

UDC 615.213:658.562.47:547.791.1

# DEVELOPMENT OF THE METHODS FOR IDENTIFICATION AND QUANTITATIVE DETERMINATION OF EPIFINE – A NEW PERSPECTIVE ANTICONVULSANT

L.O.Perekhoda

National University of Pharmacy

61002, Kharkiv, 53, Pushkinska str. E-mail: medchem@ukrfa.kharkov.ua

*Key words: pharmaceutical analysis; identification; assay; anticonvulsant*

According to the results of the pharmacological testing the substance of 4-methoxy-3-chloroanilide 1-(3'-fluorophenyl)-5-methyl-1,2,3-triazole (1H)-4-carboxylic acid has shown a pronounced anticonvulsant activity that exceeds the level of the reference drug – Depakine. It is recommended for further profound studies under conditional name Epifine. Development of methods for the quality control of a substance occupies an important place among the stages of drug introduction. For preparation of the substance to introduction we have developed the methods for identification and quantitative determination of the promising substance Epifine. Standardization has been performed in accordance with the existing requirements for development of modern methods for analysis of pharmaceutical substances possessing the pharmacopoeial quality. For identification a set of physical, physico-chemical (NMR <sup>1</sup>H, IR and UV spectroscopy) and chemical methods has been proposed. The physical and chemical properties of Epifine have been investigated, and the intervals of melting point and solubility in different solvents have been determined. As expected, in the IR spectrum the characteristic absorption bands confirming the presence of aromatic rings, in particular benzene and triazole, carbonyl groups (amide-1, amide-2), substituted amino group, methyl and methoxy groups, were observed. We have also proposed the chemical methods of identification of this compound conditioned by the presence of the anilide residue, the triazole ring, and covalently bound halogens. The non-aqueous acid-base titration has been developed for the quantitative analysis of Epifine.

## РОЗРОБКА МЕТОДИК ІДЕНТИФІКАЦІЇ ТА КІЛЬКІСНОГО ВИЗНАЧЕННЯ ЕПІФІНУ – НОВОГО ПОТЕНЦІЙНОГО АНТИКОНВУЛЬСАНТУ

Л.О.Перехода

**Ключові слова:** фармацевтичний аналіз; ідентифікація; аналіз; антиконвульсант

За результатами фармакологічних випробувань субстанція 4-метокси-3-хлораніліду 1-(3'-фторофеніл)-5-метил-1,2,3-триазол(1H)-4-карбонової кислоти виявила високу протисудомну активність, що перевищує рівень препарату порівняння Делакіну, тому вона була рекомендована для подальших поглиблених досліджень під умовною назвою «Епіфін». Серед етапів впровадження лікарських засобів важливе місце посідає розробка методик контролю якості субстанції. Для підготовки речовини до впровадження нами було здійснено розробку методик ідентифікації та кількісного визначення. Стандартизацію здійснювали відповідно до існуючих сучасних вимог до розробки методик аналізу фармацевтичних субстанцій фармакопейної якості. Досліджені фізико-хімічні властивості субстанції Епіфін, встановлені інтервали температури плавлення та розчинності у різних розчинниках. Для ідентифікації запропоновано сукупність фізичних, фізико-хімічних (ІЧ-, УФ- та ЯМР <sup>1</sup>H-спектроскопія) та хімічних методів. В ІЧ-спектрі очікувано спостерігаються характеристичні смуги поглинання, що підтверджують наявність ароматичних кілець, зокрема фенільного та триазольного; карбонільної групи (амід-1, амід-2), заміщеної аміногрупи, метильної та метоксигруп. Запропоновані хімічні методики ідентифікації цієї сполуки, обумовлені наявністю триазольного кільця, анілідного залишку, ковалентноз'язаних галогенів. Для кількісного визначення Епіфіну розроблений метод кислотно-основного титрування у неводному середовищі.

## РАЗРАБОТКА МЕТОДИК ИДЕНТИФИКАЦИИ И КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ЭПИФИНА – НОВОГО ПОТЕНЦИАЛЬНОГО АНТИКОНВУЛЬСАНТА

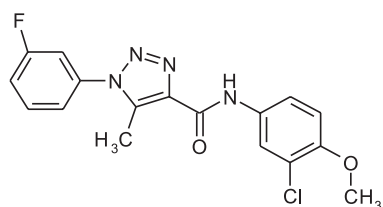
Л.А.Перехода

**Ключевые слова:** фармацевтический анализ; идентификация; анализ; антиконвульсант

По результатам фармакологических испытаний субстанция 4-метокси-3-хлоранилида 1-(3'-фторфенил)-5-метил-1,2,3-триазол (1H)-4-карбоновой кислоты показала выраженную противосудорожную активность, превышающую уровень препарата сравнения Делакина, поэтому она была рекомендована для дальнейших углубленных исследований под условным названием «Эпифин». Среди этапов внедрения лекарственных средств важное место занимает разработка методик контроля качества субстанции. Для подготовки вещества к внедрению нами была осуществлена разработка методик идентификации и количественного определения. Стандартизацию осуществляли в соответствии с существующими современными требованиями к разработке методик анализа фармацевтических субстанций фармакопейного качества. Были исследованы физико-химические свойства субстанции Эпифин, установлены интервалы температуры плавления и растворимость в различных растворителях. Для идентификации нами предложена совокупность физических, физико-химических (ЯМР <sup>1</sup>H, ИК- и УФ-спектроскопия) и химических методов. В ИК-спектре, как и ожидалось, наблюдаются характеристические полосы поглощения, подтверждающие наличие ароматических колец, в частности, фенильного и триазольного; карбонильной группы (амид-1, амид-2), замещенной аминогруппы, метильной и метоксигрупп. Также нами предложены химические методики идентификации этого соединения, обусловленные наличием триазольного кольца, анилидного остатка, ковалентно связанных галогенов хлора и фтора. Для количественного определения Эпифина разработан метод кислотно-основного титрования в неводной среде.

According to the results of the pharmacological testing the substance of 4-methoxy-3-chloroanilide 1-(3'-fluorophenyl)-5-methyl-1,2,3-triazole (1*H*)-4-carboxylic acid has shown a pronounced anticonvulsant activity that exceeds the level of the reference drug – Depakine. It is recommended for further profound studies under conditional name Epifine [1, 2].

Development of modern, unified, effective, and simple in performance methods of standardization of substances is one of the main tasks in pharmaceutical science. The current understanding of approaches to quality assurance is based on the concept that includes quality assurance of drugs from the stage of pharmaceutical development and research, through proper production, quality control, storage, sale, and provision of information to doctors and patients [3]. When introducing drugs the stage of development of the quality control procedures takes an important place. For preparation of the substance to introduction we have developed the methods for identification and quantitative determination of the promising substance Epifine. Standardization has been performed in accordance with the existing requirements for development of modern methods for analysis of pharmaceutical substances possessing the pharmacopoeial quality [4] using the standard sample of the given compound. While performing the research on identification of the substance the standard sample of Epifine repeatedly subjected to crystallization was considered as an example of this substance. Its purity and identity of Epifine was confirmed by a set of instrumental methods of analysis (NMR <sup>1</sup>H, IR and UV spectroscopy, TLC, HPLC).



For Epifine identification a set of physical, physicochemical (NMR <sup>1</sup>H, IR and UV spectroscopy) and chemical methods has been proposed; these methods allow to assess fully the nature of the substance and confirm it. In the process of our research the physical and chemical properties of Epifine were studied, and the intervals of melting point and solubility in different solvents were determined. To determine solubility standard pharmacopoeial methods were used. By its physical properties Epifine is a white crystalline powder, easily soluble in dimethylformamide, which melts at the temperature of 146-147°C. It is also soluble in dioxane and hot ethanol; however, it is insoluble in water and chloroform.

The next step was to study the possibility of Epifine identification by means of spectrophotometry in the range of IR and UV spectra, and NMR <sup>1</sup>H spec-

troscopy. For this purpose the corresponding spectra were analyzed. The absorption of the substance in the infrared region makes it possible to identify functional groups and fragments in the structure. By analyzing the infrared spectrum of Epifine a number of characteristic bands that occur due to the presence of certain features of the chemical structure can be observed. As expected, in the IR spectrum the characteristic absorption bands confirming the presence of aromatic rings, in particular benzene and triazole, carbonyl groups (amide-1, amide-2), substituted amino group, methyl and methoxy groups were observed [5]. In accordance with the generally accepted principles of interpretation of IR-spectra it has been determined that the strong absorption band intensity in the 1645 cm<sup>-1</sup> corresponds to the valence vibrations of carbonyl in position 2 ( $\nu$  C=O, amide I); the medium intensity band of deformation vibrations of the substituted amino group is at 3220 cm<sup>-1</sup> ( $\delta$  NH); the medium intensity absorption bands of the aromatic bonds C-H( $\nu$  CH) is at 3060 cm<sup>-1</sup>. The medium and strong intensity band caused vibrations by the aromatic ring present at the area of 1615-1500 cm<sup>-1</sup>. Symmetric valence vibrations of the methyl group observed in the area of 1470-1435 cm<sup>-1</sup> and at 2956 cm<sup>-1</sup> are asymmetric valence vibrations of the same group. Thus, we have concluded that the infrared spectrum of the substance proves its structure. The presence of aromatic 1,2,3-triazoles fragments and substituted aryl rings allows to use spectroscopy in the ultraviolet range of the spectrum for identification. The UV spectrum of 0.01% Epifine has two maxima of absorption at wavelengths of 228 and 282 nm (Fig.) [6].

Due to the complete substitution of the triazole ring when adding acids or alkalis redistribution of bonds is not observed; as a result, the character of the spectrum does not change. Therefore, it is recommended to assess the presence of the corresponding peaks in the spectrum, namely the wavelengths of (228±2) nm and (282±2) nm for introduction in the project of quality control procedures for Epifine substance.

The NMR<sup>1</sup>H spectrum of 4-methoxy-3-chloroanilide 1-(3'-fluorophenyl)-5-methyl-1,2,3-triazole (1*H*)-4-carboxylic acid contains all signals of hydrogen containing groups, which are characteristic for this substance. Protons of the amide group appear as a singlet signal at 10.50 ppm, and aromatic proton signals – as a multiplet at 6.94-7.85 ppm. Protons of the methoxy group and the methyl group in position 5 of the triazole cycle are observed in the NMR<sup>1</sup>H spectrum as a singlet at 3.83 ppm and 2.52 ppm, respectively [7, 8].

It should be noted that in the case of Epifine NMR<sup>1</sup>H spectroscopy does not provide information about basic pharmacophore – 1,2,3-triazoles ring, which has no protons as a result of introduction of substituents.

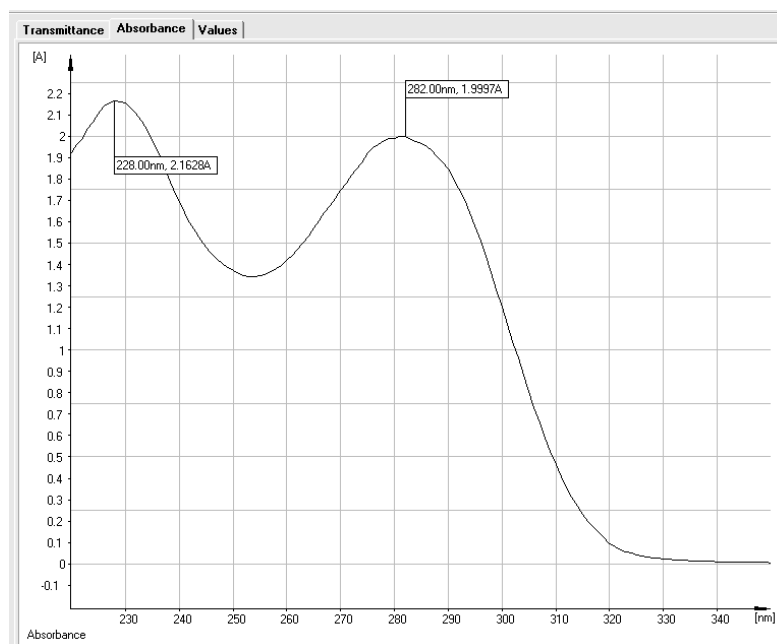


Fig. The UV absorption spectrum of Epifine.

The absence of equipment at the plant or in the quality control laboratory makes the use of this method currently unreal.

More convincing for identification of organic compounds is a combination of physical, physicochemical and chemical methods based on the properties of functional groups. Considering the Epifine structure it was logical to develop the methods for identification of this compound due to the presence of the anilide residue, the triazole ring, and covalently bound halogens (chlorine and fluorine).

Three tertiary nitrogen atoms in the molecule give a reason to expect precipitation or formation of coloured complex compounds with the general "precipitative" alkaloid reagents. The ability to form complex compounds in this case is predetermined by the presence of the undivided pair of electrons in nitrogen, which are the part of the heterocyclic triazole fragment. We used a variety of reagents and methods given in the State Pharmacopoeia of Ukraine [9]. Depending on the use of certain excipients for preparing the methods of the quality control any reagent from the SPhU can be used. They are not specific, and the result of reactions observed can characterize many nitrogen-containing organic compounds.

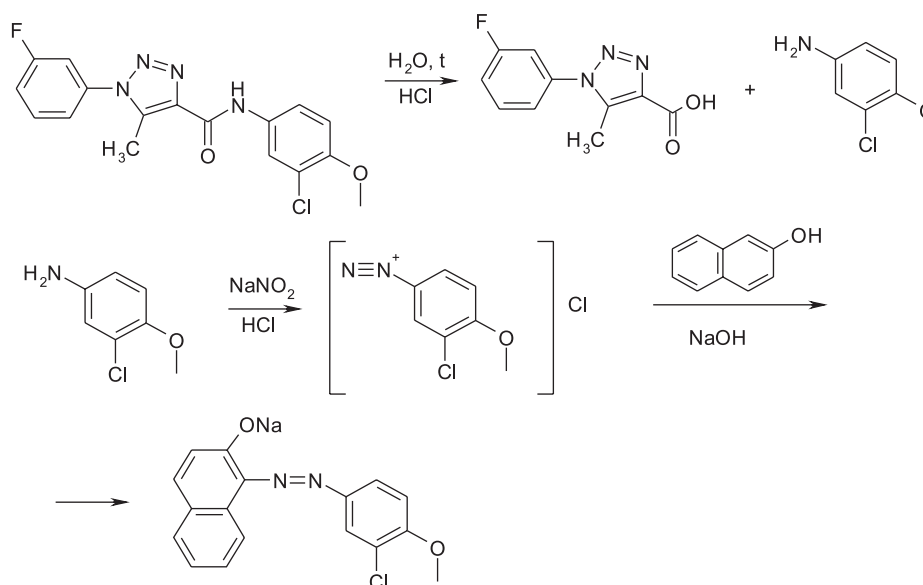
Taking into account the general monograph of the SPhU, we consider it appropriate to use potassium bismuth iodide as a reagent that is universal to determine the tertiary nitrogen. Considering the fact that a substituted anilide of chloroacetic acid was used as a starting material in the synthesis of Epifine it was logical to try to reproduce the reaction of the primary aromatic amino group after hydrolysis. To confirm the presence of the anilide residue in the molecule of Epifine the substance was subjected to acid hydrolysis, and 4-methoxy-3-chloroaniline formed in

the reaction was identified by the reaction of diazotization with the subsequent azo coupling (Scheme 1).

This test can be recommended for inclusion in the project of the methods for the quality control. To identify covalently bound halogens the reaction of chlorides and fluorides were carried out after the preliminary dry mineralization by heating the substance with anhydrous sodium carbonate and potassium nitrate. After mineralization the covalent bond is destroyed, halogens as halides are determined by ordinary reactions. Chloride ion forms a white precipitate, which dissolves in ammonia solution in the reaction with silver nitrate solution in the presence of dilute nitric acid. To determine fluoride we used the reaction with the solution of calcium chloride (with formation of a white precipitate). These reactions we recommended for inclusion into the project of the methods for the quality control. Based on the acidic properties of the amide group for quantitative determination of Epifine the acid-basic titration in the non-aqueous medium was developed. To increase the acidic properties of the substance the titration was carried out in the presence of dimethylformamide – a basic solvent increasing the acidic properties of compounds. In addition, the substance studied is easily soluble in DMF.

Pharmacopoeial solution of 0.1 M sodium methylate that standardized according to the requirements of the SPhU was selected as a titrant. The assay was performed by the reaction (Scheme 2).

To select the indicator the potentiometric titration was carried out, and inflexion points of titration (pH = 12.5) were determined. Then the experiment was repeated in the presence of the thymol blue indicator. It was found that the colour of the indicator changed within the limit inflexion point of titration, and



Scheme 1

the drop error is negligible. It was experimentally proved that 4-methoxy-3-chloroaniline 1-(2'-fluorophenyl)-5-methyl-1,2,3-triazoles (1*H*)-4-carboxylic acid reacted with sodium methylate in equimolar quantities. According to our research the acid-basic titration in the non-aqueous medium gave reproducible results close to the nominal value. The results of the quantitative determination of Epifine and metrological characteristics of this method are given below:  
 $m_1 = 0.10040$  g,  $m_2 = 0.10070$  g,  $m_3 = 0.10100$  g  
 $K_{0.1M} = 0.9990$

Found, % 99.90; 100.10; 100.20;

Metrological characteristics of the average result:  
 $\bar{x} = 99.95$ ;  $S^2 = 0.4864$ ;  $S = 0.5021$ ;  $S_{\bar{x}} = 0.2341$ ;  
 $\Delta x = 1.2140$ ;  $\Delta \bar{x} = 0.7372$ ;  $\varepsilon = 1.70\%$

Metrological characteristics demonstrate the ability to use this method and its inclusion into the project of methods for the quality control. The preliminary certification of the standard sample of Epifine is required for complete validation of the method.

### Experimental Part

The NMR spectrum was recorded on a Varian Mercury-VX-200 device, the solvent – DMSO –  $D_6$ , the internal standard – tetramethylsilane (TMS). The chemical shifts are given in the scale  $\delta$  (ppm).

#### Identification (IR spectroscopy)

The infrared absorption spectrum of the substance (previously dried to the constant mass) in disks with potassium bromide *R* (1 mg of the substance rubbed with 200 mg of potassium bromide *R*) in the

range from  $400\text{ cm}^{-1}$  to  $4000\text{ cm}^{-1}$  was recorded on a "Specord M-80" device.

#### Identification (UV spectroscopy)

Place 0.040 g of the substance into a 200 ml volumetric flask, dissolve while heating in 80 ml of ethanol *R*, cool, dilute the volume of the solution with ethanol *R* and mix. Place 10 ml of the solution obtained into a 100 ml volumetric flask and dilute with ethanol *R* and mix (Solution A). Place 1.5 ml of the solution obtained into a 10 ml volumetric flask and dilute with ethanol *R*, then mix (Solution B). Measure the optical density of the solution obtained at the wavelength of 282 nm in a cuvette with a 10 cm layer thickness using ethanol *R* as a reference solution.

#### The reaction with potassium bismuth iodide

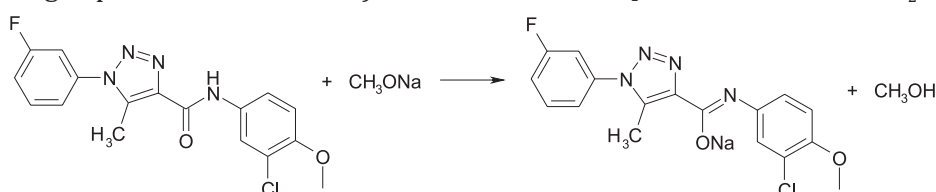
Dissolve 0.01 g in 5 ml of dilute hydrochloric acid and add 1 ml of potassium bismuth iodide solution. Immediately an orange-yellow precipitate is formed.

#### The reaction of azo coupling

Dissolve 0.1 g of the substance in 2 ml of water, add 2 ml of dilute hydrochloric acid and heat for 5 min on a water bath. After cooling add to the reaction mixture 0.2 ml of sodium nitrite and in 1-2 min add 1 ml of  $\beta$ -naphthol; an intense red precipitate is formed.

#### Determination of fluorine and chlorine

To 0.1 g of the substance add the mixture for sintering (the mixture of sodium or potassium carbonate with potassium nitrate), cool, add 5 ml of water and filter. To the filtrate add nitric acid *R* till carbon (IV) oxide stops evolving. The solution obtained divide in two parts. Add 2 ml of  $\text{CaCl}_2$  to the first part, a



Scheme 2

white precipitate is formed. Acidify the second part with dilute nitric acid *R* to pH 5 and add 0.2 ml of silver nitrate solution *R1*. A white precipitate is formed which is soluble in the excess of ammonia solution *R*.

**Assay.** Dissolve 0.300 g of the test substance in 10 ml of DMF previously neutralized by thymol blue and titrate with 0.1 M sodium methylate solution with the same indicator till blue colour appears.

Calculation of the quantitative content of active substance was performed by the formula:

$$\% = \frac{V \cdot K \cdot T \cdot 100 \cdot 100}{m_n \cdot (100 - \%_{\text{moisture}})},$$

where: *V* – is the volume of 0.1 M sodium methylate used for titration, ml; *K* – is the adjustment coefficient; *T* – is the titre of the test substance with 0.1 M sodium methylate; %<sub>moisture</sub> – is the percentage of moisture.

#### References

1. Gluschenko A. V., Rybalchenko T. L., Shtrigol S. Yu., Georgiyants V. A., Perekhoda L. O. *Ukr. biopharmats. Jrn.*, 2010, No.3(8), pp.28-34.
2. Georgiyants V. A., Gluschenko A. V., Perekhoda L. O., Zaremba O. V. *Zaporizkii Medical Journal*, 2007, No.3(42), pp.91-94.
3. Lyapunov N. A., Solovyov A. S., Stetsiv V. V. *Yezhenedelnik Apteka (Pharmacy Weekly)*, 2012, No.826 (4).
4. Breinmaier E. *Structure Elucidation by NMR in Organic Chemistry*, John Wiley @ Sons Ltd, Chichester, 2002, 258 p.
5. Perekhoda L. O. *Molecular modeling and purposeful synthesis of anticonvulsants among derivatives of five-membered di(three)azaheterocycles*, Manuscript. The thesis for the Doctor degree in Pharmacy, speciality 15.00.02 – Pharmaceutical Chemistry and Pharmacognosy, National University of Pharmacy, Kharkiv, 2013, 330 p.
6. Braun D. *Spectroscopy of organic substances*, M.: Mir, 1992, 300 p.
7. Silverstein R. M. *Spectrometric Identification of Organic Compounds – 6th ed.*, N.Y.: John Wiley & Sons, 2001, 196 p.
8. *State Pharmacopoeia of Ukraine. 1th ed.*, Kh. SOE Scientific Expert Pharmacopeial Centre, 2001, 556 p.
9. *State Pharmacopoeia of Ukraine. 1th ed., 2 revised*, Kh. SOE Scientific Expert Pharmacopeial Centre, 2008, 620 p.

Надійшла до редакції 30.03.2015 р.

1 ml of 0.1 M sodium methylate is equivalent to 36.08 mg of C<sub>17</sub>H<sub>14</sub>ClFN<sub>4</sub>O<sub>2</sub>, which should be in the range from 99.9% to 100.2% calculated with reference to the dried substance.

#### Conclusions

1. The spectral characteristics of the substance have been studied and methods of identification using UV and IR spectroscopy have been proposed.

2. The Epifine behaviour in chemical reactions has been studied and the methods of identification have been proposed. They are azo dye formation after hydrolysis, the reaction on the tertiary nitrogen with potassium bismuth iodide, and the reaction on halogens after mineralization.

3. The quantitative determination of Epifine in the non-aqueous medium by the acid-base titration has been developed.