

Recommended by Doctor of Pharmacy, Professor S. V. Kolisnyk

UDC 615.243:54.061:615.11:543.544

A. V. Myhal, M. Marksa*, O. S. Golovchenko, V. A. Georgiyants, L. Ivanauskas*

National University of Pharmacy

* Lithuanian University of Health Science

Comparison of chromatographic methods of analysis in a thin layer of the sorbent for identification of famotidine in tablets

Today three national manufacturers produce famotidine tablets, but the SPhU does not contain any monograph for this dosage form.

Aim. To verify TLC and study the possibility of using the HPTLC method for identification of famotidine in tablets.

Materials and methods. The objects of the study were three batches of famotidine tablets. TLC and HPTLC were used as the methods of the study.

Results and discussion. The possibility of using TLC and HPTLC methods for identification of famotidine tablets was confirmed in the study. The main spots of the test solutions corresponded to the size and the Rf value of the main spots of the standard solution. The Rf value for all solutions was 0.5.

Conclusions. Thus, TLC as well as HPTLC can be recommended for inclusion to the SPhU; however, HPTLC is more economically advantageous.

Key words: *famotidine; identification; verification; thin layer chromatography*

A. В. Мигаль, М. Маркса, О. С. Головченко, В. А. Георгіянц, Л. Іванаускас

Порівняння хроматографічних методів аналізу в тонкому шарі сорбенту при проведенні ідентифікації фамотидину у таблетках

Мета роботи. На теперішній час три вітчизняних виробники випускають таблетки фамотидину, однак ДФУ не містить монографії на цю форму. Метою дослідження було провести верифікацію ТШХ та вивчити можливість застосування ВЕТШХ для ідентифікації фамотидину у таблетках.

Матеріали та методи. Об'єктами дослідження було обрано три серії таблеток фамотидину. Методи дослідження – ТШХ та ВЕТШХ.

Результати та їх обговорення. У ході дослідження було підтверджено можливість застосування методик ТШХ та ВЕТШХ для ідентифікації фамотидину у таблетках. Основні плями, отримані при хроматографуванні випробовуваних розчинів, відповідають за розмірами та положенням основній плямі, отриманій при хроматографуванні розчину порівняння. Rf для всіх розчинів становить 0,5.

Висновки. Таким чином, як ТШХ, так і ВЕТШХ можуть бути рекомендовані для включення до ДФУ, однак ВЕТШХ є більш економічно вигідною.

Ключові слова: *фамотидин; ідентифікація; верифікація; тонкошарова хроматографія*

A. В. Мигаль, М. Маркса, О. С. Головченко, В. А. Георгіянц, Л. Іванаускас

Сравнение хроматографических методов анализа в тонком слое сорбента при проведении идентификации фамотидина в таблетках

Цель работы. На сегодняшний день три отечественных производителя выпускают таблетки фамотидина, однако несмотря на это, ГФУ не содержит монографии на эту форму. Целью исследования было провести верификацию ТСХ и изучить возможность использования ВЭТСХ для идентификации фамотидина в таблетках.

Материалы и методы. Объектами исследования были выбраны три серии таблеток фамотидина, методами исследования были выбраны ТСХ и ВЭТСХ.

Результаты и их обсуждение. В ходе исследования была подтверждена возможность использования методик ТСХ и ВЭТСХ для идентификации фамотидина в таблетках. Основные пятна, полученные при хроматографировании испытуемых растворов, соответствуют размерам и положению основного пятна, полученного при хроматографировании раствора сравнения. Rf для всех растворов составил 0,5.

Выводы. Таким образом, как ТСХ, так и ВЭТСХ могут быть рекомендованы для включения в ГФУ. Однако ВЭТСХ является более экономически выгодной.

Ключевые слова: *фамотидин; идентификация; верификация; тонкослойная хроматография*

Fourteen dosage forms of famotidine were registered in Ukraine as of January 1, 2017. Half of them are produced by domestic pharmaceutical plants. Famotidine tablets with the content of 20 mg and 40 mg of the active substance are produced by three companies: PJSC "Pharmaceutical Firm "Darnitsa", Pharmaceutical company "Zdorovye" Ltd., LLC "Pharmex Group", and PJSC "Kyivmedpreparat" of Arterium Corporation [1]. However, the monograph for the famotidine substance and "Famotidine tablets" were not included in the second edition of the State Pharmacopoeia of Ukraine (SPhU). Monographs on the substance and dosage forms of famotidine are presented in the world's leading pharmacopoeias [2-4]. Since the agreement on the possibility of using the text for verification and adaptation for inclusion to the national pharmacopoeia was signed between the SPhU and the United States Pharmacopoeia (USP), the more attention was paid to the methods of identification given the USP [4].

To identify the active ingredient in famotidine tablets the USP offers to use thin layer chromatography (TLC) [4] with detection in UV light at the wavelength of 254 nm. Some authors [5, 6] suggest the use of high performance thin layer chromatography (HPTLC) to separate the famotidine mixture from other active ingredients. This method has several advantages compared to TLC in terms of the time for analysis and separation efficiency, and it makes it more interesting from the economic point of view.

The aim of our study was to carry out the verification of the TLC method for identifying the active substance in famotidine tablets for drug samples of the Ukrainian producers, and to check the possibility of using the HPTLC method for famotidine identification.

Materials and methods

The object of the study was "Famotidine" tablets with the content of the active substance of 20 mg manufactured by PJSC "Kyivmedpreparat" of Arterium Corporation, Kyiv, Ukraine, batch numbers: 106810, 127728, 133634 (excipients: lactose monohydrate, potato starch; povidone, calcium stearate, colloidal anhydrous silica), they were purchased in the pharmacy.

The famotidine substance (manufactured by Nakoda Chemicals Ltd, Telangana, India, batch No. FM-1507002) was used as working standard solution (WSS). The TLC/HPTLC system Camag® (Switzerland) was used in the study. To load samples a Camag® Linomat 5 sampling device, 100 µL Linomat microsyringes, and Gaschma technical nitrogen (Jonavos raj., Lithuania) were used. Detection and documentation of chromatograms were carried out using Camag® TLC Visualizer at the wavelength of 254 nm. The WinCATS® software was used for loading samples and analyzing chromatograms.

The verification of the TLC method was carried out using the TLC Silica gel 60 F254 glass plates with the size of 20 × 20 cm (Merck, Germany) with 0.25-mm layer of the chromatographic sorbent and a Camag® Twin Trough Chamber for plates with the size of 20 × 20 cm. HPTLC Silica gel 60 F254 glass plates with the size of 10 × 10 cm (Merck, Germany) with 0.25-mm layer of the chromatographic sorbent and a Camag® Twin Trough

Chamber for plates with the size of 10 × 10 cm were used for HPTLC studies.

The following reagents were used in the study: glacial acetic acid (Fluka Chemie, Switzerland); methanol (gradient grade, for HPLC, Sigma-Aldrich GmbH, Switzerland); toluene (gradient grade, Sigma-Aldrich GmbH, Switzerland); ethyl acetate (gradient grade, Sigma-Aldrich GmbH, Switzerland); 25 % ammonia solution (Merck, Germany).

The verification of the TLC method for identification of famotidine was conducted as follows.

Test solution. Place the weighed quantity of finely powdered tablets equivalent to 40 mg of famotidine into a 10-mL volumetric flask, then dissolve in glacial acetic acid using sonication and dilute with glacial acetic acid to the volume. Centrifuge the resulting solution to obtain a clear supernatant.

Standard solution. Prepare the solution of famotidine in the glacial acetic acid with the concentration of the active substance of 4 mg/mL.

The mobile phase: ethyl acetate *R* – methanol *R* – toluene *R* – concentrated ammonia solution (40 : 25 : 20 : 2).

The sample volume: 10 µL.

The distance that the mobile phase should pass: 15 cm from the starting line.

Drying: in the air.

Detection: under UV light at a wavelength of 254 nm.

Results: the main spots of the standard solution corresponded to main spots of the test solutions in appearance and R_f values [4].

For the HPTLC study the following parameters were changed:

The sample volume: 2 µL.

The distance that the mobile phase should pass: 7 cm from the starting line.

Results and discussion

The SPhU has signed agreements with the leading world's pharmacopoeias to ensure the possibility of using the existing methods for drug analysis. The verification is necessary for the analytical methods to be included into the SPhU in order to confirm the possibility of using the method in the quality control for domestically produced medicines [7].

The verification for identification of the TLC method was carried out by reproduction of the analysis methods and comparison of the analysis results for three different batches of the drug by one manufacturer. The test was conducted by comparing spots of the active substance of the standard solution and the test solution. The study was conducted according to the requirements of the SPhU monograph "2.2.27. Thin Layer Chromatography" [7, 8]. The chromatogram obtained in the study is shown in Fig. 1.

As seen from Fig. 1, when viewed at 254 nm, the test solutions exhibited the main spots of the active substance corresponding to the spots of the active substance of the standard solution. In addition, the R_f value was calculated for each spot of the active substance in relation to the front of the mobile phase. The R_f values were 0.50 for the spot of the active ingredient of the standard solution, as well as for the spots of the test solution obtained by chromatography.

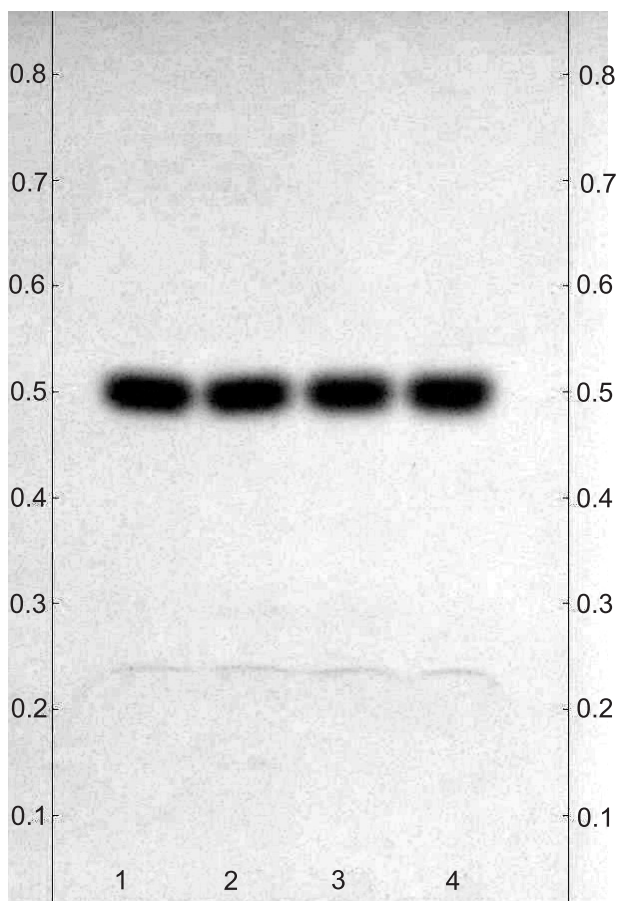


Fig. 1. The TLC chromatogram viewed at short-wavelength UV light: 1 – standard solution, test solutions of famotidine tablets manufactured by PJSC “Kyivmedpreparat” 2 – b. 106810, 3 – b. 127728, 4. – b. 133634

Thus, the correspondence of the drug batches of famotidine tablets studied to the standard solution confirms the correctness of the verified method of identification of famotidine by TLC. The method may be recommended for inclusion in the SPhU monograph for the quality control of famotidine tablets.

The HPTLC method compared to the TLC method has a number of significant advantages. The time of analysis is considerably shorter due to the use of plates with the size of 10×10 cm coated with the sorbent having the smaller grain size. The peculiarities of the sorbent structure contribute to a better resolution. Due to the high sensitivity the method requires the use of solutions with the lower concentration and the small sample volume commonly up to $5 \mu\text{L}$. Moreover, the use of small chromatographic chambers leads to reduction in the volume of the mobile phase needed for saturation of the chamber, proper resolution of plates and precision of the research [5-7].

Considering the number of significant advantages of HPTLC compared to TLC the possibility of using of this method for identification of famotidine in tablets was studied.

The methods used for preparation of the test solutions and the standard solution, test mobile phase, equip-

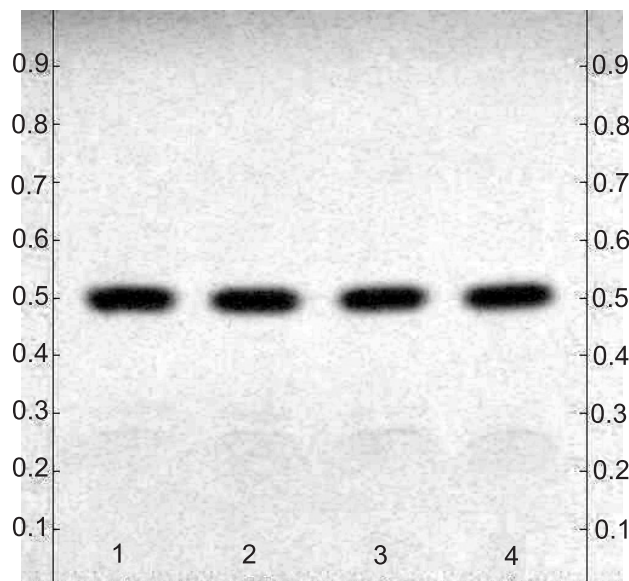


Fig. 2. The HPTLC chromatogram viewed at short-wavelength UV light: 1 – standard solution, test solutions of famotidine tablets manufactured by PJSC “Kyivmedpreparat” 2 – b. 106810, 3 – b. 127728, 4. – b. 133634

ment for applying and recording of the samples were the same as those used for the TLC study, however, some parameters were changed due to the change for more sensitive research method. The HPTLC chromatogram is shown in Fig. 2.

As seen from Fig. 2, the main spot of the active substance of the standard solution corresponded to spots of the test solutions. The R_f values calculated for each spot were 0.50.

Thus, the use of the TLC method for identification of famotidine offered by the USP can be successfully used in both TLC and HPTLC conditions with a sufficient accuracy and precision. Taking into account a number of significant advantages of the HPTLC method of analysis its application is more economically reasonable.

CONCLUSIONS

1. The method for famotidine identification in tablets in a thin layer of the sorbent has been verified to be included into the SPhU monograph.

2. The TLC method was reproduced using the ethyl acetate – methanol – toluene – ammonia solution (40 : 25 : 20 : 2) mobile phase, the plate under research was exposed to the short-wavelength UV light. The main spots of the active substance of the standard solution of famotidine and the test solutions are at the same level. The R_f values for each spot of these substances are 0.50.

3. When transferring the method from the TLC to HPTLC conditions it has been found that the main spots of the standard solution corresponded to main spots of the test solutions. The R_f values for each spots were 0.50. However, its advantages over the TLC method are as follows: much less time needed for analysis, the volumes of the mobile phase and the samples applied. Thus, it is more economically reasonable to employ this method.

Conflict of Interests: authors have no conflict of interests to declare.

REFERENCES

1. Державний реєстр лікарських засобів України [Електронний ресурс]. – Режим доступу : <http://www.drlz.com.ua/>
2. British Pharmacopoeia 2009. Volume I & II, Medicinal and Pharmaceutical Substances, Famotidine, P. 1–4 // Her Majesty's Stationery Office. – London, 2009. – Vol. 1. – 1359 p.
3. European Pharmacopoeia 9.2. 8.0 04/2013:1012 // Council of Europe : Strasbourg. – 2017. – P. 2211–2212.
4. Famotidine tablets // United States Pharmacopoeia 39 – NF34. USP Convention Inc. – 2017. – P. 3852–3854.
5. Novaković, J. High–Performance Thin–Layer Chromatography for the Determination of Ranitidine Hydrochloride and Famotidine in Pharmaceuticals / J. Novaković // J. Chromatogr. – 1999. – Vol. 846, Issue 1–2. – P. 193–198. doi: 10.1016/s0021–9673(99)00510–5
6. Development and validation of HPTLC method for estimation of ibuprofen and famotidine in pharmaceutical dosage form / Dimal A. Shah, Dixita J. Suthar, Chirag D. Nagda et al. // J. of Liquid Chromatography & Related Technologies. – 2004. – Vol. 37, Issue 7. – P. 941–950. doi: 10.1080/10826076.2013.765450
7. Державна фармакопея України : в 3-х т. – 2-е вид. – X. : Український науковий фармакопейний центр якості лікарських засобів, 2015. – Т. 1. – 1128 с.
8. Фармацевтичний аналіз : навч. посіб. для студ. вищ. фармацев. навч. закл. III–IV рівнів акредитації / П. О. Безуглий, В. А. Георгіянц, І. С. Гриценко та ін. ; за ред. проф. В. А. Георгіянц. – X. : НФаУ; Золоті сторінки, 2013. – 552 с.

REFERENCES

1. *Derzhavnyi reiestr likarskykh zasobiv Ukrainy*. Available at: <http://www.drlz.com.ua/>
2. British Pharmacopoeia. Volume I & II, Medicinal and Pharmaceutical Substances, Famotidine, P. 1–4. (2009). *Her Majesty's Stationery Office. London, 1, 1359*.
3. European Pharmacopoeia 9.2. 8.0 04/2013:1012. (2017). *Council of Europe*. Strasbourg, 2211–2212.
4. Famotidine tablets. (2017). *United States Pharmacopoeia 39 – NF34. USP Convention Inc.*, 3852–3854.
5. Novaković, J. (1999). High–performance thin–layer chromatography for the determination of ranitidine hydrochloride and famotidine in pharmaceuticals. *Journal of Chromatography A*, 846 (1–2), 193–198. doi: 10.1016/s0021–9673(99)00510–5
6. Shah, D. A., Suthar, D. J., Nagda, C. D., Chhalotiya, U. K., Bhatt, K. K. (2014). Development and validation of hptlc method for estimation of ibuprofen and famotidine in pharmaceutical dosage form. *Journal of Liquid Chromatography & Related Technologies*, 37 (7), 941–950. doi: 10.1080/10826076.2013.765450
7. *Derzhavna Farmakopeia Ukrainy, 3 (2(1))*. (2015). Kharkiv: Ukrainskyi naukovyi farmakopeinyi tsentr yakosti likarskykh zasobiv, 1128.
8. Bezuglyi, P. O., Georgiyants, V. A., Grytsenko, I. S. (2013). *Pharmatsevytchy analiz*. Kharkiv: NFAU; Zoloti storinky, 552.

Information about authors:

Myhal A. V., postgraduate student of the Pharmaceutical Chemistry Department, National University of Pharmacy. E-mail: artem.migal@gmail.com
 Marksa M., teaching assistant of the Analytical and Toxicological Chemistry Department, Lithuanian University of Health Sciences. E-mail: minzedas@gmail.com
 Golovchenko O. S., Candidate of Pharmacy (Ph.D.), associate professor of the Pharmaceutical Chemistry Department, National University of Pharmacy.

E-mail: golyas26@ukr.net

Georgiyants V. A., Doctor of Pharmacy (Dr. habil.), professor, the head of the Pharmaceutical Chemistry Department, National University of Pharmacy.

E-mail: vgeor@ukr.net

Ivanauskas L. Doctor of Biomedicine (Dr. habil.), “Pharmacy 08B” specialty, professor, the head of the Analytical and Toxicological Chemistry Department, Lithuanian University of Health Sciences. E-mail: liudas.ivanauskas@lsmuni.lt

Відомості про авторів:

Мигаль А. В., аспірант кафедри фармацевтичної хімії, Національний фармацевтичний університет. E-mail: artem.migal@gmail.com

Маркса М., асистент кафедри аналітичної та токсикологічної хімії, Литовський університет наук про здоров'я. E-mail: minzedas@gmail.com

Головченко О. С., канд. фармацев. наук, доцент кафедри фармацевтичної хімії, Національний фармацевтичний університет. E-mail: golyas26@ukr.net

Георгіянц В. А., д-р фармацев. наук, професор, завідувач кафедри фармацевтичної хімії, Національний фармацевтичний університет. E-mail: vgeor@ukr.net

Іванаускас Л., д-р біомед. наук, напрямок «Фармація 08В», професор, завідувач кафедри аналітичної та токсикологічної хімії, Литовський університет наук про здоров'я. E-mail: liudas.ivanauskas@lsmuni.lt

Сведения об авторах:

Мигаль А. В., аспирант кафедры фармацевтической химии, Национальный фармацевтический университет. E-mail: artem.migal@gmail.com

Маркса М., ассистент кафедры аналитической и токсикологической химии, Литовский университет наук о здоровье. E-mail: minzedas@gmail.com

Головченко О. С., канд. фармацев. наук, доцент кафедры фармацевтической химии, Национальный фармацевтический университет. E-mail: golyas26@ukr.net

Георгіянц В. А., д-р фармацев. наук, профессор, заведующая кафедрой фармацевтической химии, Национальный фармацевтический университет.

E-mail: vgeor@ukr.net

Иванаускас Л., д-р биомед. наук, направление «Фармация 08В», профессор, заведующий кафедрой аналитической и токсикологической химии, Литовский университет наук о здоровье. E-mail: liudas.ivanauskas@lsmuni.lt

Надійшла до редакції 21.03.2017 р.