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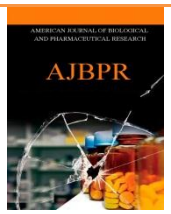

CHROMATOGRAPHIC STUDY OF THE MULTICOMPONENT ANTIFUNGAL CREAM

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ABSTRACT

In the past decade there has seen a trend towards manufacturing of multi-component medicinal products, which is associated with a modern approach to the combined pharmacotherapy – using multiple active pharmaceutical ingredients (APIs) in one dosage form. Enhancement of the pharmacological effect is achieved by the synergism or combined interactions of ingredients [1]. However, the combined dosage forms tend to be involved in various inter-component physicochemical interactions, which may affect the stability of the product during its manufacture, storage and, ultimately, its therapeutic efficacy. Therefore, the development of standardization methods for multi-component medicinal products is a relevant objective of pharmaceutical science [2-5].

INTRODUCTION

High-performance liquid chromatography is one of the universal methods to solve the above problem. The aim of this study was to develop the techniques for a qualitative and quantitative analysis of the multi-component antifungal cream suitable for pharmaceutical standardization.

MATERIALS AND METHODS

The cream containing clotrimazole, metronidazole, betamethasone dipropionate and urea in a water-in-oil base was an object of the study. The study was performed on the Agilent 1200 chromatograph with a diode-array detector (USA) under the conditions specified in the State Pharmacopoeia of Ukraine [6].

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Internal standard solution

60 milligrams of propylparahydroxibenzoate stock standard was accurately weighed into a 25-mL volumetric flask, dissolved in 10 mL of 96% ethanol and diluted to the volume with the same solvent. Then 5.0 mL of the solution was transferred to a 50-mL volumetric flask, brought to the volume with 96 % ethanol and mixed.

Betamethasone standard stock solution

23.1 milligrams of betamethasone dipropionate stock standard equivalent to 18 mg of betamethasone was accurately weighted into a 20-mL volumetric flask, dissolved in 10 mL of 96% ethanol and brought to the volume with the same solvent.

Reference solution

24.0 miligrams of standard sample clotrimazole and 15.0 mg of standard sample metronidazole were accurately weighted into a 50-mL volumetric flask and dissolved in 10 mL of 96 % ethanol. Then 2.0 mL of betamethasone standard sample and 5.0 mL of the internal

standard solution were added to the flask and the mixture was brought to the volume with 96% ethanol.

Assay preparation

3000 milligram of the cream was accurately weighed into a 100-mL flask. Then 5.0 mL of the internal standard solution and 45 mL of 96% ethanol were added. After that the flask was heated at 50°C in a magnetic stirrer until the cream completely dispersed. The content of the flask was cooled to a stable suspension by employing an ice bath. The resulting solution was centrifuged for 10 min at 6000 rpm and the supernatant was passed through a 0.45-µm Teflon filter, discarding the first mL of the filtrate.

96% ethanol and the reference solution (10 mL each) were chromatographed by liquid chromatograph under gradient conditions with UV-detector. At least 5 chromatograms were performed for each of the solutions under the conditions as follows:

- the chromatogram of 96% ethanol did not show system peaks with retention time of metronidazole, propylparahydroxibenzoate, betamethasone and clotrimazole peaks;
- the effectiveness of the chromatographic system calculated by the peaks of metronidazole, propylparahydroxibenzoate, betamethasone and clotrimazole seen in the chromatogram of the reference solution was not less than 2000 theoretical plates;
- symmetry factor of metronidazole, propyl parahydroxibenzoate, betamethasone and clotrimazole peaks was within 0.8 - 1.5;
- column size - 250 x 4.6 mm Luna C18 (2) 100 Å (Phenomenex), 5 µm particle size or equivalent, meeting the requirements of "System suitability test";
- the mobile phase flow rate - 1 mL /min;
- detector wavelength- 254 nm;
- column temperature – 30 ^{0}C.

Mobile phase A

4.28 grams of potassium dihydrogen phosphate anhydrous and 3.71 grams of sodium hydrogen phosphate anhydrous were dissolved in purified water and brought to 1000-mL volume with the same solvent. The mobile phase was filtered and degasified.

Mobile phase B

Acetonitrile for chromatography.Chromatography conditions are shown in Table 1.

Equation 1 was used to calculate the content of betamethazone (X_1) in 1 g of the cream (%)

$$X_1 = \frac{S_1 \times m_0 \times 2 \times 5 \times P}{S_0 \times m_1 \times 20 \times 5} \times \frac{392.46}{504.59} = \frac{S_1 \times m_0 \times P}{S_0 \times m_1 \times 10} \times \frac{392.46}{504.59}$$

(1), where,

S_1	betamethasone standard ratio calculated from the chromatograms of the reference solution;
S_0	betamethasone standard ratio calculated from the chromatograms of the test solution;
m_0	weight of betamethasone dipropionate taken to make the reference solution (mg);
m_1	weight of the investigated drug (mg);
P	content of betamethasone dipropionate in the standard sample taken to make the reference solution as a percentage [7, 8].

Equation 2 was used to calculate the content of clotrimazole /metronidazole (X_2) in the cream (%):

$$X_2 = \frac{S_1 \times m_0 \times 5 \times P}{S_0 \times m_1 \times 5} = \frac{S_1 \times m_0 \times P}{S_0 \times m_1}, \qquad (2),$$

where,

S_1	clotrimazole /metronidazole standard ratio calculated from the chromatograms of the reference solution;
S_0	clotrimazole /metronidazole standard ratio calculated from the chromatograms of the test solution;
m_0	weight of clotrimazole /metronidazole taken to make the reference solution (mg);
m_1	weight of the investigated drug (mg);
P	content of clotrimazole /metronidazole in the standard sample taken to make the reference solution, as a percentage.

Table 1. Chromatography conditions

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Elution mode
0-6	70	30	Isocratic mode
6-15	70 → 10	30 → 90	Linear gradient
15-19	10	90	Isocratic mode
19-20	10 → 70	90 → 30	Linear gradient
20-26	70	30	Isocratic mode

Table 2. The quantitative content of active ingredients in Betacarboclomet cream

Ingredients analysed	Labeled amount, mg/g	Found amount		Metrological characteristics
		mg/g	%	
Betamethazole dipropionate	0.65	0.653 0.658 0.646 0.654 0.658 mean value 0.655±0.01	 100.46 101.23 99.38 100.61 101.23	 X = 100.58 S(X) = 0.75 SX = 0.34 ε = ± 0.94 X ± SX = 100.58 ± 0.94
Clotrimazole	8.00	8.23 8.09 8.02 8.07 8.29 mean value 8.14±0.12	 102.87 101.12 100.25 100.87 103.62	 X = 101.74 S(X) = 1.43 SX = 0.64 ε = ± 1.75 X ± SX = 101.74 ± 1.75
Metronidazole	5.00	5.11 5.03 4.92 5.17 5.21 mean value 5.09±0.12	 102.20 100.60 98.40 103.41 104.20	 X = 101.76 S(X) = 2.32 SX = 1.04 ε = ± 2.83 X ± SX = 101.76 ± 2.83

Note. Number of measurements n = 5; P = 95%.

Figure 1. Chromatogram of the internal standard solution.

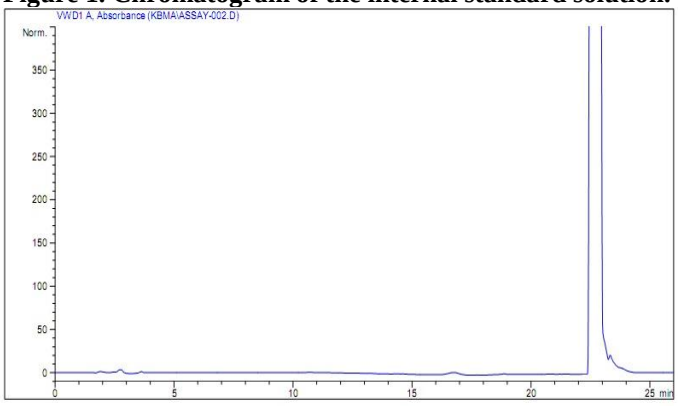


Figure 2. Chromatogram of the reference solution.

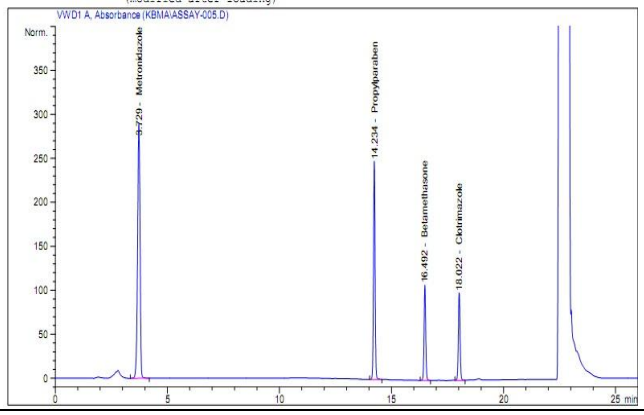
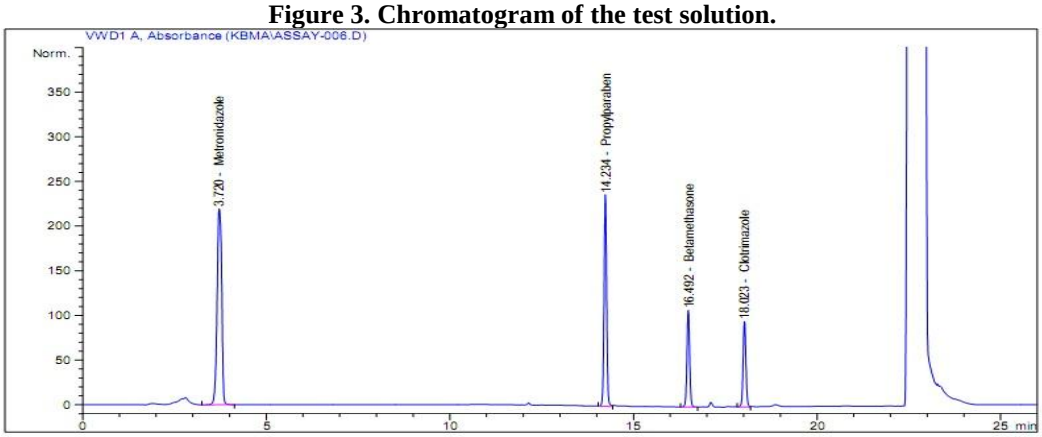


Figure 3. Chromatogram of the test solution.



RESULT AND DISCUSSION

The obtained chromatogram of the test solution shows that retention time values of the main peaks of the standard samples for clotrimazole, betamethasone dipropionate and metronidazole agree with the retention time of the peaks of the investigated samples and are 3.73, 16.49 and 18.02 min, respectively (figure 2 and 3), with the accuracy of ± 2%. The results of betamethasone dipropionate, clotrimazole and metronidazole quantification in the cream are shown in Table 1. The found content of the ingredients in 1 gram of the cream was as follows: metronidazole - 5.11 mg (normal values 4.75-5.25 mg); clotrimazole - 8.14 mg (normal values 7.60-8.40 mg); betamethasone dipropionate - 0.655 mg (normal values 0.6175-0.6825mg). The above content of betamethasone dipropionate corresponds to the content of betamethasone in 1 g of the cream (normal values: 0,4802-0,5308 mg), given the conversion factor of betamethasone dipropionate to betamethasone (392.46 / 504.59).

CONCLUSION

The chromatographic investigation carried out by HPLC showed that the described chromatography conditions provided sufficient selectivity and separation efficiency. The retention time of the main peaks of clotrimazole, metronidazole and betamethasone dipropionate solutions did not exceed 2% of the retention time of the peaks of the standard samples. The content of API in the analyzed soft drugs was within acceptable limits (± 5%). The above technique is a valuable tool to provide the quality control of the medicine.

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