СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

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CHROMATOGRAPHIC RESEARCH OF GRANULES FROM THE MEDICINAL PLANT RAW MATERIALS FOR TREATING CONSTIPATION

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In order to develop methods of the quality control of a drug in the form of granules under the conditional name «GIT-3» (Sorbosens) for treatment of constipation the research concerning identification of some characteristic plant raw material of biologically active substances in its composition have been carried out.

Numerous literary sources indicate that constipation affects 20 to 30% of the adult population. In 45-60% of cases constipation permanently or occasionally occurs in persons older than 60 and in 65-75% of cases, the constipation syndrome is accompanied by gastrointestinal or cardiac diseases. Constipation is influenced by many factors, including urban culture, fast pace of life, the lack of healthy natural environment, sedentary work, nervous tension, mental stress, permanent mental or physical fatigue, etc. [6].

Drugs based on the medicinal plant raw material (MPRM), the range of which are currently in need of expansion, are of great importance when treating constipation.

With this purpose at the department of Industrial Technology of Drugs of the National University of Pharmacy the drug in the form of granules under the conditional name «GIT-3» (Sorbosens) consisting of Indian senna (*Cassia angustifolia*) leaves, stevia leaves, wheat bran and oat powder has been developed.

An important part of the work to create a drug is development of methods of its identification with both spot tests and various chromatographic techniques. The aim of this work is the research of granules «GIT-3» (Sorbosens) using the thin layer chromatography method (TLC) to determine the possibility of their identification and further introduction to the normative documentation.

Experimental Part

The research was carried out using TLC on the plates «Sorbfil PTCCh-AF-B-UV» by the upward method; methods described in monographs [1] of the State Pharmacopoeia of Ukraine (SPU) and other scientific literature and reference sources [2, 4] were used. Detection was carried out in daylight and ultraviolet light (λ = 365 254 nm) before and after processing the chromatogram by solutions of developers.

The test solutions (alcoholic solutions of *Cassia angustifolia* and stevia leaves, wheat bran and oat) and the reference solution (alcohol solutions of stevioside, FSS of cassia extract) were prepared according to the articles or monographs on herbal drugs.

Identification of cassia leaves was carried out by SPU methods [1]. The test solution was prepared by adding 5 ml of the mixture with the equal volumes of 96% alcohol and water into 0.5 g of the powdered drug raw material. The mixture was heated to boiling and centrifuged. The supernatant liquid was used.

As a reference solution FSS of cassia extract was used; it was also dissolved in 1 ml of the mixture of equal volumes of 96% alcohol *R* and water *R*; as the mobile phase the system of solvents was used: glacial acetic acid *R* – water *R* – ethyl acetate *R* – propanol *R* (1:30:40:40). To develop the chromatogram it was sprayed with 20% solution of nitric acid *R*, heated at 120° C for 10 min, cooled and sprayed with 50 g/l solution of potassium hydroxide *R* in 50% v/v alcohol to identify zones and then examined in daylight.

As seen in Fig. 1, in the chromatogram of the test solution of cassia leaves (I), the reference solution (II) and the drug solution (III) the following zones are located at the same level: reddish-violet zones (position 5, 15) at the reddish-purple zone level (position 10) corresponding to sennoside B, reddish-violet zones (position 4, 14) at the reddish-violet zone level (position 9) corresponding to sennoside A, reddish-violet zones (position 3, 13) at the reddish-violet zone level (position 8) corresponding to sennoside D, red zones (position 2, 12) at the red zone level (position 7) corresponding to rhein-8-glucoside, and reddish-pink zones (position 1, 11) at the reddish-pink zone level (position 6) corresponding to sennoside C. This arrangement of zones is consistent with the requirements of the monograph in SPU and confirms the possibility of identifying cassia leaves in the drug studied.

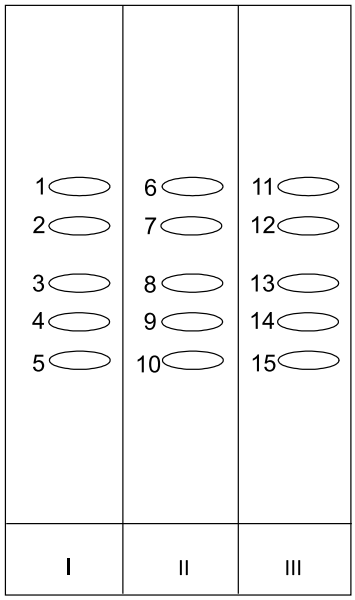

Fig. 1. TLC identification of cassia leaves in the granules «GIT-3». Test solution cassia leaves (I); reference solution (II), drug solution (III).

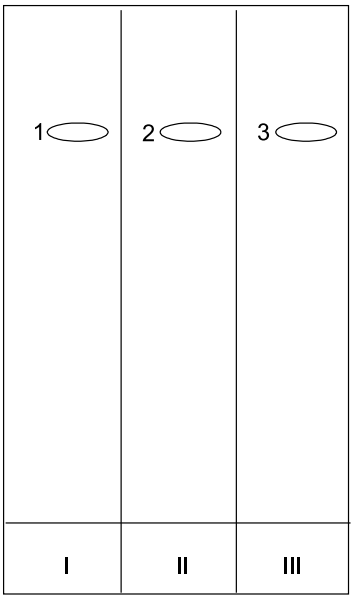

Fig. 2. TLC identification of stevia leaves in the granules «GIT-3». Test solution of stevia leaves (I); reference solution (II), drug solution (III).

Currently there is no documentation on stevia leaves. For its identification the reference and scientific literature sources have been used [2, 3]. It is known that the chemical composition of the given MPRM includes vitamins (B, K, C, folic acid), flavonols, tannins, minerals, essential oils, as well as diterpene glycosides; due to them identification was carried out.

The test solution of the drug was prepared by adding 10 ml of methanol to 1.0 g of the powdered raw material. The mixture was kept at 60°C for 40 min, followed by cooling and filtration. The resulting filtrate was applied to the starting line of the chromatographic plate. As the system of solvents the following system was used: chloroform – methanol – water (15:10:2). The reference solution was alcoholic solution of stevioside. Drying was carried out at 120°C for 10 min. The plate was sprayed with an alcoholic solution of anise aldehyde and examined in daylight.

As seen in Fig. 2, in the chromatogram of the test solution of stevia leaves (I), the reference solution (II) and the drug solution (III) at the top of the chromatogram near the finish line blue-violet zones are detected at the same level (position 1, 2, 3, respectively). This indicates the presence of stevia leaves in the composition of granules.

Wheat bran becomes increasingly an integral part of the pharmaceutical compositions, but, unfortunately, there is no standard documentation or a unique method of its determination at present. The current documents are «GOST 7169-66. Wheat bran. Specifications» and «National Standard 3016-25. Wheat and rye mixed feed» are not informative from the analytical standpoint. However, these and other literature sources [7, 8, 10, 11, 12] indicate a wide spectrum of amino acids in the composition of bran, in the presence of which identification was carried out in the composition of the drug under study.

Recently the cultivated oat is also increasingly included to pharmaceutical compositions. As in the previous case, there are no documentation developed or unique methods of its determination. The current «National Standard 49632008 Oat. Specifications» can not provide the full volume of the analytical information required.

It is known that the chemical composition of oats also includes a large range of amino acids [4, 5, 9]. As in the previous case, this fact gives reason to its identification for the presence of amino acids.

The test solution of wheat bran, oats and the solution of the drug under research were prepared by treating the sample with methanol at 60°C for 40 min. The mixture was cooled and filtered. As the reference solutions alcohol solutions of leucine, aspartic acid, arginine, valine were used for wheat bran, alcohol solutions of leucine, arginine, valine, phenylalanine were used for oats.

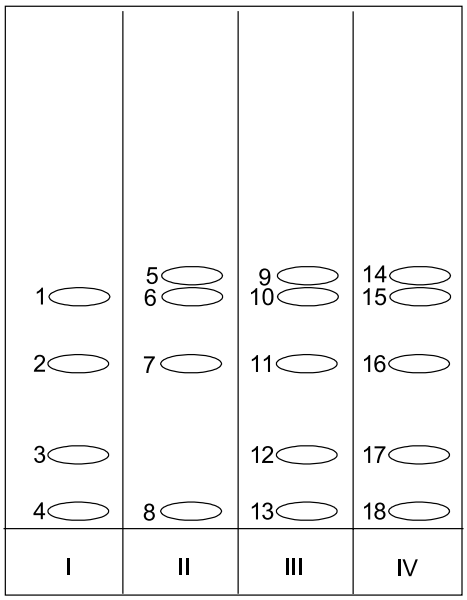

Fig. 3. TLC identification of wheat bran and oat in the granules «GIT-3» in the presence of amino acids. Test solutions of wheat bran (I) and oats (II), reference solution (III), drug solution (IV). Explanations in the text.

Ten mcl of each solution obtained was applied to the start line placing in the system of solvents: acetic acid (98%) *R* – distilled water – 1-butanol *R* in the ratio of 10:40:50 (top phase). After reaching the solvent front of 10 cm the plate was removed from the chamber, dried in the air and sprayed with 2% solution of ninhydrin in ethanol. The amino acid complex was identified by amino acid zones coloured in purple and red.

In Fig. 3 in the middle of the chromatogram at the same level there are clearly visible zones of wheat bran test solution corresponding to zones of the drug solution and the reference solution: purple zones 1 and 15 correspond to the zone of leucine 10, purple zones 2 and 16 correspond to the zone of valine 11; below there are light purple zones 3 and 17 corresponding to the zone of aspartic acid 12 and light purple zones 4 and 18 near the starting line corresponding to the zone of arginine 13. The location of the zones mentioned above confirms the presence of wheat bran in the drug composition.

The research of the cultivated oat chromatographic profile showed the presence of the following zones: in the middle of the chromatogram at the same level there are zones of the test solution corresponding to the zones of the drug solution and the reference solution. Purple zones 5 and 14 correspond to the zone of phenylalanine 9, purple zones 6 and 15 correspond to the zone of leucine 10. Below there are purple zones 7 and 16, which correspond to the zone of valine 11, purple zones 8 and 18 corresponding to the zone of arginine 13. Its confirms the presence of oats in the drug.

Thus, identification of the amino acid complex can be an integral part of the qualitative analysis of this drug.

CONCLUSIONS

1. By the method of thin-layer chromatography the research concerning identification of some characteristic plant raw material of biologically active substances in the composition of the drug in the form of granules under the conditional name «GIT-3» (Sorbosens) have been carried out.

2. The data obtained can be used when developing quality control methods for this drug.

REFERENCES

1. *Державна фармакопея України / Державне підприємство «Науково-експертний фармакопейний центр». – 1-е вид. – Доп. 2. – Х.: РІРЕГ, 2008. – 620 с.*
2. *Комиссаренко Н.Ф., Деркач А.И., Коваль И.П. и др. // Растительные ресурсы. – 1994. – Т. 30, №1-2. – С. 53-64.*
3. *Котов А.Г. Терпеноїди та фенілпропаноїди деяких видів родини ASTERACEAE: Автореф. дис. ... канд. фармац. наук: 15.00.05. – Х., 1996. – 24 с.*
4. *Маршалкин М.Ф., Саенко А.Ю., Гаврилин М.В. и др. // Вопр. питания. – 2006. – Т. 75, №3. – С. 14-16.*
5. *Никифорова Т.А., Севериненко С.М., Куликов Д.А. и др. // Вестник ОГУ. – 2010. – №5 (111). – С. 141-144.*
6. *Філіппов Ю.О. Динаміка поширеності і захворюваності на основні хвороби органів травлення в Україні за 10 останніх років / У кн. Гастроентерологія. – Дніпропетровськ: Журфонд, 2008. – Вип. 40. – С. 3-10.*
7. *Butt M.S., Qamar M.I., Anjum F.M. et al. // Internet J. of Food Safety. – 2004. – Vol. 3. – P. 15-20.*
8. *Kew M.C., Kwang S.C., Bong D.H. et al. // J. Asian-Aust. Anim. Sci. – 2005. – Vol. 18, №6. – P. 861-867.*
9. *Litwinek D., Gambuś H., Mickowska B. et al. // J. of Microbiol., Biotechnol. and Food Sci. – 2013. – Vol. 2 (Special issue on BQRMF). – P. 1725-1733.*
10. *Nadeem M.A., Gilani A.H., Khan and Mahr-Un-Nisa A.G // Int. J. Agricol. Biol. – 2005. – Vol. 7, №6. – P. 985-989.*
11. *Ribeiro F.B., Lanna E.A., Bomfim M.A. et al. // R. Bras. Zootec. – 2011. – Vol. 40, №5. – P. 939-946.*
12. *Steele E. Determination of the generally recognized as safe (gras) status of wheat bran extract as a food ingredient. – Belgium.: Fugeia NV, 2010. – 120 p.*

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ХРОМАТОГРАФИЧЕСКИЕ ИССЛЕДОВАНИЯ ГРАНУЛ НА ОСНОВЕ ЛЕКАРСТВЕННОГО РАСТИТЕЛЬНОГО СЫРЬЯ ДЛЯ ЛЕЧЕНИЯ ЗАПОРОВ

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С целью разработки методик контроля качества препарата в виде гранул под условным названием «ЖКТ-3» для терапии запоров проведены исследования по идентификации некоторых характерных биологически активных соединений ЛРС, входящих в его состав.

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ХРОМАТОГРАФІЧНІ ДОСЛІДЖЕННЯ ГРАНУЛ НА ОСНОВІ ЛІКАРСЬКОЇ РОСЛИННОЇ СИРОВИНИ ДЛЯ ЛІКУВАННЯ ЗАПОРІВ

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З метою розробки методик контролю якості препарату у вигляді гранул під умовною назвою «ШКТ-3» для лікування запорів проведені дослідження з ідентифікації біологічно активних сполук ЛРС, що входять до його складу.