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DEVELOPMENT OF THE QUANTITATIVE DETERMINATION OF THE MEDICINAL PREPARATION DERIVATIVE OF GOSSYPOL

Mamatmusaeva N. E.[1]
Ziyaev H.L.[2],
Sagdullaev B.T.[2]
Tashkent Pharmaceutical Institute[1]
Institute of Bioorganic Chemistry named after acad. A.S. Sadykov ASRUz[2]

Relevance

Plant substances with physiological activity have been used in medicine since time immemorial as effective medicines. It was found that many substances isolated from plants and playing an important role in their development and growth have IFN-inducing activity. These include purines, phenols and their derivatives (theophylline, caffeine, theobromine, tannin, etc.) [1].

As a result of numerous screenings, IFN inducers with a high immunotherapeutic effect have been identified to date. Of these, natural compounds include low-molecular polyphenols - gossypol derivatives (megasin, kagocel, savrac, gozalidone, rogasin) [2,3].

To date, the incidence of influenza and other acute respiratory diseases (ARI) exceeds the total incidence of all other infections. During the influenza epidemic, they account for 10-50% of the temporary disability of the population. In other years, influenza and acute respiratory infections account for up to 40% of all adult diseases registered in polyclinics, more than 80% of all infectious diseases and more than 60% of diseases among children. Mortality from influenza during epidemics in different age groups ranges from tens to hundreds of cases, and during a pandemic, the rate can reach 1,000 cases per 100,000 population [4,5].

Polyphenols are the main raw material for the creation of broad-spectrum drugs due to their high biological activity and low toxicity. Based on them, antiviral, anti-inflammatory, antiulcer and antitumor drugs have been created. A derivative of gossypol - the drug Rometin (megosin complex with N-polyvinylpyrrolidone) is a development of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan.

The results of previous clinical studies of the drug Rometin preparation at a dose of 100 mg indicate a pronounced antiviral and immunotropic (interferon inducer) activity in influenza [6].

Objective of the Research

Development of a method for quantitative determination of the Rometin preparation using the HPLC method. The use of the HPLC method for the analysis of the Rometin preparation is considered the most optimal, since this method makes it possible to simultaneously identify the compound by retention time - a specific characteristic for a natural compound under the same standard conditions, and simultaneously carry

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out their quantitative determination in the substance and dosage form. An objective prerequisite for improving the method of quantitative analysis of the substance and dosage form of Rometin is the availability of a standard sample (SS) of rometin developed at the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan [7,8,9,10,11].

Samples were used in the analysis: the substance and the finished capsule dosage form of rometin. To obtain the capsule form of rometin, a number of technological operations were carried out: in a dry, clean mixer, they are sequentially loaded in the form of pre-sifted powders (active and excipient) and thoroughly mixed until smooth, after which they are encapsulated into hard gastric or enteric capsules. Then the dust-free and selected capsules are packed on a blister-packing machine according to No.10 and packed in consumer packaging.

Materials and Methods

Approximately 60 mg (accurately weighed) of a standard sample of rometin (USP reference standard or working standard) are placed in a 50 ml volumetric flask, dissolved in the mobile phase, the volume of the solution is adjusted to the mark with the mobile phase and mixed. 5.0 ml of the resulting solution is transferred into a volumetric flask with a capacity of 50 ml, the volume of the solution is adjusted to the mark with the mobile phase and mixed. 2 ml of the solution is filtered through a membrane filter with a pore diameter of 0.45 μm. A Durapore hydrophilic filter or a Pall Pharmalab Nylon 66 membrane filter was used as a filtering device. The shelf life of the standard solution is no more than 24 hours when stored at room temperature and no more than 9 days in the refrigerator.

In parallel, under the same chromatographic conditions, the standard sample was analyzed. About 1.0 mg of rometin standard sample (USP reference standard or working standard) and about 1.0 mg of rometin sulfone are placed in a 10 ml volumetric flask, dissolved and the volume of the solution is adjusted to the mark with the mobile phase and mixed. The shelf life of the permissive solution is not more than 9 days in the refrigerator. The test solution was prepared as follows: the contents of 20 capsules were emptied and weighed. The contents of the capsule were weighed - a powder equivalent to 60 mg of rometin and placed in a volumetric flask with a capacity of 50 ml, about 30 ml of the mobile phase was added, sonicated for 10 minutes, the volume of the solution was brought to the mark with the mobile phase and mixed. 5.0 ml of the obtained solution was transferred into a volumetric flask with a capacity of 50 ml, the volume of the solution was brought up to the mark with the mobile phase and mixed. 2 ml of the solution was filtered through a Durapore hydrophilic membrane filter or a PallPharmalabNylon 66 membrane filter. The shelf life of the test solution is no more than 24 hours when stored at room temperature and no more than 9 days in the refrigerator.

Quantitative determination of rometin by HPLC. The determination is carried out by HPLC [12,13].

Chromatography Conditions

Instrument: Liquid chromatograph equipped with a 215 nm UV detector
Column: C8, Prodigy C8, 150 x 4.6 mm, 5 µl, packed with octadecylsilane bound to porous silicon or ceramic microparticles 3-10 µm in diameter (an alternative column may be used that meets the suitability requirements of the chromatographic system).
Flow rate: 0.8 ml/min.
Column temperature: Room.

Preparation of buffer solutions

About 1.40 g of sodium hydrogen phosphate is placed in a volumetric flask with a capacity of 1000 ml, dissolved in water and the volume of the solution is adjusted to the mark with water, mixed. Adjust pH to 7.9 with phosphoric acid, filter through a 0.45 µm Durapore hydrophilic membrane filter or 0.45 µm PallPharmalabNylon 66 membrane filter. The pH of the buffer should be 7.9.
Mobile phase. Mix buffer pH 7.9 and acetonitrile (HPLC grade) in a ratio (71:29, v/v) and sonicate for 10 minutes.

Carrying out chromatographic analysis

Introduce 40 µl of the resolving solution, the standard solution (five times), the tested solution into the liquid chromatograph, register the chromatograms and measure the areas of the main peaks.
On the chromatogram of the standard solution, the efficiency of the chromatographic column, calculated from the peak of rometin, should be at least 5000 theoretical plates.
Note: The retention time for the non-retained compound (maleic acid) and percent yield for the C8 column, Prodigy C8, 150 x 4.6 mm, 5 µm are 1.9 minutes, respectively.
The specificity of this analytical procedure was proven by comparing the analyte and the standard sample.
On the obtained chromatograms, the retention time of the main peak of rometin in the chromatogram of the tested solution is observed to correspond to the retention time of the rometin peak in the chromatogram of the standard solution (Fig. 1 and 2).

The amount of rometin in the capsule (X) as a percentage of the declared amount is calculated by the formula:

$$X = \frac{At \cdot Ws \cdot 5 \cdot 50 \cdot 50 \cdot Aw \cdot P \cdot 100}{As \cdot 50 \cdot 50 \cdot Wt \cdot 5 \cdot 100 \cdot L} = \frac{At \cdot Ws \cdot Aw \cdot P}{As \cdot Wt \cdot L} \quad (1)$$

where,
At is the area of the rometin peak on the chromatogram of the tested solution;
As is the average peak area of rometin in the chromatogram of the standard solution;
Ws - weight of standard sample of rometin, in milligrams;
Wt - weighed sample of the drug, in milligrams;

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P is the purity of the rometin standard, calculated as;
Aw is the average weight of the contents of the capsule, in milligrams;
L is the declared amount of rometin in one capsule, in milligrams

Results and Discussion

The specificity of this analytical procedure was proven by comparing the analyte and the standard sample. Based on the results of the obtained chromatograms, it can be seen that the retention time of the main peak of rometin in the chromatogram of the tested solution corresponds to the retention time of the rometin peak in the chromatogram of the standard solution (Fig. 1 and Fig. 2).

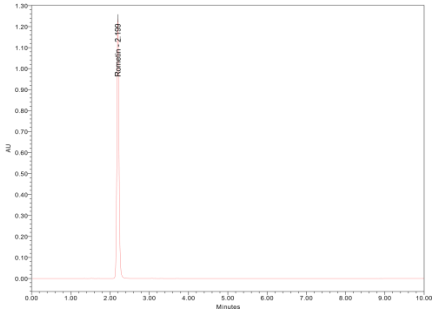

Fig. 1. Chromatogram of rometin standard solution

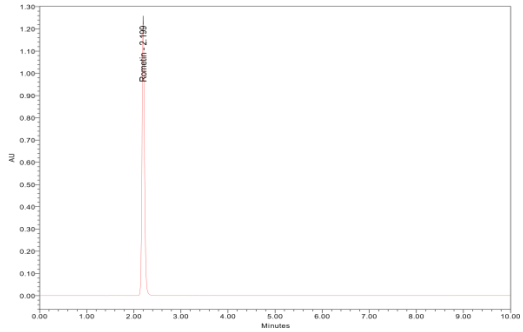

Fig. 2. Chromatogram of rometin tested solution

The linearity of the method was determined at five concentration levels: 80%, 90%, 100%, 110%, 120% of the working concentration of the analyte (in triplicate). For 100% working concentration, the value of the rometin content of 50 µg/ml was taken.

Table 1 Determination of linearity method

% of working concentration	№	Rometin content, mcg/ml	Peak area, mAU	Linear equation, correlation coefficient
80	1	80	3085483	y = 360532x + 3E+06 R = 0.9991
	2		3075946	
	3		3090865	
90	1	90	3496312	
	2		3489264	
	3		3485995	
100	1	100	3808284	
	2		3810647	
	3		3799565	
110	1	110	4151843	
	2		4148371	
	3		4155800	
120	1	120	4557462	
	2		4549682	
	3		4560909	

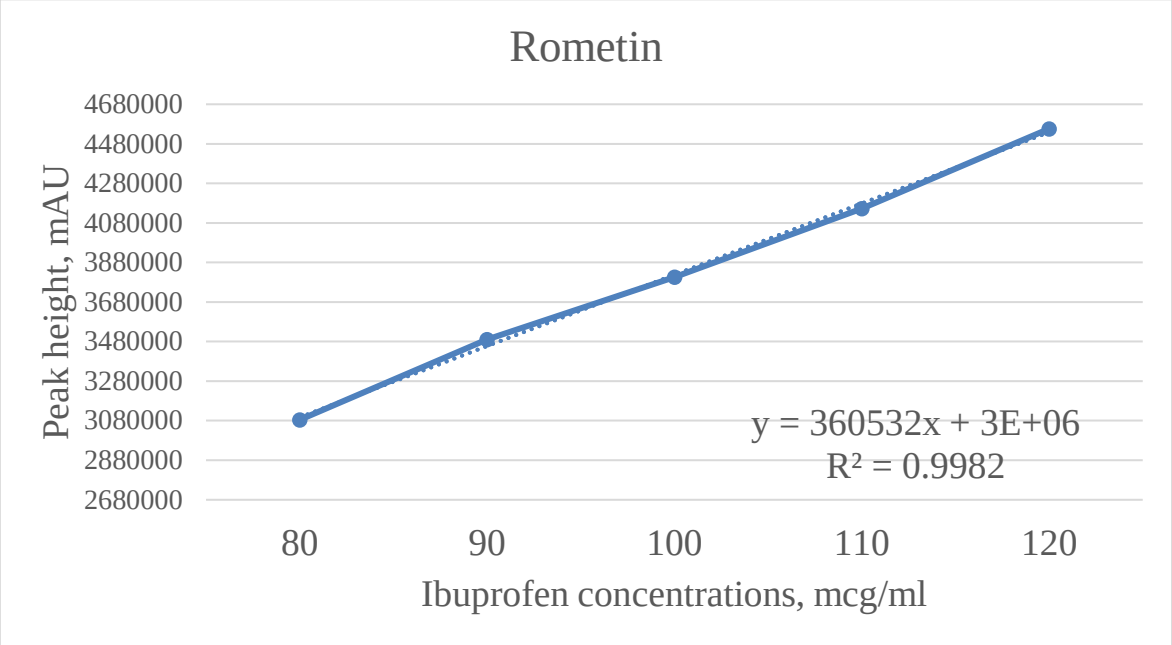

Fig.3. Schedule of rometin tested solution

The presented graph shows the presence of a well-defined linear relationship with a correlation coefficient of 0.9991.

Acceptance criterion: the correlation coefficient must be at least 0.990.

The correctness of the analytical procedure was confirmed on nine prepared standard sample solutions in the concentration range from 80 to 120% of the working concentration of the analyte.

Calculation of the deviation of the results obtained by the formula:

$$\delta = \frac{(X_{av} - X_c)}{X_c} \cdot 100 \quad (2)$$

where: δ - relative value of systematic error, %;

X_{av} - average concentration, in milligrams per milliliter;

X_c - calculated concentration from the target, in milligrams per milliliter.

Table 2 Measurement results

% of working concentration	Rometin taken, mcg/ml	Peak height, mAU.	Found Rometin, mcg/ml	Rometin yield value, %	Average value, %	SD	RSD, %
80	80.05	3085483	80.09	100.05	100.01	0.24	0.24
	80.05	3075946	79.84	99.74			
	80.05	3090865	80.23	100.22			
100	100.04	3808284	100.62	100.58	100.52	0.15	0.15
	100.04	3810647	100.68	100.64			
	100.04	3799565	100.39	100.35			
120	119.98	4557462	119.94	99.97	99.93	0.13	0.13
	119.98	4549682	119.74	99.80			
	119.98	4560909	120.03	100.04			
Average value=100.15; SD=0,0,18; RSD =0,0,17 %; x̄-μ=0.13; t_c=0,35; tp,f=0,0,46, δ = 0.29 %							

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The results obtained by this method are not burdened by the systematic error ($t_c<t_{p,f}$) the confidence interval of the true (100 ± 0.13) and the relative standard deviation (RSD) < 2.0%, and the value of the yield of the analytical procedure is in the range of 90 up to 110%, which corresponds to the accepted criterion. The chosen technique is characterized by good repeatability of results.

Determining the repeatability of a method. The test was performed by one analyst by multiple repetitions with the same homogeneous sample (9 repetitions). The rutin content of 0.05% was taken as 100% working concentration.

Calculation of the variation coefficient

$$V = RSD \cdot 100 \quad (3)$$

where: V - coefficient of variation (dispersion) %;

RSD - relative standard deviation.

$$RSD = S/Xav \quad (4)$$

where: S- standard deviation;

Xav - average concentration of all definitions.

$$S = \sum_{i=1}^{n}(X - X_{av})^2/(n-1) \quad (5)$$

where: S - standard deviation;

X - concentration obtained as a result of the experiment, %;

X_{av} - average concentration of all determinations;

n - number of determinations.

Table 3 Measurement results

№	Sample of the preparation, g	Rometin peak height, mAU	Rometin content, mg/caps
1	0.2043	3808284	98.69
2	0.2051	3810647	99.14
3	0.2081	3799565	100.30
4	0.2009	3856422	98.27
5	0.2043	3800947	98.50
6	0.2054	3869565	100.82
7	0.206	3792191	99.09
8	0.2104	3822765	102.02
9	0.2039	3889936	100.61
Average value	0.2054	3827813.5556	99.7153
SD	0.0027	35197.9501	1.2750
RSD, %.	1.3071	0.9195	1.2787

According to the results of Table 2, it can be seen that the coefficient of variation does not exceed 2%. Acceptance criterion: coefficient of variation should not exceed 2%.

The developed method for the quantitative determination of rometin in the form of a

substance and a capsule dosage form has a high sensitivity, a small relative error of determination (2% and 2%), and high reproducibility.

Conclusion

Based on the research, HPLC method for the quantitative determination of rometin in the form of a substance and a capsule dosage form was developed. The developed unified method allows for a qualitative analysis of the retention time of rometin, as well as its quantitative determination of both the substance and the dosage form.

References

1. Ershov F.I. Medicines used in viral diseases: [Text] / Ershov F.I., Romantsov M.G. // Guide for doctors. Moscow: GEOTAR-Media; 2007. p.363.
2. Kiselev O.I. Influenza pandemic 2009/2010: antiviral therapy and treatment tactics. [Text] / Kiselev O.I., Ershov F.I., Bykov A.T., Pokrovsky V.I. // St. Petersburg, Moscow, Sochi: A-Print; 2010. https://elibrary.ru/item.asp?id=17948247.
3. Isakov V.A. The effectiveness of Cycloferon® in the treatment and prevention of influenza and acute respiratory infections. [Text] / Isakov V.A., Romantsov M.G., Kabolova I.V., Erofeeva M.K., Vodeyko L.P., Smagina. // Russian Medical Journal (RMJ). Respiratory diseases. 2011; 23: 1420. Available at: http://www.rmj.ru/articles_7894.htm.
4. Mamatmusaeva N.E., Ermatov A.M., Ziyaev Kh.L., Sagdullaev B.T. Interferon inducers of local origin// The process and dynamics of the scientific path. I International Scientific and Theoretical Conference.-Vol.2, 26 February, 2021. Athens, Hellenic Republic, - pp. 141-143.
5. Interferon inducers of plant origin. [Text] / Sayitkulov A.M. // Abstract. diss. Doctor of Biological Sciences, Moscow, 1995. p.50.
6. Shamansurova E.A., Makhkamova G.T. The effectiveness of the drug rometin in acute respiratory infections. // Infection, immunity and pharmacology. T. 2021.- pp. 196-203.
7. Mamatmusaeva N.E., Sh.O.Khakimjonova, Kh.L.Ziyaev. Development of the composition and technology of Rometin capsules. Pharmaceutical Bulletin of Uzbekistan. -2020. Tashkent. No. 1. pp. 33-36.
8. Mamatmusaeva N.E., Faizullaeva N.S., Ziyaev H.L., Ermatov A., Sagdullaev B.T. //Optimization of the composition and technology of the solid dosage form "Romethine". Natural Volatiles & Essential Oils. Turkey. 2021; 8(5). pp.12096-12108.
9. Mamatmusaeva N.E., Fayzullaeva N.S., Ziyaev Kh.L., Sagdullaev B.T.// Development of Rometin capsule composition using mathematical modeling.International Conference on Multidimensional Innovative Research and Technological Analyzes. Spain. 2022. pp.135-142 http://www.conferenceseries.info/index.php/ICMRITA
10. Mamatmusaeva N.E., Abdugafurova D.G., Vypova N. L.,Ziyaev H.L.,Sagdullaev B.T. Study of the effect of the substance Megosin and its dosage forms in the form of

gastro-soluble and enteric capsules on non-specific ulcerative colitis (NUC) in rats. Interdisciplinary research: scientific horizons and perspectives. III International Scientific and Theoretical Conference. - Vol.3, May 6, 2022. Vilnus, Republic of Lithuania, - pp. 62-65.
11. Mamatmusaeva N.E., Abdugafurova D.G.,Vypova N. L.,Ziyaev H.L.,Tagayalieva N. A.Abrekova N. N., Sagdullaev B.T. Anti-ulcer effect of megosin in a model of acute experimental colitis in RATS. International Journal of Health Sciences, (2022). 6(S7), 47349–47356.
12. State Pharmacopoeia of the Russian Federation: in 3 volumes. M.: FEMB, 2015. V.3. pp. 426-427.
13. Draft BPA 42-0113-09. Validation of analytical methods. M., 2009. p.12.

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