazolo[4,3-a]pira-zin-8(7h)-ono kiekybinio nustatymo metodas naudo-jant nevandeninį potenciometrinį titravimą. Siūlomo metodo tinkamumas patvirtintas ištyrus pagrindines charakteristikas – tiesiškumą ir tikslumą.