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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
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: PrJSC “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 Chemikals 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 Gaschem 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 (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 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 Rf 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 Rf value was calculated for each spot of the active substance in relation to the front of the mobile phase. The Rf 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.
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 µL. 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, equipment 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 spot 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.
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