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VALIDATION OF THE METHOD FOR THE QUANTITATIVE ASSESSMENT OF TANNINS IN THE ABOVE-GROUND PART AND ROOTS OF *LIMONIUM GMELINII* AND SUBSTANCE "LIMONIDIN" OBTAINED ON THEIR BASIS

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Abstract. Quantitative assessment of the basic groups of biologically active constituents in herbal medicines is one of the key indicators of their quality and ensures a constant composition of the total products. Analytical monitoring of certain drugs or their ingredients is required in order to ensure their shelf-life safety and efficacy. Validation of analytical methods is conducted in order to ensure that the analytical procedure takes rightful place in the system of quality assurance. Tannin content in the above-ground part and roots of industrially important plant *Limonium gmelinii* and substance "Limonidin" obtained on its basis, as one of the major indicators of their quality, must meet the proper requirements. Present work was designed with the purpose of comparing the methods of quantitative assessment of tannins in all studied objects, namely complexometric titration and spectrophotometric method. Latter one was validated in terms of linearity, range of application, liability and precision. It meets the specific requirements for a particular application and can be used for the study of tannins in *Limonium gmelinii* and products created on its basis.

Key words: quantitative, study, tannins, method, validation.

Limonium gmelinii is one of the medicinal plants, which meets the requirements of biological activity, resource provision on the territory of Kazakhstan, conditions of harvesting and cultivation, simplicity of technological processes for production of medicinal products on its basis, as well as economic and environmental viability. Roots and the above-ground part of *L.gmelinii* were introduced into the medicine and as specific monographs into the State Pharmacopoeia of Kazakhstan [1].

Quantitative assessment of the basic groups of biologically active compounds in herbal drugs is one of the major quality indicators, ensuring a constant composition of the total products. The procedure of validation of analytical methods is conducted in order to ensure that the analytical procedure takes rightful place in the system of quality assurance, fitting its purposes and guarantying reliable and accurate results of analysis [2].

Present work was designed with the purpose of comparing the methods of quantitative assessment of tannins in the above-ground part and roots of *L.gmelinii* and substance "Limonidin" obtained on their basis in accordance with the GMP requirements [3].

Tannin content in the above-ground part and roots of industrially important *L.gmelinii* plants and substance "Limonidin" obtained on their basis is one of the

major quality indicators, which must meet up the proper requirements. The method of complexometric titration was selected for the quantitative assessment of tannins in both raw material and derived medicinal forms, based on the fact that this method is officinal and therefore validated. However, it should be noted that each individual plant has its own chemical composition, and accordingly it is necessary to confirm that the selected method is suitable for the analysis of a particular plant and drugs produced on its basis. Spectrophotometric method was selected as an alternative for assessment of tannins in all of these objects primarily due to its simplicity and promptness.

Previously, it was noted that the UV spectrum of "Limonidin" substance contains absorption bands (λ_{max1} = (268±2), λ_{max2} = (363±2) nm), which are shown on the Figure 1.

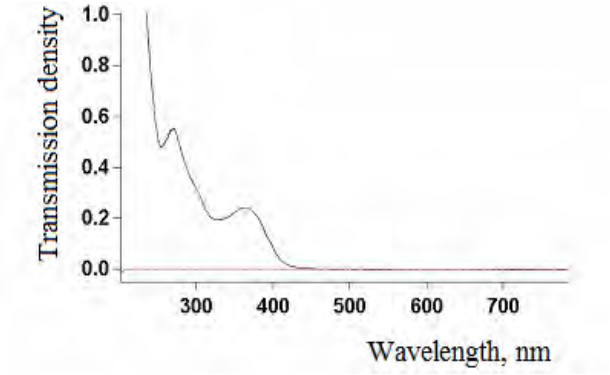

Figure 1 – UV spectrum of the substance "Limonidin"

As a result, measurement of the optical density of solution of the substance was performed at a wavelength of 270 nm in relation to 30% ethanol. Tannin solution was used as a reference standard. The total amount of tannins in conversion to substance (X, %) was calculated by the formula:

$$X_4 = \frac{D_x \times M_{cm} \times 100 \times 50 \times 25 \times 2{,}5 \times 100}{D_{cm} \times M_x \times 5 \times 2{,}5 \times 50 \times 25} = \frac{D_x \times M_{cm} \times 2000}{D_{cm} \times M_x},$$

where M_{cm}– weight of tannin, g; M_x – weight of substance, g; D_{cm} – absorption of tannin; D_x –absorption of sample under measurement [4].

Similarly, measurements were performed in extracts obtained from *L.gmelinii* roots and above-ground part. Results of quantitative assessment of tannins in all of those objects are shown in Table.

The quantitative content of tannins in *L.gmelinii* plants and substance "Limonidin"

Group of BAS	Amount of BAS in roots, %	Amount of BAS in the above-ground part, %	Amount of BAS in the substance isolated from the roots, %	Amount of BAS in the substance isolated from the above-ground part, %
Tannins	15.89	8.21	37.29	19

Validation is an action of proving and documenting that any process, procedure or method truly and consistently leads to the expected results. Validation of any analytical method should include as appropriate: precision, linearity and selectivity, limits of detection and quantitation, recovery and reproducibility [2].

In particular, linearity indicates the ability to produce results, which are directly proportional to the concentration of analyte in the sample. Linearity of spectrophotometric assessment of tannins is established within 25-150% of a given concentration, and the correctness of the data of linear dependencies for the quantitative assessment is confirmed by correlation coefficient, which value must be over 0.995.

The blander existence was established by calculation of sample range, the difference between the maximum and minimum values. The table value Q_n is a critical value, which was compared with the test statistics, what is necessary for detecting the errors during the experiments. After these calculations the homogeneity of samples was confirmed, which allowed using the measured values in order to establish linearity using linear regression, shown on Figure 2.

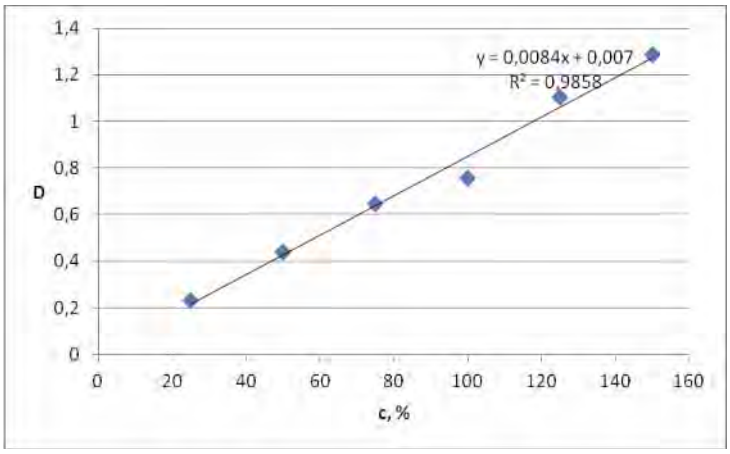

Figure 2 – Graph of the linear regression function indicating the average value of the analytical signal inputted from the amount of substance, %

According to the obtained regression the accuracy of the linear dependence is confirmed by the correlation coefficient: r = 0.9858> 0.9950. The range of application for the quantitative assessment of tannins in substance “Limonidin” is detected as 0.0025 to 0.02 g.

Taking into account that the precision of the analytical method is ascertained by carrying out the analysis as per the procedure and as per normal weight taken for analysis, we calculated the % assay, mean assay, % of deviation and % of relative standard deviation. As known the relative standard deviation of the mean value should not exceed 2.0%, and it is calculated as 0.7% for tannins content.

The difference in dispersion values of average results of two samples obtained under within-laboratory reproducibility in determining the content of

tannins, are insignificant, and their ratio does not exceed the value of the Fischer's table coefficient. The difference between two averages is insignificant, since the calculated value of the Student's coefficient is less than the table t-test value. On this basis it can be considered that within-laboratory reproducibility is confirmed.

Thus, we have performed a validation of the spectrophotometric method for assessment of tannins in accordance with the validation procedures. Validation characteristics and their correlation with acceptable criteria are defined.

The spectrophotometric method of quantitative assessment of tannins is validated; it meets the specific requirements for a particular application and can be used for the analysis of *L.gmelinii* plants and products on their basis.

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Резюме

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LIMONIUM GMELINII ӨСІМДІК ТАМЫРЫ МЕН ШӨБІНЕ МЕН «ЛИМОНИДИН» СУБСТАНЦИЯСЫ НЕГІЗІНДЕ АЛЫНАТЫН ТЕРІ ИЛЕГІШ ЗАТТАРДЫ САНДЫҚ АНЫҚТАУ ӘДІСТЕРІНІҢ ВАЛИДАЦИЯСЫ

Өсімдік субстанция құрамындағы биологиялық белсенді заттардың мөлшері соңғы өнім сапасының негізгі көрсеткіші болғандықтан оның тұрақты құрамын қамтамасыз етеді. Ал тиімділігі мен сақтау мерзімін анықтау үшін белсенді құрамдасының сандық сараптамасын жүргізеді. Аналитикалық әдістеменің валидациясы осы берілген әдістеме сапа өнімін бақылайтынын көрсетеді. Өндірістік маңыздылығы бар *Limonium Mill* өсімдіктің тамыры мен жер үсті бөлігі және «Лимонидин» субстанция құрамындағы тері илегіш заттардың мөлшері олардың сапаларының негізгі көрсеткіші ретінде аталынып, белгілі талаптарға сай болуы тиіс. Осы жұмыстың мақсаты – жоғарыда көрсетілген заттар құрамындағы тері илегіш заттардың сандық сараптама әдістемелерін, яғни комплексометриялық титрлеу мен спектрофотометрия салыстыру. Соңғысы сызықтық, презициондылық, орындалуы, қолдану аймағы сияқты көрсеткіштері бойынша валидациядан өтіп, *Limonium Mill* өсімдігі және оның негізіндегі препараттарға қолданылуы мүмкін.

Тірек сөздер: сандық, бағалау, таниндер, әдіс, валидация.

Резюме

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ВАЛИДАЦИЯ МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ДУБИЛЬНЫХ ВЕЩЕСТВ В ТРАВЕ И КОРНЯХ РАСТЕНИЙ *LIMONIUM GMELINII* И В ПОЛУЧАЕМОЙ НА ИХ ОСНОВЕ СУБСТАНЦИИ «ЛИМОНИДИН»

Количественное определение основных групп биологически активных веществ, являющихся действующими в лекарственных средствах растительного происхождения, является одним из основных показателей их качества и гарантирует постоянный состав суммарных

препаратов. Аналитический контроль лекарственных средств или определенных ингредиентов в препарате необходим для того, чтобы гарантировать их безопасность и эффективность на протяжении всего срока годности. Для того, чтобы аналитическая методика заняла достойное место в системе обеспечения качества, предусмотрена процедура валидации аналитических методик. Содержание дубильных веществ в траве и корнях исследуемых растений и получаемой на их основе субстанции «Лимонидин» является одним из главных показателей их качества и поэтому оно должно удовлетворять установленным требованиям.

Целью данной работы было сравнение методов количественной оценки дубильных веществ в исследуемых объектах, а именно комплексометрического титрования и спектрофотометрического метода. Последний показал более качественные результаты в плане линейности, диапазона применения, надежности и точности. Он соответствует особым требованиям для конкретного применения и может быть использован для изучения дубильных веществ в *Limonium gmelinii* и средств, созданных на его основе.

Ключевые слова: количественная, оценка, танины, метод, валидация.