SIMULTANEOUS ASSAYING OF FAMOTIDINE, CALCIUM CARBONATE AND MAGNESIUM HYDROXIDE IN THE EXTEMPORANEOUSLY PREPARED SUSPENSION

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ABSTRACT

INTRODUCTION: The share of extemporaneously prepared drugs on the pharmaceutical market has been on the increase lately. The suspension of famotidine with calcium carbonate and magnesium hydroxide is one of such drugs.

AIM: The aim of our study is to develop the assay methods for each component in the simultaneous sample of the suspension in order to decrease the volume of the medical form and the time for analysis.

MATERIALS AND METHODS: The object of study is the extemporaneously prepared suspension with the content of active substances per 5 ml: famotidine 10 mg; calcium carbonate 400 mg; magnesium hydroxide 120 mg. Tablets with famotidine content (20 mg) were used for suspension preparation.

The substance of famotidine was used as the working standard sample (WSS). The “Evolution 60S” spectrophotometer, the AB 204 S/A Mettler Toledo analytical balances, class A measuring vessels as well as reagents that meet the requirements of the State Pharmacopeia of Ukraine were used in the study.

RESULTS: Complexometric titration for calcium carbonate and magnesium hydroxide using murexide and Eriochrome Black T as indicators and UV-spectrophotometric determination of famotidine at $\lambda_{\max}=265$ nm wavelength were carried out in the simultaneous sample of the suspension. The metrological characteristics confirm the correctness of the proposed methods.

CONCLUSIONS: The developed methods can be used to determine the active compounds in the simultaneous sample of the suspension. The uncertainty of the average results for calcium carbonate quantitative determination was 0.94%, for magnesium hydroxide – 1.14%, and for famotidine – 0.75%.

Keywords: famotidine, antacids, suspension, complexometry, UV-spectrophotometry

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INTRODUCTION

The share of extemporaneously prepared drugs on the pharmaceutical market has been on the increase lately. Its main advantage in comparison to drugs of industrial production is the possibility of taking into account individual characteristics and the patient's needs (1). The use of finished dosage forms is common in the world's pharmaceutical

compounding. One of the basic conditions of preparing drugs in pharmacies is the presence of precise, easily reproducible and economically accessible assay methods for each component included in the formula. Taking into account the conditions of economic availability, the physical and chemical methods of analysis offered by the leading international Pharmacopoeias (2-4) are not acceptable for the majority of Ukrainian pharmacies because of the high price of standard samples, expensive equipment, etc. (5). Therefore, chemical methods of analysis, which are more accessible for pharmacies, should be suggested to confirm the proper quality of pharmaceutical drug production.

Famotidine is an antiulcer drug of the new drugs generation that belongs to the group of pharmacological antagonists of histamine H_2 -receptors. Nowadays it is prescribed because of its higher efficiency, lower toxicity and lower doses than its predecessors (1). In the gastroenterological practice famotidine is used independently as well as in combined dosage forms, for example, with carbonates and hydroxides of alkaline earth metals. Such combined dosage drugs are aimed at a rapid and lasting acid-lowering effect due to a combination of active ingredients. The famotidine suspension with calcium carbonate and magnesium hydroxide is an example of such combined dosage form. However, a dosage form with such composition requires developing the methods of quantitative determination for active substances different in their nature. This assay method should give the pharmacist-analyst the opportunity to set the quantitative composition of active substances using common methods with the minimum consumption of the compounded drug needed for analysis.

AIM

The aim of our research is to develop the methods for determining the stability of the components in the suspension, which would reduce the time for sample preparation and analysis and would require taking only one aliquot of the dosage form.

MATERIALS AND METHODS

Extemporaneously compounded suspension containing the active compounds per 5 ml: famotidine 10 mg; calcium carbonate 400 mg; magnesium

hydroxide 120 mg was chosen as the object of the study (6,7). Suspension was prepared in accordance with the following prescription:

Rp.: Tab. Famotidine 20 mg/5 tabl.
Calcii carbonatis 4 g
Magnesii hydroxide 1.2 g
Aquae purificatae 50 ml.

The method of the suspension preparation: 5 tablets of famotidine containing 20 mg of the active ingredient were thoroughly pulverized in a mortar, then 4 g of calcium carbonate and 1.2 g magnesium hydroxide, carefully ground in a mortar to homogeneity in a dry form were added. Afterwards half of the amount of purified water was added and carefully ground to ensure maximum dispersion. Subsequent to that, the remaining parts of water were added and transferred to the bottle, washing the mortar from the walls, trying to quantitatively transfer the dispersed compounds.

Famotidine tablets with the active substance content of 20 mg (manufactured by Kyivmedpreparat PJSC, Arterium Corporation, Kyiv, Ukraine) were used for the suspension preparation. Calcium carbonate and the magnesium hydroxide (Czech Republic) of analytical purity grade were used.

The famotidine substance (manufactured by Nakoda Chemicals Ltd, Telangana, India) was used as the working standard sample (WSS).

The "Evolution 60S" Spectrophotometer (USA), the "AB 204 S/A METTLER TOLEDO" analytical balances (Poland) as well as class A measuring vessel and reagents that conform to the State Pharmacopoeia of Ukraine (SPhU) (2) which is a permanent member of the Commission of the European Pharmacopoeia (Ph.Eur.) and fully harmonized with regard to the quality requirements for pharmaceutical products were used (4).

Quantitative determination of active substances in the investigated suspension

Calcium carbonate: 5 ml (exact weight amount) previously shaken and the suspension free from air bubbles, equivalent to 400 mg of calcium carbonate, were placed in a beaker, adding 20 ml of water R and 10 ml of diluted hydrochloric acid solution. The obtained sample was heated in a water bath for 30 minutes, cooled, quantitatively transferred to a 100 ml

volumetric flask and the volume of the solution was adjusted up to the mark with water R.

Of the resultant solution 20 ml were transferred into a conical flask; the volume of the solution was adjusted to 100 ml with water R and 20 ml of the diluted sodium hydroxide solution were added to pH 12-13, then 100 mg of murexide indicatory mixture were added. The solution was titrated with the 0.1 M sodium edetate solution until the colour changed from pink to violet (2, 4, 5, 8, 9 and 10).

Total amount of calcium carbonate and magnesium hydroxide: 20 ml of the resultant solution, obtained from the previous sample preparation, equivalent to about 120 mg of magnesium hydroxide, were placed in a conical flask, the solution was adjusted to the 100 ml volume with water P, 20 ml of ammonia buffer solution pH 10.0 was added to pH 9-10 of the resultant solution, then 100 mg of Eriochrome Black T indicatory mixture were added. The solution was titrated with 0.1 M sodium edetate solution until the colour changed from violet to deep blue (2,4,9-11).

The difference between the titrant's volumes that were spent for titration of the total amount of the metal cations and those were spent on the titration of calcium carbonate, was used to calculate the content of magnesium hydroxide (4, 9, 10,11):

$$V_{\text{EDTA}(\text{Mg})} = V_{\text{EDTA}(\text{Mg}+\text{Ca})} - V_{\text{EDTA}(\text{Ca})},$$

where $V_{\text{EDTA}(\text{Mg})}$ – calculated volume of 0.1 M sodium edetate solution spent for titration of magnesium hydroxide, ml;

$V_{\text{EDTA}(\text{Mg}+\text{Ca})}$ – volume of 0.1 M sodium edetate solution spent for titration of the total amount of calcium carbonate and magnesium hydroxide, ml;

$V_{\text{EDTA}(\text{Ca})}$ – volume of 0.1 M sodium edetate solution spent for titration of calcium carbonate, ml.

The quantitative content of the active substances, determined by the complexometric method, was calculated according the formula (5):

$$x, g = \frac{V_{\text{EDTA}} \cdot K_{\text{EDTA}} \cdot T_{\text{EDTA}/\text{subst.}} \cdot V_{\text{v.r.}} \cdot m_{\text{recipe}}}{m_{\text{analysis}} \cdot V_p}$$

where V_{EDTA} – volume of 0.1 sodium edetate solution spent for titration of the test solution, ml;

K_{EDTA} – correction coefficient of 0.1 M sodium edetate solution;

$T_{\text{EDTA}/\text{subst.}}$ – titre of 0.1 M sodium edetate solution, mg/ml;

$V_{\text{v.f.}}$ – volumetric flask volume, ml;

V_p – pipette volume, ml;

m_{analysis} – weight amount of the sample for analysis, g;

m_{recipe} – suspension weight amount by the recipe, g.

Famotidine. Absorption spectrophotometry in the UV part of the spectrum

Sample solution: 10 ml of the resultant solution from the experiment for quantitative determination of calcium carbonate were transferred into a 100-ml volumetric flask, the solution volume was adjusted to the mark with 0.1 M hydrochloric acid solution. The solution was mixed thoroughly (concentration of the sample solution was 0.02 mg/ml).

Standard solution: 10 mg (accurate weight amount) was placed in a 100-ml volumetric flask; 40 ml of 0.1 M hydrochloric acid solution was added. If necessary, it could be treated with ultrasound. The solution volume was adjusted up to the mark with the same solvent. The solution was mixed thoroughly. After that, 10 ml of the obtained solution were transferred to a 50-ml volumetric flask and the volume of the solution was adjusted to the mark with the same solvent.

The measurement was conducted on a spectrophotometer at the wavelength of famotidine absorbance maximum at $\lambda_{\text{max}} = 265 \text{ nm}$ with the cuvette replace for three times. The average value was used for calculations. The amount of hydrochloric acid solution used as the compensation solution was 0.1M (4,9,12,13,14,15,16).

The content of the active compound was calculated according to the formula:

$$x, g = \frac{A_{\text{inv}} \cdot m_{\text{st}} \cdot P \cdot 10 \cdot 100 \cdot 50 \cdot m_{\text{prescription}}}{A_{\text{st}} \cdot m_{\text{analysis}} \cdot 100 \cdot 100 \cdot 50 \cdot 10}$$

where A_{inv} – absorbance of the sample solution;

A_{st} – absorbance of the standard solution;

m_{analysis} – weight amount of the sample for analysis, g;

m_{st} – weight amount of the WSS, g;

P – content of active compound in the famotidine WSS, %;

$m_{\text{prescription}}$ – suspension weight amount by the prescription, g.

lution of 0.1 M hydrochloric acid solution. Results of the measurements are shown in Table 3.

Table 1. Results of calcium carbonate and magnesium hydroxide titration

Substance	Sample weight amount, g	Titrant volume, ml	Quantitative content	
			x, %	x_{aver} , %
CaCO ₃	0.4152	8.3	100.05	99.97
	0.4062	8.1	99.80	
	0.4001	8.0	100.07	
Mg(OH) ₂	0.1237	3.6	99.84	100.68
	0.1222	3.6	101.06	
	0.1221	3.5	101.14	

Table 2. Results of the titration of calcium carbonate and magnesium hydroxide, total amount

№	Sample weight amount, g		Volume of titrant, ml			Quantitative content, x, %	
	CaCO ₃	Mg(OH) ₂	for CaCO ₃ titration	for the total amount of substance titration	volume difference	CaCO ₃	Mg(OH) ₂
1	0.4152	0.1237	8.2	11.8	3.6	100.05	99.84
2	0.4062	0.1222	8.1	11.7	3.6	99.80	101.06
3	0.4001	0.1221	8.1	11.6	3.5	101.32	98.34
aver.	-	-	-	-	-	100.39	99.75

RESULTS

The results of calcium carbonate and magnesium hydroxide titration are shown in Table 1 and those of the determination of their total amount are presented in Table 2.

The use of the photometrical methods for quantitative determination was based on the Beer-Lambert law. To verify the compliance of the famotidine solution with the Beer-Lambert law, the graph of dependence of the absorbance (A) of the test solution on its concentration (C) in the 0.0005-0.005% concentration range was built (Fig. 1).

Famotidine was measured in model mixtures in the presence of calcium carbonate and magnesium hydroxide in the quantities that correspond to its content in the suspension. The measurement of the standard solution absorbance was carried out at the same time. Absorbance was measured three times removing the cell against the compensation so-

Approbation of testing the assay method on the drug sample

The determination of the quantitative content of the active compounds such as calcium carbonate, magnesium hydroxide and famotidine was conducted in the suspension extemporaneously prepared. The results of the investigations are shown in Ta-

Fig. 1. The plot of linear dependence of absorbance on the concentration of famotidine in 0.1 M hydrochloric acid solution

bles 4 and 5. The metrological characteristics of the average results of the quantitative determination of the active compounds in the extemporaneously prepared suspension are shown in Table 6.

Table 3. The results of famotidine measurements in model mixtures

Sample	The weight amount of the famotidine sample, g	Absorbance, $A_{st} = 0.694$	Quantitative content, %
1	0.099	0.689	99.3
2		0.689	99.3
3		0.690	99.4

DISCUSSION

Complexometric determination of calcium carbonate and magnesium hydroxide in the simultaneous sample.

The SPhU as well as the world's leading pharmacopoeias (2, 3, 4 and 5) recommend complexometric determination for drugs containing alkaline earth metals and heavy metals salts. After analyzing a number of literary sources and the properties of the substances under study, we decided to develop methods for determining the simultaneously present amounts of calcium carbonate and magnesium hydroxide, based on certain differences in the acid-base properties of the indicated substances.

Some literary sources (4, 8 and 9) suggest determining the total amount of magnesium and cal-

Table 4. The results of calcium carbonate and magnesium hydroxide titration in suspension

№	Volume of aliquot, ml	The weight amount of the sample, g	Volume of the titrant, ml			Quantitative content, x, g	
			For CaCO_3 titration	For the total amount of substances titration	Volume difference	CaCO_3	Mg(OH)_2
1	5	5.0361	7.4	11.1	3.7	4.06	1.18
2		5.0543	7.3	11.0	3.7	3.99	1.18
3		5.0239	7.2	11.0	3.8	3.96	1.22
aver.	-	-	-	-	-	4.00	1.19

Table 5. The results of the quantitative determination of famotidine in suspension

Sample	Volume of aliquot, ml	The weight amount of the famotidine sample, g	Absorbance, $A_{st} = 0.694$	Quantitative content, %
1	5	5.0361	0.581	9.9
2		5.0543	0.578	9.8
3		5.0239	0.569	9.7
average	-	-	-	9.8

Table 6. Metrological characteristics of the average result of quantitative determination of calcium carbonate, magnesium hydroxide and famotidine in extemporaneously prepared suspension ($P=95\%$, $t(P,v) = 2,5706$)

Substance	n	$\bar{x}$	S^2	S	$S_{\bar{x}}$	Δx	$\Delta \bar{x}$	$\bar{\epsilon}\%$
Calcium carbonate	5	4.00	$2.1067 \times 10(-3)$	0.0459	0.0187	0.0925	0.0378	0.9431
Magnesium hydroxide	5	1.19	$4.2667 \times 10(-4)$	0.0207	0.0084	0.0416	0.0170	1.4239
Famotidine	5	9.8	$8.0000 \times 10(-3)$	0.0894	0.0365	0.1802	0.0736	0.7508

cium cations at pH 10 of the reaction medium in the presence of Eriochrome Black T indicator mixture, which forms complexes purple with the calcium and magnesium ions. Adding of the EDTA causes gradual displacing of the indicator from the complexes. After all, there is only one blue colour of free indicator remaining. But the change of the indicator's colour is gradual. So, the titration has to be carried out to totally change the colour, using overtitrated sample as the colour reference.

For calcium cations titration there is another indicator – murexide should be used, which forms red-pink complexes with calcium in the medium with pH level 12 and above. It should be noted that at this pH level magnesium ions are precipitated as hydroxides (5,9,10,11). Thereby, to determine calcium and magnesium in simultaneous presence, two titrations should be conducted: one of them at pH level 10 using the Eriochrome Black T indicating mixture to determine the total amount of calcium and magnesium, and another one is titration at pH level 12 using the murexide indicating mixture for calcium cations determination.

To verify the suitability of the methods, studies were carried out on the initial substances of the active ingredients in the suspension.

Weight amounts of substances were taken on an analytical balance in the amounts equivalent to 400 mg calcium carbonate and 120 mg for magnesium hydroxide. The volumes of solutions were adjusted to the volumes stated in the assay method of the suspension. The solution of diluted hydrochloric acid was used to acidify the reaction medium. Then the solutions under study were heated for 30 minutes, cooled up to the room temperature, quantitatively transferred into a 100-ml volumetric flask, washing off the remains from the beaker walls with the solvent, and the solution volume was adjusted up to the mark with the water.

As the pH value is an important condition for complexometric titration, we paid particular attention to this question while we developed the method.

The necessary 12-13 pH level has been achieved by adding the diluted sodium hydroxide solution and 9-10 pH level has been achieved by adding the ammonia buffer solution with pH 10.0. Acidity was controlled by putting the drop of the solution on the uni-

versal indicatory paper and comparing colour changes of the paper with the pH value scale.

The mechanisms of the complexometric determination of calcium cations and the total amount of calcium and magnesium cations are shown in Fig. 2 and Fig. 3:

Fig. 2. Mechanism of calcium cations interaction during complexometric determination of Ca^{2+}

Fig. 3. Mechanism of metal cations interaction with titrant during determination of the total amount of Mg^{2+} and Ca^{2+}

As shown in Table 1, the substances meet the requirements of the SPHu in terms of quantitative con-

tent. Moreover, the data from Table 2 confirm the possibility of separate assaying of calcium carbonate and magnesium hydroxide in the simultaneous presence with the reproducible results.

UV-spectrophotometric determination of famotidine on model mixtures

The method of absorption spectrophotometry in the ultraviolet part of the spectrum was suggested to determine famotidine in the suspension. As shown on Fig. 1, the graph of dependence of absorbance on the concentration of famotidine in the 0.0005-0.005% concentration range is linear. From the data obtained in the measurement of famotidine in model mixtures we can conclude about a slight undercalculation of the research results within one per cent compared with the famotidine standard sample. However, the error of the result is within the tolerances according to the SPhU, therefore, the method can be used for the quantitative determination of famotidine in suspension in the suggested conditions.

Approbation of testing the assay method on the drug sample

The data obtained during the analysis of the dosage form meets the requirements of the norms of deviation according to the order of the Ministry of Health of Ukraine number 812 from 17.10.2012, and the SPhU (2,17).

So, statistically processed, the experimental data indicate that the developed assaying method is characterized by precision, can be reproducible in conditions of laboratories for quality control of medicinal products and compounding pharmacies, and used to analyse famotidine suspension with calcium carbonate and magnesium hydroxide.

CONCLUSION

1. During the research the methods of assaying famotidine, calcium carbonate and magnesium hydroxide in the suspension in simultaneous presence at one sample were suggested for the first time.
2. The conditions of complexometric titration for quantitative determination of calcium carbonate and magnesium hydroxide in combined presence were chosen. Determination of calcium carbonate was conducted with murexide and the total amount of calci-

um carbonate and magnesium hydroxide was determined with Eriochrome Black T with the following calculation of the difference of titrant volumes spent for titration of calcium cations. The presence of famotidine does not prevent the determination.

3. The method of the absorption spectrophotometry in UV part of the spectrum, by the standard method, was proposed for the quantitative determination of famotidine.
4. The developed methods were tested on the model mixtures and on the dosage form. The obtained data meet the requirements of the deviation norms of the SPhU and the order of Ministry of Health of Ukraine № 812 from 17.10.2012.

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