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RESEARCH ARTICLE

Spectrophotometric determination of 2-[5-(furan-2-yl)-4-phenyl-4n-1,2,4-triazol-3-ylthio]-1-(4-chlorophenylethanone)

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ABSTRACT:
Currently, the potential of 1,2,4-triazole derivatives in the development of anticonvulsants is attracting special attention of researchers, as such compounds can modulate the activity of receptors responsible for the development of seizure syndrome. 1,2,4-triazole derivatives can affect the GABAergic system and ion channels, which play a key role in the mechanisms of seizure development. It is known that 2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenylethanone) has anticonvulsant activity. Therefore, it is extremely important to develop sensitive and specific methods of analysis on the way to introducing this substance. Development and validation of a simple, economical and eco-friendly UV-spectrophotometric method for the quantitative determination of 2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenylethanone). A working standard sample of 2-[5-(furan-2-yl)-4-phenyl-4n-1,2,4-triazol-3-ylthio]-1-(4-chlorophenylethanone), ethyl alcohol of grade chemically pure" and the following analytical equipment were used in the study: Specord 250 Plus spectrophotometer, ABT-120-5DM electronic balance, Sonorex Digitec DT100H ultrasonic bath, class A measuring glassware. The optimal parameters for the quantitative determination of 2-[5-(furan-2-yl)-4-phenyl-4n-1,2,4-triazol-3-ylthio]-1-(4-chlorophenylethanone were investigated - the solvent was chosen, the stability of the analyzed solutions in time was studied, and the concentration limits at which the Bouguer-Lambert-Beer law is observed were determined. It was found that the maximum value of light absorption of the substance under study is observed at a wavelength of 280 nm in the medium of ethyl alcohol. On the basis of the obtained data, a spectrophotometric method was developed for the quantitative determination of 2-[5-(furan-2-yl)-4-phenyl-4n-1,2,4-triazol-3-ylthio]-1-(4-chlorophenylethanone by the standard method. The linear dependence within the range of application of the proposed method (80-120%) was estimated. The calculated metrological characteristics confirm the linearity of the method in the concentration range of the substance under study from 0.64 to 0.96 mg/100 ml. The possibility of using the spectrophotometric method in other laboratories with obtaining reliable results by predicting the total uncertainty of the method was proved. As a result of the study, a simple and reliable spectrophotometric method for the quantitative determination of 2-[5-(furan-2-yl)-4-phenyl-4n-1,2,4-triazol-3-ylthio]-1-(4-chlorophenylethanone) by intrinsic absorption was developed. The results were statistically processed, which indicates a high reproducibility of the developed method. The practicality of the proposed method and minimal environmental impact were confirmed using modern analytical tools.

KEYWORDS: Spectrophotometry, 1,2,4-triazole derivatives, assay, epilepsy.

INTRODUCTION:

Epilepsy and other seizure disorders remain one of the pressing challenges in modern medicine, as a significant number of patients fail to achieve adequate seizure control with currently available medications [1, 2]. In this context, the development of new compounds with anticonvulsant activity has become an important area of focus in pharmaceutical chemistry. 1,2,4-Triazole and its derivatives are well known for their broad spectrum of biological activity, including anti-inflammatory, antibacterial, antifungal, and anticancer properties, while

also exhibiting low toxicity [3–7]. Particular interest has been directed toward their potential as anticonvulsant agents, as these compounds may modulate receptor activity involved in the development of seizure syndromes. 1,2,4-Triazole derivatives can influence the GABAergic system and ion channels, which play a crucial role in the pathophysiology of seizures. As previously noted, 2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone exhibits anticonvulsant activity. This compound may help reduce side effects compared to traditional anticonvulsants such as carbamazepine or valproic acid. Its structural characteristics may provide improved pharmacokinetics, potentially prolonging the duration of drug action (Figure 1). When combined with other agents, this may lead to enhanced therapeutic outcomes.

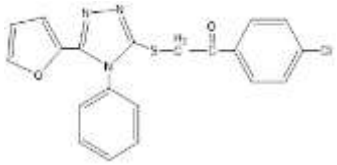

Figure 1. Structural formula of 2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone.

The development of a spectrophotometric method for determining 2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone is a critical step toward the implementation and wider use of this compound. Every new biologically active substance requires the creation of sensitive and specific analytical methods that enable its quantitative determination in various environments—including in pure form, biological fluids, and pharmaceutical formulations. Spectrophotometry is one of the most suitable and reliable methods for this purpose. It stands out for its accessibility, requiring fewer reagents compared to techniques such as HPLC or mass spectrometry, and it does not demand complex or expensive equipment [8, 9]. Spectrophotometry also enables rapid determination of compound concentrations across different media, as well as the assessment of stability and optimal storage conditions [10, 11].

MATERIALS AND METHODS:

Object of study: Working standard sample of 2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone.

Solvent: ethanol, analytical grade (pure for analysis).

Analytical equipment: Specord 250 Plus spectrophotometer, ABT-120-5DM electronic balance, Elmasonic E60/H ultrasonic bath, class A volumetric glassware

The study was conducted at the Educational and Scientific Medical Laboratory Center of the Zaporizhzhia State Medical and Pharmaceutical University.

QUANTITATIVE DETERMINATION PROCEDURE FOR THE ANALYZED COMPOUND:

An accurate sample of the substance (0.0200 g) is placed into a 250.0ml volumetric flask, dissolved using an ultrasonic bath for 1minute, then brought to volume with ethyl alcohol and thoroughly mixed. From this stock solution, 1.00ml is transferred into a 10.00ml volumetric flask, brought to volume with ethyl alcohol, and mixed well. The optical density is measured against the solvent at a wavelength of 284nm.

RESULTS AND DISCUSSION:

To develop the spectrophotometric method, optimal parameters for the quantitative determination of 2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone were established. When selecting a solvent, factors such as solubility of the compound, availability, and environmental safety were considered. The solvents tested included ethyl alcohol, methyl alcohol, acetonitrile, and DMF (dimethylformamide).

Ethyl alcohol was selected as the most suitable solvent. The maximum absorbance was observed at a wavelength of 284 nm (Figure 2).

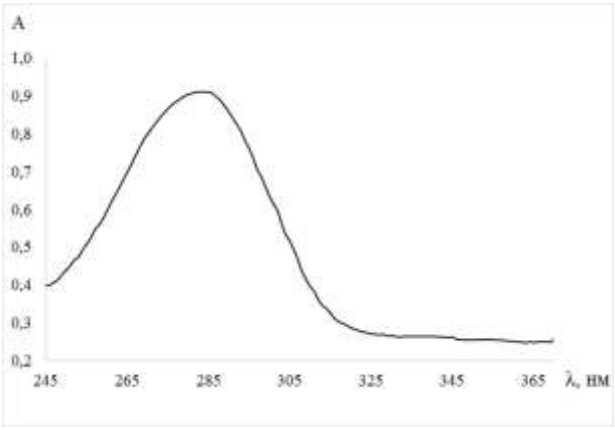

Figure 2. Absorption spectrum of 2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone.

At the next stage of the study, the stability of the analyzed solution over time was evaluated. To assess this, the optical density of the solution was measured every 15minutes over the course of 1 hour. The relative standard deviation (RSDt%) and confidence interval (Δt%) were calculated. The obtained data are presented in Table 1.

Table 1. Stability of the analyzed solution over time

t, хв	0	15	30	45	60	Середнє	RSD_t%	$Δ_t$%	*max δ, %*

A	0,9028	0,9005	0,9059	0,8992	0,9033	0,9023	0,285	0,61	0,64

Table 2. Specific absorbance values and concentration range within which the Beer–Lambert–Bouguer law is observed

Compound	*λ_{max}, nm*	*Concentration range, mg/100 ml*	*Specific absorbance (A_{1%1cm})*
2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone	284	0,64 – 0,96	***1041±15***

During the study, the specific absorbance value was also determined, and the concentration range within which the compound follows the Beer–Lambert–Bouguer law was established. The results are presented in Table 2.

Based on the obtained results, a spectrophotometric method for the quantitative determination of 2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone was successfully developed.

According to the proposed method, nine measurements were carried out within the specified range: three concentrations were analyzed, each with three replicate determinations, using the standard method. The results were expressed as percentages, and the following statistical parameters were calculated: mean value ($\hat{Z}$, %), relative standard deviation (Sz, %), relative confidence interval (Δ%,) The results are presented in Table 3.

Table 3. Results of the quantitative determination of the analyzed compound

Analyzed compound	*Metrological Characteristics*			
	$\bar{Z}$*%*	*S_z%*	*Δ%*	*max*Δ_{As}*%*
2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone	100,24	0,77	***1,43***	***2,0***

The assessment of linearity for the developed method was carried out by diluting the stock solution and measuring the optical density within the application range of the method (80–120%). A graph of absorbance versus concentration of the analyzed compound was plotted in normalized coordinates (Figure 3).

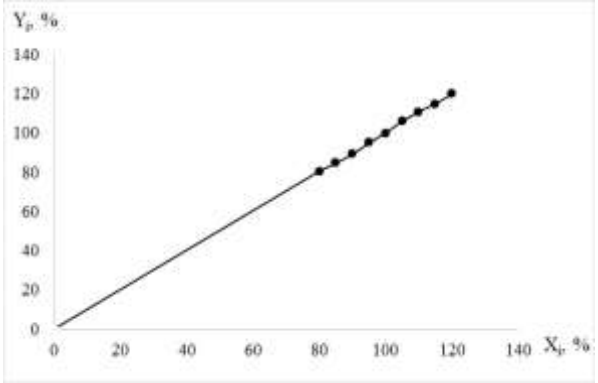

Figure 3. Graph of the dependence of optical density on the concentration of the analyzed compound.

The method of least squares was used to process the obtained results (Table 4), which confirm that the method meets the acceptability criteria established by the State Pharmacopoeia of Ukraine (SPhU) [12].

Table 4. Metrological characteristics of the linear dependence

Parameter	Value	Criteria	Conclusion
$b \pm (s_b)$	0,9935±(0,0153)	–	–
$a \pm (s_a)$	0,5199±(1,5542)	$\lvert a \rvert \leq \Delta a = t(95\%;7) \cdot s_a = 2{,}945$	complies
$s_{x,0}$(%)	0,6034	$\leq \Delta_{As}(\%)/t(95\%;7) = 1{,}056$	complies
r	0,9991	≥ 0,9970	complies

The calculated limit of detection (LOD) and limit of quantification (LOQ) demonstrate a "margin of robustness" for the proposed method:
LOD (MB) = 3.3 × Sa / b = 3.3 × 1.5542 / 0.9935 = 5.16
LOQ (MKB) = 10 × Sa / b = 10 × 1.5542 / 0.9935 = 15.64

To confirm the reproducibility of the developed method in other laboratories, a forecast of the total uncertainty was performed [13]. The calculations of sample preparation uncertainty were carried out in accordance with the requirements of the State Pharmacopoeia of Ukraine (SPhU) concerning the maximum permissible errors for volumetric glassware, balances, and instruments. As a result, it was found that the greatest uncertainty in sample preparation is contributed by steps 1 and 3 (Figure 4).

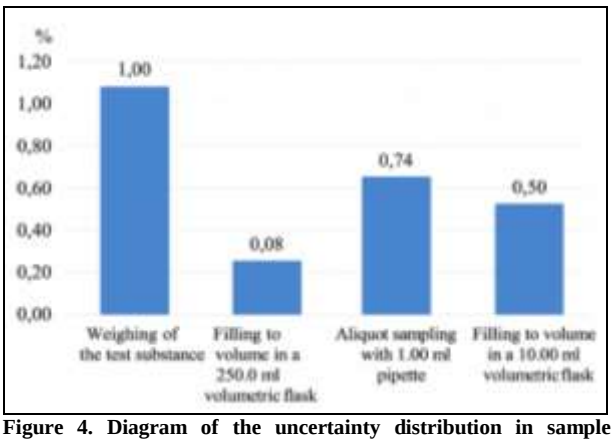

Figure 4. Diagram of the uncertainty distribution in sample preparation for the quantitative determination method of the analyzed compound.

The sample preparation uncertainty (ΔSP) and the predicted total uncertainty of the analysis results were calculated. The results showed that the forecasted uncertainty does not exceed the maximum permissible uncertainty of analysis results (max ΔAs = 2.0%), confirming the acceptability and robustness of the developed method:

$$\Delta_{SP} = \sqrt{1{,}00^2 + 0{,}08^2 + 0{,}74^2 + 0{,}50^2} = 1{,}34\%$$

$$\Delta_{As} = \sqrt{\Delta_{SP}^2 + \Delta_{FAO}^2} = \sqrt{1{,}34^2 + 0{,}70^2} = 1{,}51\%$$

Thus, the developed method can be successfully applied for the analysis of the investigated compound in other laboratories, ensuring the reliability and accuracy of the results obtained.

ASSESSMENT OF THE ENVIRONMENTAL FRIENDLINESS AND PRACTICALITY OF THE DEVELOPED METHOD:

To evaluate the environmental sustainability of the developed analytical method, the Analytical GREEnness calculator (AGREE) software was used. This tool quantitatively assesses parameters such as reagent consumption, waste generation, energy use, and impact on human health/environment, based on the 12 principles of green chemistry. The outcome of the assessment is a pictogram with an overall score from 0 to 1, where higher values indicate a more environmentally friendly analytical procedure [14]. The AGREE score for the proposed method was 0.78, indicating a minimal environmental impact (Figure 5).

Figure 5. Result of the environmental assessment of the developed method using the AGREE tool

Using the Blue Applicability Grade Index (BAGI) software, the analytical practicality of the proposed method was assessed. The BAGI tool introduces a novel metric that takes into account factors such as simplicity, speed, cost-effectiveness, and ease of use. The assessment output is presented as an asteroid-shaped pictogram, with a numerical score displayed in the center, ranging from 25 to 100. A score of 60 or above is considered the threshold for a method to be deemed "practical"[15]. The obtained score of 80.0 indicates a satisfactory level of practicality of the developed method (Figure 6).

Figure 6. Result of the practicality assessment of the developed method using the BAGI tool

CONCLUSIONS:

- The feasibility of using spectrophotometry for the development of quantitative determination methods for 1,2,4-triazole derivatives has been demonstrated.
- Optimal parameters for the quantitative determination of 2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone were established, including solvent selection, assessment of solution stability over time, and identification of the concentration range within which the Beer–Lambert–Bouguer law is obeyed.
- A simple, economical, and reliable spectrophotometric method for the quantitative determination of the compound based on its intrinsic absorbance was developed.
- Statistical analysis of the obtained results confirmed the high reproducibility of the developed method.
- The environmental friendliness and practicality of the method were validated using the AGREE and BAGI tools.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

PROSPECTS FOR FURTHER RESEARCH:

The developed method can be applied to the quantitative determination of 2-[5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone in pharmaceutical formulations, as well as for stability studies, dissolution testing, and other quality control procedures.

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analysis," state registration No. 0123U103937 (2023–2028).

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