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DEVELOPMENT OF A DESIGN RESEARCH FOR DETERMINING THE QUALITY INDICATORS OF POTENTIAL API. 1. NEWLY SYNTHESIZED SUBSTANCES FOR PRIMARY PHARMACOLOGICAL SCREENING

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Постійне зростання у світі лікарських засобів синтетичного походження обумовлює пошук, цілеспрямований синтез і фармакологічні дослідження нових біологічно активних речовин. Встановлення будови речовини і вивчення фізико-хімічних властивостей потребує використання низки методів і випробувань, які дозволяють отримати речовини з «фармакопейною якістю» вже на етапі синтезу потенційних АФІ. Зміна в подальшому умов синтезу, розчинників для кристалізації тощо може призвести до зміни профілю домішок та їх кількості, одержання інших поліморфних модифікацій, ізомерів тощо і внаслідок цього – до зміни фармакологічних властивостей. Для запобігання цьому вимоги до субстанцій, що передаються для фармакологічного скринінгу мають бути уніфікованими.

Мета. *Метою роботи є узагальнення відомостей щодо методів встановлення структури і фізико-хімічних властивостей нових біологічно активних речовин, оцінка їх відповідності фармакопейним вимогам до якості і формулювання обов'язкових вимог до стандартизації уперше синтезованих речовин для їх передачі для проведення первинного фармакологічного скринінгу у вигляді структури первинного «сертифікату якості».*

Матеріали і методи. *Для виконання досліджень використано збір та аналіз даних, наведених у сучасній науковій літературі та документах регуляторних органів.*

Результати. *Визначені відповідність досліджень зі встановлення структури вперше синтезованих речовин фармакопейним показникам якості субстанцій, запропоновано структуру «сертифікату їх якості», виділено основні принципи забезпечення стабільних показників якості при синтезі АФІ.*

Обговорення. *Обґрунтовано обов'язкове визначення для вперше синтезованих речовин таких показників як температура плавлення, розчинність у розчинниках різної полярності (ліпофільності), елементний склад та/або молекулярна маса. З фізико-хімічних методів обов'язковими є УФ-, ІЧ-, і як мінімум ПМР-спектроскопія, для встановлення чистоти обов'язковим є використання хоча б одного з хроматографічних методів – ТШХ з використанням речовин-свідків, або ВЕРХ/МС (переважно, оскільки крім чистоти дозволяє оцінити кількісний вміст речовини та профіль домішок).*

Висновки. *Узагальнені підходи до особливостей встановлення будови і вивчення властивостей нової синтезованої речовини з передбачуваної біологічною активністю за допомогою фізичних, фізико-хімічних і хімічних методів. Уніфіковано методи встановлення будови БАР, які повністю характеризують структуру, надають інформацію щодо чистоти і кількісного вмісту сполуки на первинному етапі фармакологічних випробовувань. Виділено основні принципи забезпечення стабільних показників якості в синтезі потенційних АФІ*

Ключові слова: *активний фармацевтичний інгредієнт, стандартизація, вимоги до якості, дизайн досліджень, методи аналізу*

1. Introduction

The range of new active pharmaceutical ingredients is constantly updated due to the synthesis of new substances, the improvement of the molecules of existing medicinal substances by studying the products of their metabolism, the pharmacological properties of isomeric and polymorphic modifications, etc. [1–3]. The Chemical Abstracts Service registration system contains information about 138 million organic and inorganic substances described in literature since the beginning of the XIX century [4]. More than 7,000 potential active substances for efficiency and security are thoroughly checked at various stages of the life cycle, requiring an average of 10 to 15 years [5–7]. The search for and development of new innovative medicines is the only way to improve the effectiveness of pharmacotherapy and remains the key driving force of the global pharmaceutical market. Every year, pharmaceutical companies bring dozens of new drugs to the market, investing heavily into the research. Thus, according to the European federation of pharmaceutical industries and associations (EFPIA), € 36.500 million was invested in 2018 for R & D in Europe only, and the number of specialists engaged in pharmaceutical development reached 115,000 people [3]. In 2016, global BAS development was expected to be about $35.2 billion worldwide, with an estimated growth

of up to $71 billion by 2025 [4]. During 2014–2018 the world pharmaceutical market was brought innovative medicines by manufacturers of USA – 125, Europe – 67, Japan – 34, other countries – 41 [8].

The first stage of the search of new biologically active substances (BAS), which can later be used in medical practice, is their synthesis, synthesis products are transmitted for pharmacological screening, which includes the study of specific activity and acute toxicity. The choice of a leader substance to establish a specific pharmacological action on various disease models in laboratory animals compared to known drug preparations and subsequent pre-clinical trials of substance [9]. For biological screening and pharmacological research, synthesized substances must have a proven structure with certain physical and chemical properties [10].

It is known that only 1–2 from several hundred chemical substances that have been screened are selected in in-depth studies [11], therefore, all screening substances may not normally be subject to Pharmacopoeia standardization and fully meet the requirements of real API [12]. One of the problems of potential API is the involvement of classical organic chemists into the search for potential API, which does not fully understand the importance of pharmacopoeial approaches to the quality of API candidates. And it is necessary to understand, that at reproduction of synthesis in the future should be maximally reproduced all conditions - not only amount of incoming substances, temperature and time of reaction. The same should be solvents – both those used in synthesis, and for recrystallization. All this can affect not only the output of the final substance, but also some of its characteristics as the shape and size of crystals, solubility, optical activity, purity and etc. Changes in synthesis techniques, the use of reagents of other purity, the replacement of the path of separation of the substance from the reaction mixture, the solvent for recrystallization can lead to the appearance of undesirable impurities that can affect biological activity, and also strengthen the side effect of the substance [13-15]. In turn, the consequence of these changes can be changes in pharmacological activity, and in this case resynthesizes for in-depth pharmacological studies the substance may not show the qualities that were found in the primary screening. [16, 17]. To prevent this, it is necessary to identify the mandatory requirements that are key for the first time synthesized substances for transmission to primary pharmacological screening and can guarantee acceptable quality of potential API.

The purpose of the work is to summarize information on methods of establishing the structure and physico-chemical properties of new biologically active substances, assessment of their compliance with pharmacopoeial quality requirements and formulation of mandatory requirements for standardization of first synthesized substances for their transmission for primary pharmacological screening in the form of the structure of the primary «certificate of quality».

2. Planning (methodology) of research

To increase the level of conformity of the new synthesized BAS to the functional purpose - to have stability, stability, purity, constant quantitative content, to show reproducible results in pharmacological studies - leads to the need to standardize each stage of research. Of course, the pharmacopoeial requirements for substance quality at this stage are exaggerated and very "expensive pleasure", so it is necessary to choose such methods that will allow to estimate directly or indirectly not only the identity of the substance, but also its purity and quantitative content.

To summarize and form standardized quality requirements for the first synthesized chemicals for their transmission for primary pharmacological screening, we have identified the main stages:

1. Analysis of properties of substances, established during synthesis of new substances and their harmonization with pharmacopoeial quality requirements.

2. Determination of the methods of proving the structure which are mandatory at this stage for the identification of substance.

3. Discussion of approaches to the assessment of purity and quantitative determination.

4. Generalization of requirements of quality requirements for the first synthesized chemicals for their transfer for primary pharmacological screening, formulation of the structure of the "certificate of quality".

5. Formulation of basic principles for ensuring stable quality indicators in the synthesis of potential API.

3. Materials and methods

The basic legislative act of regulation of quality control of medicines in Ukraine at all stages of production, vacation, storage, etc. is the Law of Ukraine "On medicines".

To perform the research according to the established methodology, collected and analyzed data in modern scientific literature and regulatory documents are used to carry out research on the established methodology (ICH [19], State Pharmacopeia of Ukraine [20], European Pharmacopeia [21], Pharmacopeia USP [22], installations ST-N MZU 42-3.5: 2016 "Medicines. Process Validation." that is harmonized with EMA / CHMP / CVMP / QWP / BWP / 70278/2012-Rev. 1 [23], guidelines on the rules for production, distribution, storage, pre-clinical and clinical studies of medicines [24, 25].

4. Results

For synthesized substances that undergo primary pharmacological screening, standardization consists in the implementation of methods and results of establishing the structure and physico-chemical properties of the substance to form the primary "pharmacopoeial" specification, helps to eliminate the risks that can arise in subsequent attempts to resynthesize the substances.

Among the physico-chemical properties and methods of establishing the structure of substance, the ones specified in Fig. 1 are most commonly used.

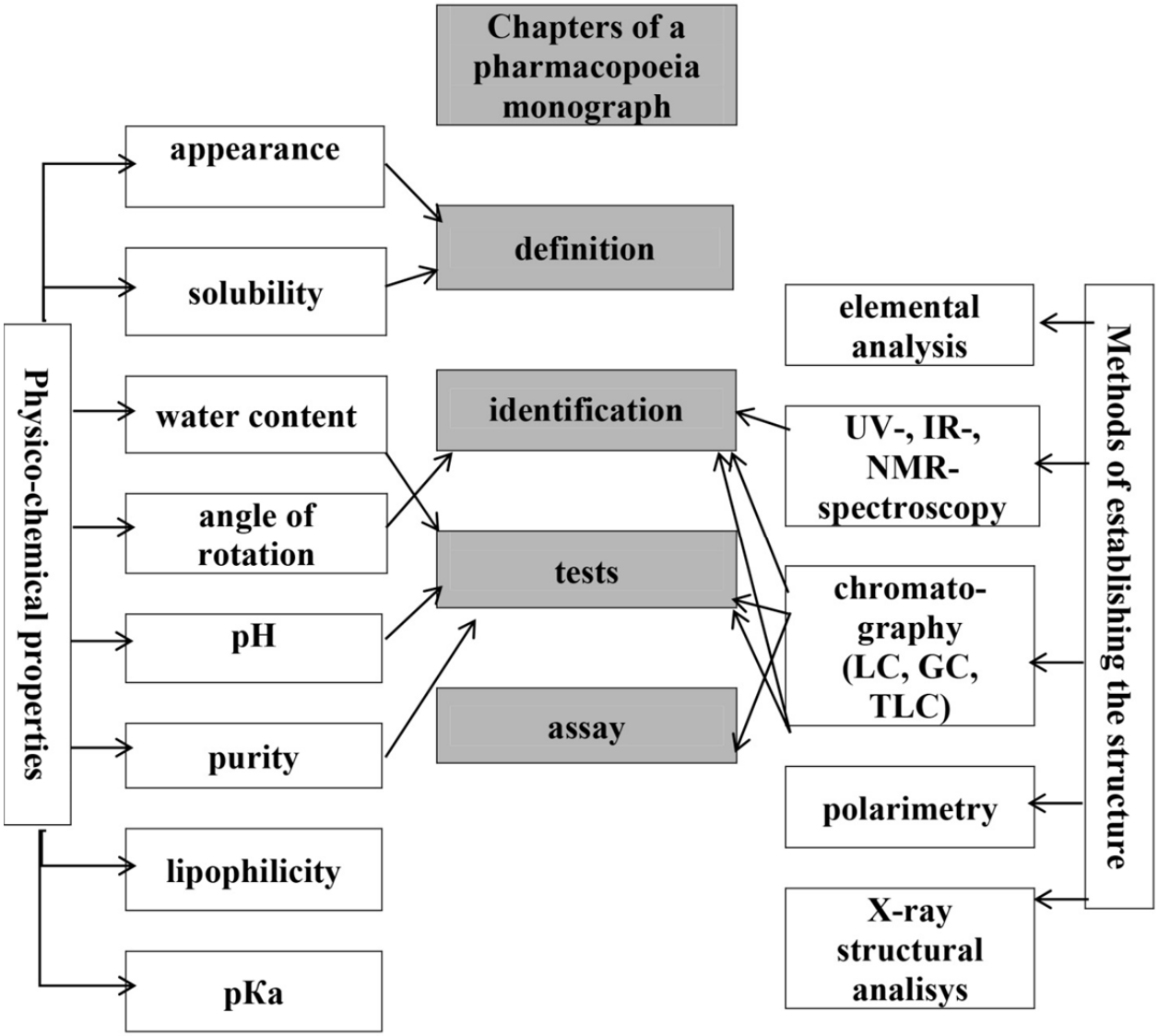

Fig. 1. Correlation of methods of structure proving and study of physico-chemical properties of BAS with pharmacopoeial quality indicators

Certificate of quality of BAS (project)

Chemical name

Structural formula of compound

Molecular formula **Molecular weight (calculated)**

Indicator		**Methods**	**Supporting documents**
Description (color, shape of crystals, etc.)		Visual, organoleptic, microscopy	working journal (WJ)
		X-ray structural analysis	data from the program
Solubility		pharmacopoeial	WJ
Identification	**Melting point**	capillary method	WJ
	elemental analysis[1]	electron spectroscopy	a printout from the program
	molecular mass[1]	Mass-spectrometry, LC/MS	Mass-spectrum
	angle of rotation[2]	polarimetry	WJ or a printout from the program
	spectral characteristic	IR-spectroscopy	IR-spectrum with signal assignment
		UV / Vis - spectroscopy	absorption spectrum
		NMR-spectroscopy [3]	NMR-spectrum (spectra) with signal assignment
	chemical reactions	express reaction for synthesis control	WJ
Tests	**pH**[4]	potentiometric	WJ, a printout from the program
	content of related and associated impurities[5]	TLC	photography
		LC	chromatograms
		LC/MS	chromatograms + MS spectra
Assay		LC/MS	LC/MS chromatograms

Fig. 2. The structure of the "certificate of quality" of the newly synthesized substance for transfer for primary pharmacological screening (mandatory tests are marked with gray):
[1] one of two indicators is enough;
[2] for optically active substances;
[3] must be at least ^{1}H NMR;
[4] for soluble substances with predicted basic or acidic properties;
[5] one of the listed methods

Principles of ensuring stable quality indicators in the synthesis of potential API	directed synthesis;
	clear regulation of conditions of synthesis and purification (quantity of initial substances, solvents, temperature, time of synthesis, etc.);
	improvement of synthesis method by reducing the stages of material production (to reduce the amount of impurities)
	implementation of the "green chemistry" principles in order to reduce the necessary changes in the further transfer to industrial production;
	use of high purity reagents and solvents to prevent impurities from forming;
	control of the synthesis process to increase the output of the target product and prevent the occurrence of adverse reactions;
	increase the degree of purification of the substance by selecting the appropriate solvent for crystallization;
	establishing the structure and possible modifications of the synthesized substance by modern objective physical, physico-chemical and chemical methods

Fig. 3. Basic principles for ensuring stable quality indicators in the synthesis of potential API

5. Discussion

Nowadays researchers in the field of pharmaceutical (medicinal) chemistry almost always carry out directed synthesis of substances with certain pharmacological properties. In the synthesis of new substances, the scientist does not always know in advance that this substance will be transferred for pharmacological screening. The idea of further research can be initiated by pharmacologists or studies *in vitro* and *in silico*. But today, the requirements for structure proving even for publication in scientific journals and for registering with databases (assigning CAS numbers, etc.) are quite high. We also believe that these studies should be comprehensive at the initial stage, as it is the substance synthesized for primary screening that becomes a prototype for the production of a reference standard.

In order to form the requirements for a "certificate of quality" of a potential API, they should be divided into those that establish properties, prove the structure (identify), provide information about quantitative content and purity. We tried to generalize these indicators in accordance with the structure of the pharmacopoeia monograph (Fig. 1).

Definition: When new substances are received, scientists always describe their *appearance*. In the study of the appearance, chemists usually indicate the color of the substance and the shape of the crystals, which can vary depending on the change in the synthesis conditions or solvent chosen for the recrystallization of the substance (temperature, pressure, the nature of the solvent, concentration, crystallization speed, presence of a crystallization center, presence and concentration of impurities, etc.) and further influence the pharmacological action (polymorphic modifications) [26]. When receiving amorphous or oil-like substances, it is also noted. It is mandatory at this stage to specify the solvent that was used to recrystallize the substance.

Crystalline structure and isomerism. Phenomenon of polymorphism is one of the most studied in pharmaceutical development for today [27]. It is established that the pharmacological activity and bioavailability depend on the crystalline structure [28]. If the substance is subject to polymorphism, it is necessary to determine which polymorphic modification is transmitted for pharmacological studies, and how the conditions of synthesis and / or crystallization can affect its modification. The spatial structure and isomerism of molecules [29] are equally important. A reliable method of establishing a crystalline and spatial structure is the method of X-ray-structural analysis. This method is desirable for assessing the characteristics of the structure of the substance.

Solubility, as in pharmacopoeia studies, is very approximate. Usually, this indicator is investigated when choosing a solvent for crystallization, which allows to maximally purify the target product from the accompanying impurities - semiproduct and by-products of synthesis. The solubility indicators describe the purity of the substance in a third way. The presence of impurities can improve or worsen the solubility in certain solvents. The solubility of the substance gives general information about their lipofilicity (hydrophilicity), the importance of which for pharmacological tests it is impossible to exaggerate, so at this stage it is advisable to use solvents with a wide polarity and lipofilicity to establish solubility.

pH. It is advisable to be determined for soluble substances that may have acidic or basic properties.

Identification. Since a large number of physico-chemical methods are used to prove the structure, they are in our opinion sufficient for the identification of the substance and is the basis for further obtaining a standard sample.

Melting point. Chemists-synthetics determine it by various methods - capillary, instant fusion and etc. The instant fusion method gives express information on the proper course of synthesis, while the determination of melting point by capillary method gives information not only on identity, but also on the purity of the substance. The presence of impurities, depending on their nature and amount, can lead to an increase in the melting point interval or to its depression.

Elemental analysis is a primary way of bringing the structure, quite reliable when using modern analytical equipment, informative only when used with other methods of proving the structure. It is mandatory in the absence of a mass spectrum.

Molecular weight. Today, mass spectrometry is widely used to prove the structure of organic substances and gradually replaces elemental analysis. The study of mass spectra gives information not only about the molecular weight of substances, according to the results of fragmentation of molecules and the analysis of signals, it is possible to obtain information about its structure. Recently, the method of chromato-mass spectrometry is used, which allows simultaneously to obtain information about the purity of the substance at the same time.

Angle of rotation, specific rotation (optical purity). Optical isomerism is an important characteristic of organic substances. Many cases of various pharmacological properties of optical isomers are known, often only one of the optical isomers is active. It is known that optical activity depends to a large extent on the conditions of synthesis, as well as minor changes, for example, the temperature regime can lead to the racemization of the target product. Therefore, the setting of the rotation angle or calculation on its basis of specific rotation for optically active substances is mandatory and is simultaneously an indicator of the identity and purity of the substance. To determine the purity of optically active substances, the method of polarimetry is used.

UV/Vis spectra. Recently, absorption spectroscopy in the UV and visible areas is used to prove the structure rarely, mainly when the electron nature of the substance change during in the synthesis, chromophores appears / disappear, maxima displacement occurs etc. If the absorption spectrum of the substance is characterized by one or more absorption maximums, the specificity of the procedure is enhanced by determining the specific absorbance or by finding the values the ratio of optical densities at various maxima. The ratio of optical densities and the magnitude of the specific absorbance value indicate not only the individuality of the substance, but also the absence of related substances. In addition, solvents changing are possible to preset the chemical properties (for example, the acidic, basic or amphoteric character of the substance) of aromatic and heterocyclic organic substances, that is very useful for pharmacological tests.

Since the analysis of absorption spectra at the presence of maxima, optical absorbance, the ratio of optical densities, etc. can give information about the identity and purity of the newly synthesized substances, therefore we consider this method to be one of the key in the complex standardization of substances having chromophore groups in the structure.

IR spectra. IR spectroscopy is a pharmacopoeial method of identification of substances for the first identification and is mandatory for manufacturers. At the stage of srtucture proving, it allows to identify almost all functional groups, especially that ones cannot be determined by the NMR1H spectroscopy (for example, carbonyl, nitrile groups). Since there is no standard sample at this stage of the study and therefore the IR spectrum of the standard sample, it is advisable to give signal assignment for all characteristic absorption bands.

The method is mandatory for all newly synthesized substances.

NMR spectra. Since the end of the 20th century, the method of NMR^{1}H spectroscopy is mandatory for proving the structure of the substance. It allows to identify all functional groups containing hydrogen. Nowadays this method is included to the majority of pharmacopoeias as mandatory for the proving of the structure for new substances, but it is usually not introduced into monographs [30-32]. According to the results of NMR spectroscopy at hydrogen, oxygen, carbon, nitrogen, phosphorus nuclei structure of a large amount of organic substances with a complex structure can be established [30]. Recently, the most influential scientific journals require NMR spectra on carbon nuclei.

Without at least PMR spectrum, the structure of the substance is considered as unproven. In addition, NMR spectra provide approximate information about the purity of the substance.

Chemical identification methods. At this step, chemical methods are used only for confirmation of the reaction running, for example, Belstein test for confirmation of halogenation, a reaction with bromine water to assess the completeness of the reactions of double bond attachment, etc. [21]. In the presence of a sufficient complex of physical and chemical studies are not mandatory.

Tests (purity). *Moisture and volatile solvents.* To determine the presence in the structure of the substance of water (solvate, hydrate), thermal stability and remnants of volatile solvents at this stage, thermal analysis methods are used, among which differential scanning calorimetry (DSK), thermogravimetry (TGA), thermomicroscopy [33].

Impurities. In this study, we do not discuss any impurities from equipment, reagents, or the environment. In the case when synthesis is carried out on the same equipment, their content and profile will be the same. The use of glass vessels in the laboratory synthesis reduces the risks of contamination of the substance with metal ions, which in the future can get from industrial equipment.

More important at this stage is to assess the content and profile of the semi-products or by-products of synthesis which can influence pharmacological activity and toxicity of the substance, transferred for pharmacological screening [34].

Methods of indirect determination of impurities.

Indirect and primary information about the presence of accompanying impurities (related substances) can be provided by physical / physico-chemical characteristics (melting point / boiling point, solubility) and the physico-chemical methods discussed above (UV, IR, NMR spectroscopy). But this information is insufficient, because it can only characterize the presence of foreign substances in a sufficiently large amount. For example, the PMR spectrum can detect impurities if their content approaches to 10 %. In addition, it is almost impossible to give information about the profile of these impurities. Since both the content and profile of impurities that are key to determining the purity of the compounds, these methods are not enough.

Chromatographic methods are the most reliable for the determination of related substances and are therefore mandatory for the primary evaluation of the purity of the newly synthesized substance.

Synthetic chemists widely use the method of chromatography in the thin layer of sorbent (TLC). This method allows to control the synthesis progress and to determine the individuality of substances synthesized. When quantitative approaches and aliquots with different concentrations are used, it is possible to reliably estimate the content of impurities. In addition, the use of raw materials and synthesized semi-products/possible side products as standards allows to assess the content and profile of the related substances. In such modification this method is acceptable for primary standardization for purity test.

Currently, the universal method of chromato-mass spectrometry (LC/MS) is increasingly used to prove the structure and purity of substances. This method is reliable and unconditional for information on purity and quantitative content, due to peaks square and corresponding mass spectra the identity and content of the basic substance, molecular masses and the content of impurities can be determined [35]. It is recommended and preferrable for primary standardization.

Assay. The development of methods of quantitative determination is rather laborious, confirmation of correctness requires carrying out validation studies [36, 37]. In addition, the use of physico-chemical methods at this stage is impossible due to the lack of a standard sample. The presence of impurities in the amount, less than 1 %, has been experimentally established, and the results of spectral studies give extraneous information on the content of the main substance. Application of chromato-mass spectrometry (LC / MS) method, which gives objective estimation of quantitative content of the basic substance and related impurities is recommended.

Thus, the physico-chemical properties and methods of proving the structure of the synthesized substance at the stage of pharmacological screening are largely correlated with pharmacopoeial quality requirements and they are the basis for the development of the specification - the primary "quality certificate", which includes signal indicators, which identify and prove the structure of the substance: description, solubility, identification, related substances, assay and other tests that depend on the synthesis scheme and properties of substance (scheme 2).

To ensure the stability of these indicators, common rules should be followed during the development of synthesis method (scheme 3). Compliance with these rules allows, along with the focus of synthetics on increasing the output of the target product, to carry out measures to reduce the probability of contamination of the synthesized substance by accompanying and foreign impurities, to predict changes that can be made when the synthesis is transferred into industrial conditions and to achieve standard stable parameters of the quality of the substance when it is re-synthesized.

6. Conclusions

The re-synthesis of prospective API, showed activity in primary pharmacological screening sometimes leads to the uniqueness of the results of pharmacological studies. This can be the result of changes in the crystalline structure, isomerism, purity and quantitative content of the main substance. Methods of establishing the structure of potential API at the initial stage have not only fully characterize its structure, but also provide primary information on the content and profile of impurities, the quantitative content of the substance. At the same time, you should strive to obtain substances with "pharmacopoeial" purity. The conformity of the elements of the structure of synthesized substances proving with pharmacopoeial quality indicators has been established. At the initial stage the melting point, solubility in solvents of different polarity (lipophylicity), elemental analysis and / or mass-spectrometry are mandatory for the characteristics of substances. For optically active substances it is mandatory to determine the rotation angle, for substances subject to polymorphism – X-ray structural analysis. Among physico-chemical methods of identification, UV, IR, PMR spectroscopy are mandatory, the use of one of chromatographic methods - TLC with the use of test substances, or LC/MS is mandatory for purity estimation. LC/MS method is preferrable, because in addition to purity, it allows to estimate the quantitative content of the substance and the profile of impurities). The importance of detailed research is also explained for the further production of a standard sample of the substance. To prevent change and ensure sustainable results of the quality indicators of potential API in repeated synthesis, standard synthesis conditions should be provided. The main principles of ensuring stable quality indicators in the synthesis of potential API are highlighted.

Conflict of interest

Authors declare no conflict of interest.

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STANDARDIZATION OF ORIGINAL MEDICINE ANTI-ALCOHOL ACTION ON ASSAY OF GLYCIN

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Мета. *Розробка та валідація доступної методики кількісного визначення гліцину в новому оригінальному лікарському засобі, що застосовується при алкогольній залежності.*
Методи. *Для кількісного визначення гліцину в препараті в формі порошку шипучого для приготування орального розчину була розроблена і валідована спектрофотометрична методика з використанням спектрофотометра Specord 200 фірми «Analytik Jena».*
Результати. *В результаті проведеного дослідження було розроблено модифікований чутливий спосіб кількісного визначення гліцину спектрофотометричним методом. Обрані оптимальні умови проведення реакції гліцин – нінгідрин з метою отримання стабільних результатів аналізу: аналітична довжина хвилі – 568 нм; нагрівання реакційної суміші проводять в киплячій водяній бані протягом 30 хв; об'єм буферного розчину – 4 мл, обраний pH буферного розчину 6.8 і введений відновник – аскорбінова кислота. Встановлено, що в методиці відсутня систематична похибка, відносна невизначеність для ймовірності 95 % не перевищує максимально допустиму невизначеність результатів аналізу (1,77 % ≤ 2,4 %). Для методики кількісного визначення гліцину були вивчені такі валідаційні параметри як специфічність, лінійність, правильність, прецизійність і робасність. Встановлено, що всі розраховані валідаційні параметри відповідають необхідним критеріям прийнятності.*
Висновки. *Розроблена і валідована доступна чутлива спектрофотометрична методика, заснована на здатності продуктів взаємодії гліцину з нінгідрином поглинати у видимій області спектра. Всі валідаційні параметри відповідають критеріям прийнятності*
Ключові слова: *стандартизація, спектрофотометрія, валідація, гліцин, препарат антиалкогольної дії*

1. Introduction

According to WHO data, about 2.3 billion people around the world consume alcohol, and more than 76 million suffer from alcohol dependence (AD). Currently, mortality from alcoholism and directly related diseases is in third place, just after mortality from cardi-