Zdoryk Oleksandr, Candidate of pharmaceutical science, Associate professor, Pharmaceutical chemistry department, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002
E-mail: oleksandr_zdoryk@ukr.net

Georgiyants Viktoria, Doctor of pharmaceutical sciences, Professor, head of the department, Pharmaceutical chemistry department, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002
E-mail: vgeorg@ukr.net

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DEVELOPMENT OF METHODS FOR DETERMINATION OF PHENOLIC ACIDS AND FLAVONOIDS IN CAPSULES CONTAINING CORYLUS AVELLANA L. DRY EXTRACT

© N. Blyznyuk, Yu. Prokopenko, V. Georgiyants

The questions of standardization and quality control of both herbs and herbal remedies remain relevant, because it is well-known that product quality standards are essential, whether consumer using herbs or drugs. The necessity of the standardization methods development for the initial herbal material and capsule dosage form for the further quality control under manufacturing conditions remains relevant.

Aim. *The aim of our research was to develop simple, specific, accurate and reproducible methods for identification of flavonoids and phenolic acids in capsule dosage form containing Corylus avellana L. dry extract.*

Methods. *The samples of gelatine capsules containing Corylus avellana L. dry extract for oral administration were analyzed. The analysis was carried out using Camag HPTLC system.*

The absorption spectroscopy determination of the sum of flavonoids was carried out using THERMO Scientific Evolution 60S Spectroscope in wavelength range of 300–600 nm.

Results. *As a result of HPTLC research rutine and quercitrine have been identified in capsule dosage form containing Corylus avellana L. dry extract. Among phenolic acids, neochlorogenic and chlorogenic acids have been identified.*

Under the given conditions, the spectrum of the test solution had a maximum absorption at wavelength 406 nm. The analysis of flavonoids total content in gelatine capsules containing Corylus avellana L. dry extract calculated as rutine has shown the content of 1,7 %.

Conclusion. *Effective HPTLC and absorption spectroscopy methods for determination of flavonoids and phenolic acids in capsule dosage form containing Corylus avellana L. dry extract have been developed. It has been found that described methods are promising enough for standardization of capsules with Corylus avellana L. dry extract and may be suggested for the quality control of the dosage form under manufacturing conditions*

Keywords: *capsules, Corylus avellana L., extract, HPTLC, absorption spectroscopy, phenolic acids, flavonoids*

Питання стандартизації та контролю якості як рослин, так і лікарських засобів рослинного походження набуває актуальності, враховуючи той факт, що стандарти якості продукції є вкрай важливими, незалежно від того, чи вживає споживач лікарські рослини або лікарські засоби. Необхідність розробки методик стандартизації для вихідної рослинної сировини та капсульовано лікарської форми для подальшого контролю якості в умовах виробництва залишається актуальною.

Мета. *Метою нашого дослідження була розробка простої, специфічної, точної та відтворюваної методики ідентифікації флавоноїдів та фенольних кислот у капсульованій лікарській формі з сухим екстрактом Corylus avellana L.*

Методи. *Для дослідження використовували зразки желатинових капсул з сухим екстрактом Corylus avellana L. для орального застосування. Аналіз проводили з використанням системи Camag для ВЕТСХ.*

Визначення вмісту суми флавоноїдів методом абсорбційної спектроскопії здійснювали за допомогою спектрометра THERMO Scientific Evolution 60S у діапазоні хвиль 300–600 нм.

Результати. *В результаті ВЕТСХ аналізу у капсульованій лікарській формі з сухим екстрактом Corylus avellana L. були ідентифіковані рутин та кверцитрин. Серед фенольних кислот були ідентифіковані неохлорогенова та хлорогенова кислоти.*

В умовах проведення спектрофотометричного дослідження спектр випробовуваного розчину мав максимум поглинання за довжини хвилі 406 нм. Вміст суми флавоноїдів у желатинових капсулах з сухим екстрактом Corylus avellana L. у перерахунку на рутин становив 1,7 %.

Висновки. *З метою визначення флавоноїдів та фенольних кислот у капсульованій лікарській формі з сухим екстрактом Corylus avellana L. були розроблені ефективні методики ВЕТСХ та абсорбційної спектрофотометрії.*

Було визначено, що описані методики є достатньо перспективними для стандартизації капсул з сухим екстрактом Corylus avellana L. та можуть бути запропоновані для проведення контролю якості лікарської форми в умовах виробництва

Ключові слова: *капсули, Corylus avellana L., екстракт, ВЕТСХ, абсорбційна спектрофотометрія, фенольні кислоти, флавоноїди*

1. Introduction

The use of herbs with medical purpose is the oldest form of healthcare known to humanity and it has been used in all cultures throughout human's history [1]. Herbal remedies occupy a leading position in global pharmaceutical markets.

That's why one of the priority areas of modern pharmaceutical science and practice is the development of new herbal remedies, due to their low toxicity, the breadth of therapeutic action, and low risk of side effects during prolonged use. Generally, all medicines, irrespective of their origin, should meet the basic requirements of being at least safe and effective.

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

Nevertheless, questions of standardization and quality control of both herbs and herbal remedies remain relevant, because it is well-known that product quality standards are essential, whether consumer using herbs or drugs [2]. Therefore, the given question of the search, development and standardization is still pressing and requires solutions.

3. Analysis of recent studies and publications in which a solution of the problem and which draws on the author

Standardization of any herbal remedy involves regulating the given remedy to a defined content of a constituent or a group of substances with established and proved therapeutic activity [1, 2].

Earlier, the anticonvulsant properties of Corylus avellana L. dry extract have been determined; it was the reason to develop the original dietary supplement for nerve health. The screening of the role of biologically active substances or fractions from different Ukrainian flora herbs in realization of the anticonvulsant activity has shown that one of the main groups of compounds responsible for mechanisms of anti-seizure activity were flavonoids [3, 4]. Besides, wide spectrum of pharmacological activity of flavonoids gives certain reasons to analyze their content in dosage forms without reference to application of the given remedy [5, 6].

Analytical methods used in drug analysis are diversified and are being constantly improved to find better solutions to satisfy manufacturers and institutions that test drug quality [7]. According to the literature data, there are several analytical techniques for the identification and quantification of maker compounds in herbal dosage forms [8, 9].

As a rule, pharmaceutical manufacturers recommend various analytical techniques, but spectroscopy and chromatography methods still playing a significant role in pharmaceutical analysis. Due to the complex compound of herbal remedies, given methods are commonly used for the quality control and standardization of both herbs and herbal products [7, 8].

HPTLC (High performance thin layer chromatography) method has been widely used for the standardization and qualitative analysis of herbs and herbal dosage forms due to its simplicity, high speed, and relatively low cost.

The use of HPTLC plates in combination with automated sample applicators and development chambers, high resolution cameras, and computing software allows more control over experimental conditions and greater data analysis capabilities [10]. Moreover, HPTLC method can run many samples simultaneously, requires small volumes of solvents, and gives rather instant visual results [11]. For example, individual bands of different active compounds may act as additional characteristics for their better identification: compounds with native fluorescence are observed as bright zones on a dark background under UV light. While natural substances with no fluorescence (no chromophore groups) or colour can be visualized after post-chromatographic derivatization [2, 12, 13].

Absorption spectroscopy is another effective method for identification and assay of flavonoids [14]. As a rule, $AlCl_3$ reagent is used. Adding $AlCl_3$ reagent allows eliminating the influence of another biologically active substances having polyphenol structure. Reference compounds for recalculations are being chosen generally after TLC or HPTLC identification tests. Subsequently, the content of the sum of flavonoids in the given herb or remedy will be calculated and regulated as the chosen reference standard [5, 14].

Thus, both HPTLC and absorption spectroscopy methods remain the most flexible and reliable, methods ideally suited for the analysis of herbs and herbal remedies. When used with standardized procedures, described methods guarantee reproducible results, a vital element in the routine analysis of plant extracts and pharmaceutical products during manufacturing process.

4. Allocation of unsolved parts of the general problem, which is dedicated to the article

The necessity of the standardization methods development for the initial herbal material and capsule dosage form for the further quality control under manufacturing conditions remains relevant.

5. Formulation of goals (tasks) of Article

Considering the facts given above, the aim of our research was to develop simple, specific, accurate and

reproducible methods for identification of flavonoids and phenolic acids in capsule dosage form containing Corylus avellana L. dry extract.

6. Statement of the basic material of the study (methods and objects) with the justification of the results

HPTLC identification of phenolic acids and flavonoids. The samples of gelatine capsules containing Corylus avellana L. dry extract for oral administration were analyzed.

The capsules filling was removed and placed into a volumetric flask, and the ultrasound extraction was allowed to run using methanol as a solvent in a ratio 1 to 10 at room temperature for 20 minutes. After centrifugation, the supernatant was collected and directly applied onto HPTLC plates.

The analysis was carried out using Camag HPTLC system equipped with a semi-authomatic TLC sampler Linomat 5, Visualizer, integrated software WinCATS 1.4.9., and Videoscan.

TLC plates Si 60 F_{254} (Merck, Germany) of 0.2 mm layer thickness were used. TLC separation was carried out using mobile phase of water, ethyl acetate, anhydrous formic acid and anhydrous acetic acid (17,5:67,5:7,5:7,5 V/V/V/V).

After the mobile phase draw up the plate until it is approximately 0,5 cm from the end, the plate was dried, proceed with 2-aminoethyl diphenylborinate and macrogol, and after that, examined in UV-light at 365 nm.

Flavonoids and phenolic acids reference solutions were used.

The scheme of the obtained chromatogram is presented on Fig. 1.

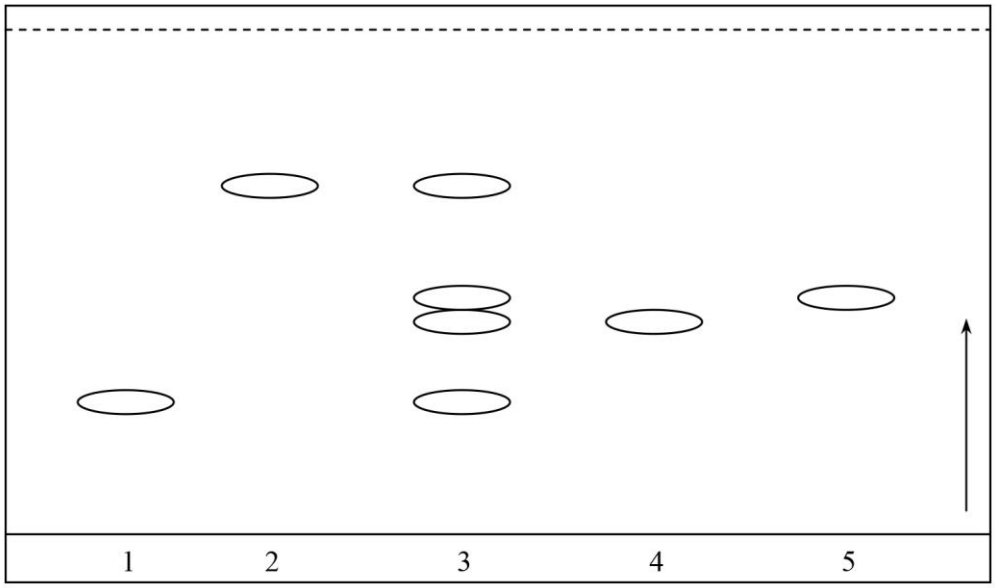

Fig. 1. HPTLC chromatogram scheme obtained from capsule dosage form containing Corylus avellana L. dry extract (rutine (1), quercitrine (2), test solution from capsule dosage form (3), neochlorogenic acid (4), chlorogenic acid (5)).

As a result of HPTLC research rutine (1) and quercitrine (2) have been identified in capsule dosage form containing Corylus avellana L. dry extract (3). Among phenolic acids, neochlorogenic (4) and chlorogenic (5) acids have been identified.

Considering the necessity of the reference compound chose for the further quantitative analysis of the sum of flavonoids, rutine reference standard was chosen, which is consistent at obtained HPTLC research results and the literature data.

The absorption spectroscopy determination of the sum of flavonoids. The analysis was carried out using THERMO Scientific Evolution 60S Spectroscope.

Test solution for determination of the sum of flavonoids has been prepared as follows: the capsules filling was removed and placed into a volumetric flask. Ethanol 50% was added, and the ultrasound extraction was allowed to run at room temperature for 30 minutes. After centrifugation, the obtained solution was filtered.

1 ml of the obtained solution was put into a volumetric flask; ethanol 50 % and then $AlCl_3$ ethanol solution 50 g/l were added. Then, in 10 minutes the acetic acid solution 50 g/l was added, and the solution was brought up to the mark with ethanol 50 %.

The mixture was left for 10 min at room temperature and then subjected to spectral analysis in the range of 300–600 nm against the blank, where $AlCl_3$ solution was substituted by ethanol solution 50 %.

Rutine reference sample solution of 100 μM was used.

The content of the sum of flavonoids was calculated acoording to the formula:

$$X = \frac{A_1}{A_0} \cdot \left(\frac{m_0 \cdot 1}{100 \cdot 25} \right) \cdot \left(\frac{50 \cdot 50}{m_1 \cdot 1} \right) \cdot \frac{100}{(100 - d)},$$

where A_1 – the test solution absorption coefficient;

A_0 – the reference solution absorption coefficient;

m_0 – reference standard sample weight to prepare reference solution;

m_1 – the capsules filling sample weight to prepare test solution;

d – loss on drying, %.

Under the given conditions, the spectrum of the test solution had a maximum absorption at wavelength 406 nm (Fig. 2). The similar spectrum of rutine reference solution had a maximum absorption at wavelength 408 nm.

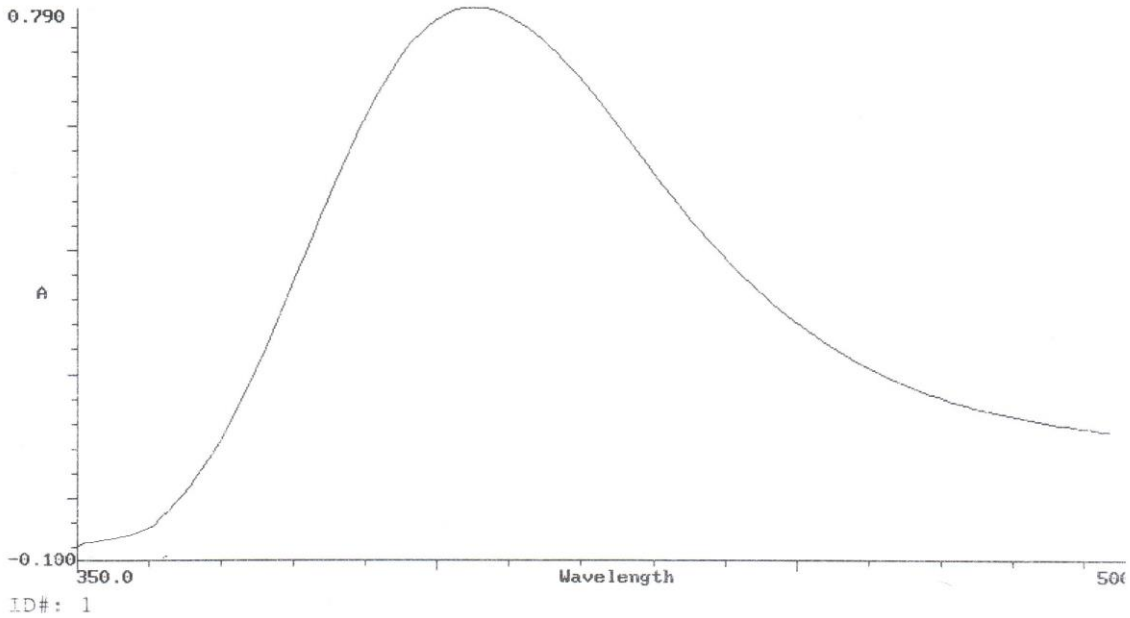

Fig. 2. The UV-spectrum of the test solution obtained from capsule dosage form containing Corylus avellana L. dry extract

The analysis of flavonoids total content in gelatine capsules containing Corylus avellana L. dry extract calculated as rutine has shown the content of 1,7 %.

7. Conclusion

Effective HPTLC and absorption spectroscopy methods for determination of flavonoids and phenolic acids in capsule dosage form containing Corylus avellana L. dry extract have been developed. As a result of research work, neochlorogenic and chlorogenic acids have been identified in capsule dosage form containing Corylus avellana L. dry extract, as well as flavonoids rutine and quercitrin. The analysis of flavonoids total content in gelatine capsules containing Corylus avellana L. dry extract calculated as rutine has shown the content of 1,7 %.

It has been found that described methods are promising enough for standardization of capsules with Corylus avellana L. dry extract and may be suggested for the quality control of the dosage form under manufacturing conditions.

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Blyznyuk Nataliya, Quality, standardization and certification department, Institute of Pharmacy Professionals Qualification Improvement of National University of Pharmacy, Pushkinskaya str., 53, Kharkiv, Ukraine, 61002
E-mail: natasha.bliznyuk@mail.ru

Prokopenko Yuliya, PhD, Associate Professor, Department of quality, standardization and certification of medicines, National University of Pharmacy, Pushkinskaya str., 53, Kharkiv, Ukraine, 61002
E-mail: yuliya.prok@gmail.com

Georgiyants Victoriya, Doctor of pharmaceutical sciences, Professor, head of Department, Department of Pharmaceutical chemistry, National University of Pharmacy, Pushkinskaya str., 53, Kharkiv, Ukraine, 61002
E-mail: vgeor@ukr.net