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Research Article

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Substantiation of the methods of quality control for a substance with the anticonvulsant action

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ABSTRACT

The data concerning substantiation and development of the methods of quality control for a new biologically active substance with the anticonvulsant action are presented in the article. Identification of the substance under research has been performed by physical and chemical methods (IR-, UV- and PMR-spectroscopy) and by chemical reactions. For quantitative determination of dimetpyrazine the method of acid-base titration in the non-aqueous medium has been developed. The relative uncertainty of the average is 0.51%.

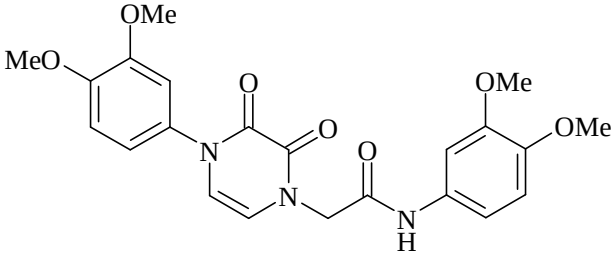
Key words: Pharmaceutical analysis; Identification; Quantitative determination; Acid-base titration; Anticonvulsants.

INTRODUCTION

A great number of modern medicines are heterocyclic compounds, among them derivatives of diazine, namely folic acid, riboflavin, tetrahydropterin, xanthopterin are widely used [1]. Currently the synthesis of compounds in series of diazine is in progress, and their pharmacological properties are studied [2,3,4]. The researchers of the National University of Pharmacy carry out the search of promising biologically active substances among derivatives of diazine [5].

In the process of our work [6] it has been found that 3,4-dimethoxyphenylamide-4-[(3,4-dimethoxyphenyl)-2,3-dioxo-1,4-dihydropyrazine-1-yl]acetic acid exhibits the anticonvulsant activity and is promising in terms of creating a new medicine on its basis. Development of analytical documentation for the quality control of a biologically active substance is one of the necessary conditions of its introduction into medical practice.

The State Pharmacopoeia of Ukraine (SPhU) has certain requirements to development of analytical normative documentation for a substance [7]. We took them into account while studying physical and chemical properties of 3,4-dimethoxyphenylamide-4-[(3,4-dimethoxyphenyl)-2,3-dioxo-1,4-dihydropyrazine-1-yl]acetic acid (the conditional name is dimetpyrazine):



dimetpyrazine

The aim of the work was to develop the methods of quality control for analytical normative documentation in order to prepare the substance to introduction. Standardization was performed according to the current requirements for development of methods of analysis of pharmaceutical substances with the pharmacopoeial quality [7].

EXPERIMENTAL SECTION

Experiments were carried out using chromatographically pure sample of the substance (the impurities content was 0.5%). The measuring glassware of class A, reagents meeting the requirements of the SPhU, "AXIS" analytical balance, a "Specord M-40" spectrophotometer were used for the work. The IR-spectrum of the substance was recorded on a "Specord M-80" device in KBr tablets with the concentration of the substance of 1%. The NMR-spectrum was recorded on a Bruker DRx500 device, the solvent was DMSO–d_6, the internal standard was tetramethyl silane (TMS), the working frequency was 500 MHz. Chemical shifts are given in scale δ (ppm). The melting point was determined according to the requirements of the SPhU [7].

Identification
The IR absorption spectrum of the substance must correspond to the spectrum of the reference standard of 3,4-dimethoxyphenylamide-4-[(3,4-dimethoxyphenyl)-2,3-dioxo-1,4-dihydropyrazine-1-yl]acetic acid (SPhU, 2.2.24).
Dissolve 0.10 g of the substance in methanol *R*, add 10.0 ml of 0.1 M solution of sodium hydroxide and dilute the solution to 100.0 ml with methanol *R*. Dilute 1.0 ml of the solution obtained to 100.0 ml with methanol *R*. The UV-spectrum of absorption (2.2.25) of the solution obtained in the region from 250 nm to 380 nm must have the maximum at the wavelength of 285 nm and shoulder at the wavelength of 312 nm.

To 0.05 g of the substance add 2 ml of water *R*, 2 ml of hydrochloric acid *R* and heat on a water bath for 5 min. After cooling the solution obtained gives the reaction for primary aromatic amines (2.3.1) with formation of a red colour.

Place 0.05 g of the substance into a porcelain cup, moisten with 2 drops of concentrated nitric acid; a red-brownish colour appears.

Place 0.05 g of the substance into a porcelain cup, moisten with 2 drops of concentrated sulphuric acid; a bright yellow colour appears.

Assay. Dissolve 0.400 g of the test substance in 10 ml of dimethylformamide and titrate 0.1 M solution of sodium methylate with the same indicator till the blue colour.

Simultaneously the control experiment was performed.

One ml of 0.1 M solution of sodium methylate corresponds to 44.14 mg of $C_{22}H_{23}N_3O_7$, which must be from 98.5% to 101.5% calculated with reference to a dried substance (the loss on drying is not more than 0.5%).

RESULTS AND DISCUSSION

Physical, physicochemical and chemical properties were used for identification of the substance. By its physical properties dimetpyrazine is a white crystalline powder. It is readily dissolved in dimethylformamide, poorly dissolved in alcohol, insoluble in water.

The requirements to the melting range are generally accepted. The melting temperature of the substance under research is from 182 to 184 ^oC [7].

Recently mostly physical and chemical methods are used for identification of organic substances with the complex structure. The UV-, IR- and NMR-spectra of 3,4-dimethoxyphenylamide-4-[(3,4-dimethoxyphenyl)-2,3-dioxo-1,4-dihydropyrazine-1-yl]acetic acid were investigated in order to study possibilities of their application for identification. Due to the presence of conjugated double bonds dimetpyrazine can be identified by the method of spectroscopy in the ultraviolet region of the spectrum. 0.001% Solution of the substance in the mixture of methanol and 0.1 M solution of sodium hydroxide in the range from 250 nm to 380 nm must have the maximum at the wavelength of 285 nm and shoulder at 312 nm (Fig. 1).

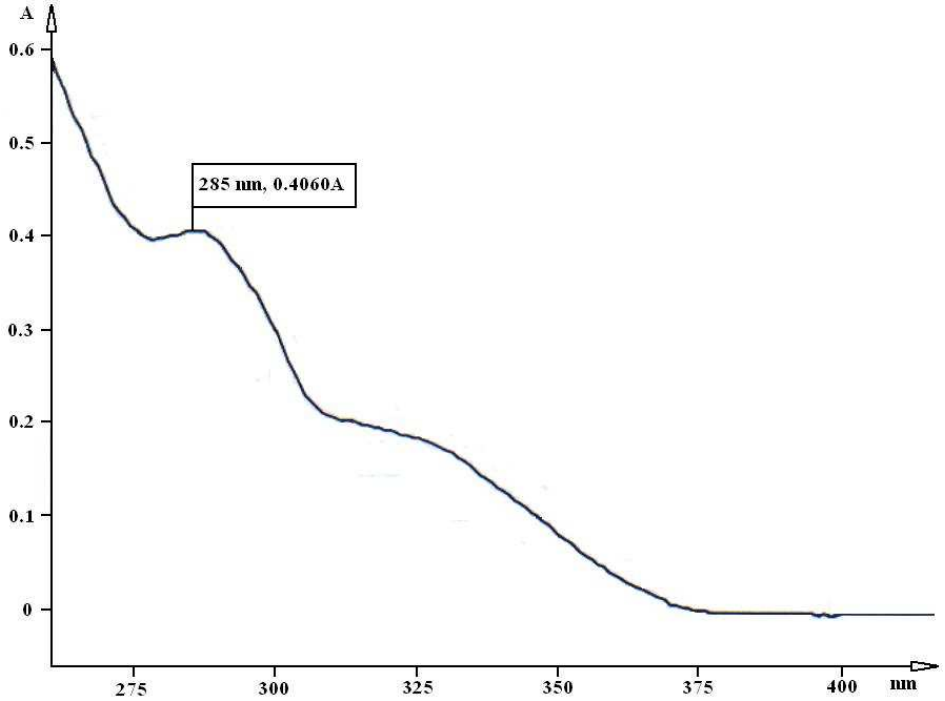


Fig. 1. The UV-spectrum of 3,4-dimethoxyphenylamide-4-[(3,4-dimethoxyphenyl)-2,3-dioxo-1,4-dihydropyrazine-1-yl]acetic acid (dimetpyrazine)

The most common method of substances identification is the method of absorption spectrophotometry in the IR region; we recommend to use it comparing to the pharmacopoeial reference standard (Fig. 2).

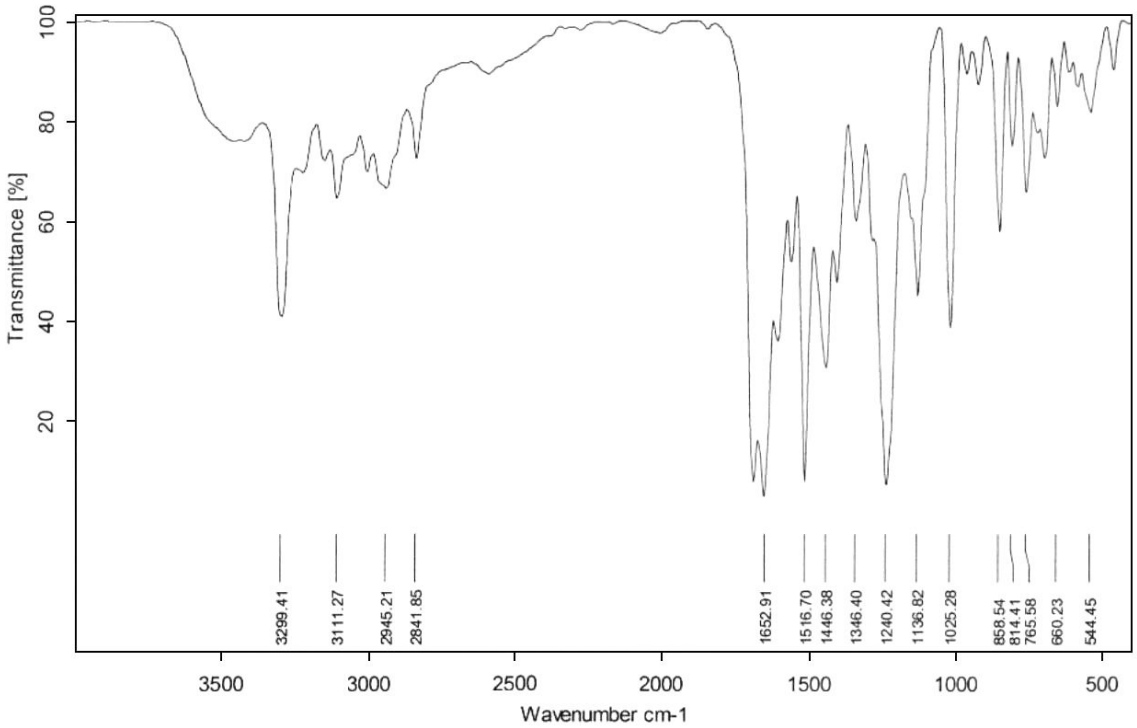


Fig. 2. The IR-spectrum of 3,4-dimethoxyphenylamide-4-[(3,4-dimethoxyphenyl)-2,3-dioxo-1,4-dihydropyrazine-1-yl]acetic acid (dimetpyrazine)

The IR-spectrum of absorption recorded in potassium bromide disks has characteristic bands due to the presence of stretching vibrations of NH-groups (3299 cm^{-1}), stretching vibrations of CO amide group (amide-I) (1653 cm^{-1}), bending vibrations of amide-II (1517 cm^{-1}), bending vibrations of methyl groups in methoxy groups (1446 cm^{-1}) bending vibrations of aromatic and heterocyclic CH (1240 cm^{-1}, 1025 cm^{-1}) (Fig. 2) [8,9].

The method of NMR spectroscopy is included to the SPhU, it can be also used for identification of medicinal compounds although it hardly can be considered routine. Besides, it is a reliable tool to confirm the structure at the stage of pharmaceutical development.

The NMR-spectrum of the substance under study is characterized by the presence of proton signals corresponding to the main structural fragments: a singlet signal of amide group protons at 10.18 ppm; proton signals of the aromatic ring as a multiplet in the region of 6.60-7.35 ppm; a singlet signal of methylene group protons at 4.60 ppm and a singlet signal of methoxy group protons at 3.78 ppm. All these signals have the appropriate intensity and allow confirming the structure objectively and in a certain way the absence of admixtures [10,11] (Fig. 3).

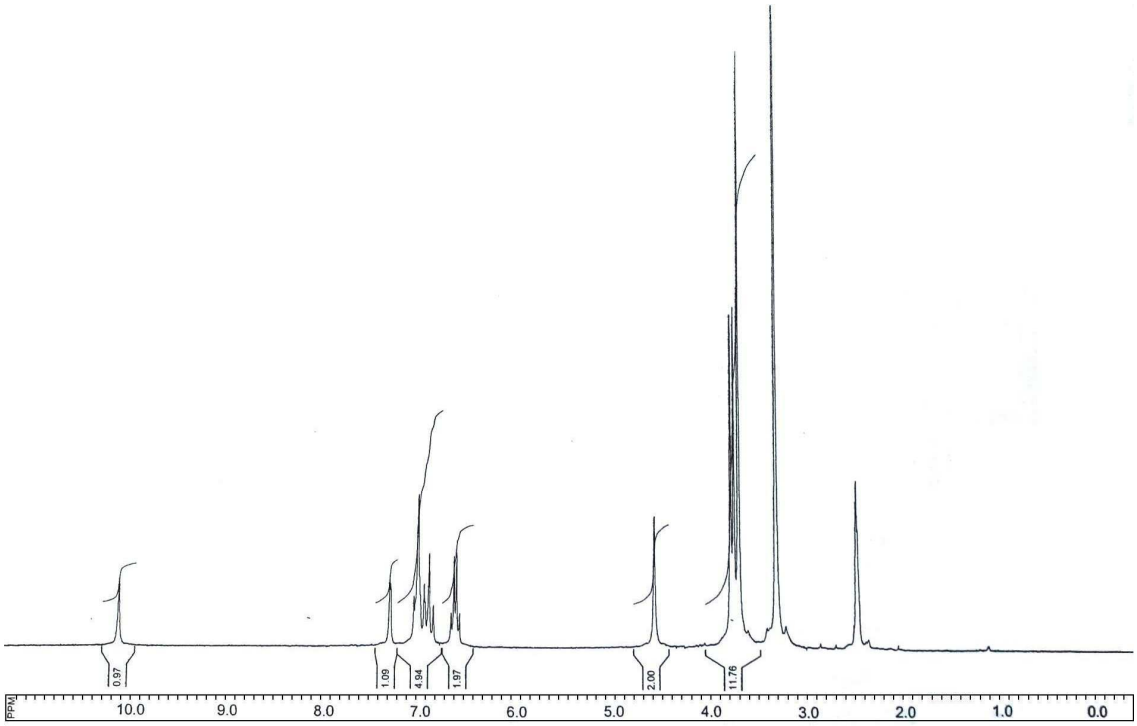


Fig. 3. The NMR-spectrum of 3,4-dimethoxyphenylamide-4-[(3,4-dimethoxyphenyl)-2,3-dioxo-1,4-dihydropyrazine-1-yl]acetic acid (dimetpyrazine)

The chemical methods of organic substances identification are based on determination of certain functional groups in the molecule. 3,4-Dimethoxyphenylamide-4-[(3,4-dimethoxyphenyl)-2,3-dioxo-1,4-dihydropyrazine-1-yl]acetic acid has in its structure phenyl radicals, methoxy groups and heterocyclic nitrogen atoms [12,13].

The presence of tertiary nitrogen atoms in the molecule of 3,4-dimethoxyphenylamide-4-[(3,4-dimethoxyphenyl)-2,3-dioxo-1,4-dihydropyrazine-1-yl]acetic acid causes reactions with special reagents (total alkaloid reagents). For example, under the action of concentrated nitric acid the substance becomes red-brownish in colour. Under the action of concentrated sulphuric acid a bright yellow colour is formed. The residue of 3,4-dimethoxyaniline in the compound was identified after acid hydrolysis by the reaction of diazotization with further azocoupling.

Among nonspecific impurities that are recommended to identify in the given substance there are heavy metals, iron and chlorides, as well as acidity and alkalinity of water extraction and residual amounts of dimethylformamide. Identification of these impurities is regulated by the State Pharmacopoeia of Ukraine [7].

The assay for dimetpyrazine was carried out by the method of acid-base titration in the non-aqueous medium due to the presence of two tertiary nitrogen atoms in the molecule. For intensification of basic properties the compound was dissolved in dimethylformamide and titrated with 0.1 M solution of sodium methylate using thymol blue as an indicator. Simultaneously the control experiment was performed.

The quantitative content of the active substance was calculated with reference to a dried substance.

Table 1 presents the results obtained and the metrological characteristics of the method.

Table 1. Quantitative determination of dimetpyrazine by acid-base titration in the non-aqueous medium

No.	T, g/ml	K 0.1M CH_3ONa	m, g	Found, %	Metrological characteristics of the average
1.	0.04414	0.9902	0.4011	100.76	$\overline{x} = 100.09$
2.			0.4002	99.88	$S^2 = 0.2354$
3.			0.4004	100.38	$S = 0.4852$
4.			0.3997	99.46	$S_{\overline{x}} = 0.1981$
5.			0.4008	99.73	$\Delta x = 1.2469$
6.			0.4005	100.36	$\Delta\overline{x} = 0.5091$
					$\varepsilon = 1.25\%$
					$\overline{\varepsilon} = 0.51\%$

Therefore, the relative uncertainty of the average is 0.51%, and it indicates the sufficient accuracy of the method.

CONCLUSION

Based on the study of physical, physicochemical and chemical properties of 3,4-dimethoxyphenylamide-4-[(3,4-dimethoxyphenyl)-2,3-dioxo-1,4-dihydropyrazine-1-yl]acetic acid the methods of its identification by IR-, UV- and PMR-spectroscopy and by chemical reactions have been proposed. The method for quantitative determination of dimetpyrazine by acid-base titration in the non-aqueous medium has been developed; the relative uncertainty of the average is 0.51%.

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