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Development and validation of UV spectrophotometric method of detection of quinolin-8-ol sulfate and 2-mercaptobenzothiazole determination in pharmaceutical dosage form

Разработка и валидация УФ-спектрофотометрической методики определения сульфата 8-оксихинолина и 2-меркаптобензотиазола в лекарственной форме

Abstract

The simple, sensitive and low cost UV-vis spectrophotometric methods have been developed and validated for the quantification of 2-mercaptobenzothiazole and quinolin-8-ol sulfate in the antifungal ointment. The valid of maximum absorbance for the detection of 2-mercaptobenzothiazole was recorded at 324 nm (0.0024-0.0064% solution of ointment in water-methanol (1:1)) with LOD of 0.0008 g and LQD of 0.025 g of dosage form with r^2=0.9984. The quinolin-8-ol sulfate was detected at the maximum absorbance of 505 nm after reaction with Fast Red GL Salt to form red colored solution with concentration in the range of 0.0072-0.0312% (solution of ointment in water-ethanol solution (5:1) with LOD of 0.125 g and LQD of 0.38 g of dosage form, while linearity was r^2=0.9956). The proposed technique can be successfully used for the quality control of the proposed ointment in laboratories possessing UV-vis spectrometer.
Keywords: Antifungal, 2-mercaptobenzothiazole, pharmaceutical dosage forms, quinolin-8-ol sulfate, UV-spectrophotometry, validation.

Резюме

Лекарственные формы с выраженным противогрибковым действием, содержащие сульфат 8-оксихинолина и 2-меркаптобензотиазол, привлекают внимание для фармакологических исследований. При этом известны методы только их индивидуального количественного определения. Целью исследования было разработать простые, точные, воспроизводимые и недорогие УФ-спектрофотометрические методики определения сульфата 8-оксихинолина и 2-меркаптобензотиазола при их совместном присутствии в мази. Экспериментально были подобраны условия определения и концентрация исследуемых растворов. Так, для сульфата 8-оксихинолина линейность (r^2=0,9956) для максимальной оптической плотности и концентрации в соответствии с законом Бугера – Ламберта – Бера была обнаружена в диапазоне концентраций 0,0072–0,0312% мази в растворе дистиллированная вода-этанол (5:1) при 505 нм

после образования красного раствора по реакции с диазолем красным ЖЛ. Для 2-меркаптобензотиазола аналитическая длина волны составляла 324 нм, линейность (r^2=0,9984) была найдена для 0,0024–0,0064% раствора мази в смеси дистиллированная вода-этанол (5:1). Также методики были валидированы по следующим критериям: точность, правильность, прецизионность и робастность, и могут быть успешно использованы для контроля качества предлагаемой мази в лабораториях UV-vis спектрофотометрически.

Ключевые слова: сульфат 8-оксихинолина, 2-меркаптобензотиазол, валидация, УФ-спектрофотометрия.

■ INTRODUCTION

The spread of fungal diseases becomes more dangerous and rapid nowadays, especially, when it is reported, that even Candida parapsilosis and Candida tropicalis are developing resistance to triazoles and echinocandins [1].

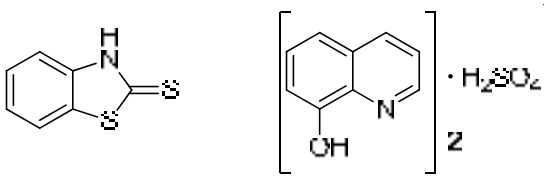
2-Mercaptobenzothiazole (2-MBT, benzothiazole-2-thiol, CAS 149-30-4) is known to have antibacterial, antifungal and fungicide properties (Fig. 1) [2].

In the review of its biological activities by M.A. Azam and B. Suresh it said about various antifungal properties, namely, anti-Candida activity was studied against 15 Candida strains [3] and the results showed 50% growth inhibition at concentrations between 1 and 78 mg*L^{-1}. The effects against Aspergillus niger was found in 33 mg*L^{-1}, and similar results were described for the fungus Trichophyton rubrum. To completely inhibit the growth of Microsporum gypseum and Epidermophyton floccosum the 2-MBT concentration had to exceed 50 mg*L^{-1}.

Among proven mechanisms of its activity 2-MBT is antagonist of the androgen receptor (AR) signaling pathway – cell viability counter screen, disruptor of the mitochondrial membrane potential, agonist of the antioxidant response element signaling pathway and the peroxisome proliferator-activated receptor gamma or antioxidant response element signaling pathways [4].

Also, despite the allergic properties, 2-MBT is not mutagenic or carcinogenic, according to a comprehensive report by the German Federal Institute for Occupational Safety and Health (Fig. 1) [5]. Moreover, reevaluation of human toxicity within the EU REACH Community Rolling Action Plan concluded, that there is no need for a proposal for harmonized classification and 2-MBT labeling.

Besides, quinolin-8-ol sulfate (8-Q) and its derivatives are also in our area of interest, because they are found to be antifungal, antibacterial, antiproto-



A **B**

Fig. 1. 2-Mercaptobenzothiazole (2-MBT, A) and quinolin-8-ol sulfate (Q-8, B) structures

zoic [6–8], antituberculotic [9–11], antineoplastic, antiasthmatic, antiplatelets [12–17], potential HIV-1 integrase inhibitors [18–21] and anti-cancer drugs [22] (Fig. 1). 8-Q is reported to be inhibitor of human Jumonji domain containing 2E (JMJD2E), human tyrosyl-DNA phosphodiesterase 1 (TDP1): qHTS in cells in presence (absence) of CPT and ROR gamma transcriptional activities [23].

So, complex pharmaceutical dosage forms with both 8-Q and 2-MBT is very interest object for pharmacological studies. And while they are done, methods of quantity determination should be developed.

Quantitative investigations of 2-MBT are much more studied, that of 8-Q, namely, in our pervious article there are mentioned some of its chromatographic, mass-spectrometry or electrochemical detections, with development and validation of simple, but accurate UV-spectrophotometric method by maximum absorbance at the 324 nm [24].

There are much lesser papers dealing with quinolin-8-ol studies. Hence, the transfer of divalent metal ions ($Mn^{2+,}$ Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}) facilitated by 8-Q at the nitrobenzene–water interface was studied by polarographic and voltammetric techniques [25]. A series of the same 3d metal complexes, but with 5-chloroquinolin-8-ol has been synthesized and characterized by elemental analysis, IR spectroscopy, TG-DTA thermal and X-ray analysis [26]. Also molybdenum (0–2.5 μg) may be determined spectrophotometrically at 385 nm run after flow-injection extraction into chloroform the chelate of molybdenum(IV) with 8-Q by flow-injection spectrophotometric determination [27].

Considering the same goal as in the previous work, namely developing simple, sensitive, accurate and reproducible method for simultaneous estimation of 2-MBT and 8-Q, the above mentioned methods need special and expensive equipment, which are not widely presented in the chemical labs. That's why accurate and valid UV spectrophotometric determinations of both ingredients were developed to be low cost, fast and widely used in the common laboratories.

■ EXPERIMENTAL SECTION

Instrumentation

Substances were weighed using analytical balances Kern ABT 120-5DM, KERN&Sohn GmbH, Germany. UV spectra were recorded on Analytic Jena UV-*vis* spectrophotometer Specord 200 (190–1100 nm), Germany.

Reagents and solutions

The solvents were of the highest purity available from the LAB-SCAN (Ireland) and were used without any further purification. The distilled water was used throughout the experiments. Investigated substances were purchased from "Orgsintez", Berezniki, Perm' region, Russia.

The investigated ointment consisted of: 2-mercaptobenzothiazole – 10.00 g, quinolin-8-ol sulfate – 3.00 g, benzoic acid – 10.00 g, salicylic acid – 10.00 g, Helianthus annuus oil – 15.0 g, polyethylene oxide 400 – 30.00, emulgator № 1 (Ph. Art. # 42-2121-92, mixture of alcohols of C 16-20 with sodium salts of the same alcohols sulfoethers) – 7.50 g, MGD (Distilled Monoglycerides, Techn. Cond. # 10-04-02-89) – 4.00 g, Tween-80 – 5.00 g, distilled water till 100.00 g.

Validation

The calibration curve of 2-mercaptobenzothiazol was constructed by a UV-visible spectrophotometer absorbance data monitored 5 times for each sample at the wavelength of maximum absorbance at the 324 nm in 3 mL cuvette with 1 cm layer in comparison to water – methanol (1:1) solution. And at the 505 nm – for quinoline-8-ol sulphate in comparison to control solution.

The regression equation was obtained by the method of least squares for n=6. Regression equation: Y = slope*x + intercept. Slope, intercept and correlation coefficient were determined from the regression analysis' calculations in Microsoft Excel 2007 [28]

Using this linear equation, regression coefficient (r^2) and the detection limits were determined. Accuracy: mean ± SD; Linearity (lowest – highest concentration while curve is linear); SE of intercept: √ of ∑(y-y′/n), where y – standard concentration, y′ – found concentration; SD of intercept: SE of intercept*√n.

The limit of detection (LOD): 3.3*(SD of intercept / slope); and the limit of quantitation (LOQ): 10*(SD of intercept / slope). The LOD was defined by the concentration with a signal-to-noise ratio of 3. The analyte peak in the LOQ sample should be identifiable, discrete, and reproducible with a precision of ±20% and accuracy within 80%–120%. The deviation of standards other than LOQ should not be more than ±15% of the nominal concentration.

Precision (repeatability of the method) was evaluated by repeated absorbance detection and the results were expressed as the mean standard deviation (SD) and the percent relative standard deviation RSD (%) = SD/Mean. For intra-day analysis the samples were analyzed six times a day at 09:00 am, 11:00 am, 01:00 pm, 03:00 pm, 05:00 pm, and 07:00 pm, while for inter-days stability they were analyzed for 6 consecutive days at 09:00 am.

Working solutions

Working solutions of 2-mercaptobenzothiazol were prepared by dissolving 0.0200 – 0.0700 g of ointment in the 100.0 mL flask with water - methanol (1:1) solution with thorough physical stirring for 20 min. Then 1.00 mL was quantitatively transferred to 100.0 mL flask in the same manner and stirred for 10 min.

Working solutions of quinoline-9-ol sulphate were prepared by dissolving 0.2000-0.8000 g of ointment in the 100.0 mL flask with 10.00 mL of 95% ethanol, with consequent addition of distilled water to the flask line. Stirred for 20 min. The 1.00 mL of obtained solution was quantitatively transferred into the 25.00 mL flask, then 10.00 mL of ethanol 95% was added, 5.00 mL of freshly prepared 0.1% water solution of Fast Red GL Salt (CAS No. 85223-03-6, 4-methyl-2-nitrobenzenediazonium naphthalene-1,5-disulphonate (1:1)), 0.50 mL of 1.0% water solution of K_2CO_3, and distilled water to the flask line. Solution was stirred for 10 min. In parallel, all reagents were placed into the 25.00 mL flask in the same way, but without ointment addition to make control solution.

To prepare 0.004% standard solution, 0.1000 g of 8-Q was placed in 100.0 mL flask, dissolved with 10.00 mL of 95% ethanol and diluted with distilled water to the flask line. Stirred for 20 min. The 1.00 mL of obtained solution was quantitatively transferred into the 25.00 mL flask with subsequent addition of all the above mentioned reagents and distilled water. All solutions were stored at 18–22 °C.

RESULTS AND DISCUSSION

2-Mercaptobenzothiazole determination

Firstly, the optimal concentrations of ointment solution, different solvents and their ratios were investigated to obtain maximum absorbencies in the range of 0.6–0.9 of active ingredients to be valid for quantitative calculations. Methanol-water (1:1) solution was found to be the best choice, due to formation of solution, when diluted the second time, and presence in each laboratory.

During preparation the various concentrations, among the range of the accurate ones appeared to be 0.004% solution of ointment for 2-MBT detection at the 324 nm, because there was practically no absorption of 8-Q at this wavelength (Fig. 2). Moreover, the less valid concentration is taken, the less impact the absorption of 8-Q in this area does.

Validation of the methods was prepared in accordance to the analytical methods validation parameters [29].

The six working standard solutions were made by dissolving 0.0200–0.0700 g of ointment to obtain 0.002–0.006% final water-methanol (1:1) solutions, and their absorbancies were measured at the 324 nm.

The appropriate accuracy and recovery data are given in the Table 1.

The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis. The accuracy of the method was proven by recovery of six different concentrations in the calibration range – 99.99±1.95% (Table 1). The mean percent of recoveries was found to be 99.99±1.78%.

The calibration curve had good linearity (R^2=0.9984) for ointment samples mass between 2.7–7.9 cg for first dilution (Table 1, Fig. 3).

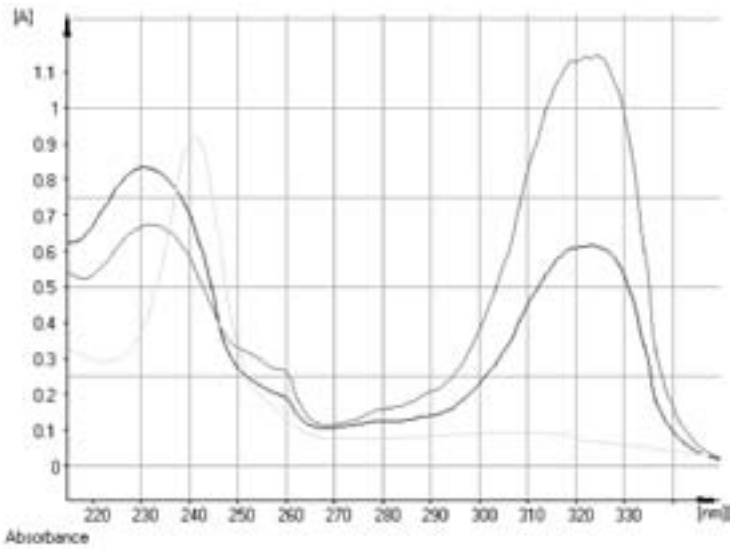


Fig. 2. UV spectra of: the first graph – 0.004% solution of ointment in water-methanol (1:1), the second one – 0.0008% solution of 2-MBT in water-methanol (1:1), the third one – 0.0008% solution of 8-Q in water-methanol (1:1)

Table 1
Concentration of working 2-MBT solutions in water – methanol (1:1) mixture, their absorbencies, recovery and accuracy data

#	Sample of dosage form, g	ΔA	[1] Theoretical concentration, cg in sample	[2] Calculated concentration, cg in sample	[3] Recovery
1	0.0270	0.3325	2.70	2.6418	97.8458
2	0.0304	0.3895	3.04	3.0707	101.0109
3	0.0401	0.5307	4.01	4.1332	103.0719
4	0.0522	0.6623	5.22	5.1234	98.1494
5	0.0613	0.7915	6.13	6.0956	99.4382
6	0.0788	1.0337	7.88	7.9180	100.4820
Mean (n=6)					99.9997
SD					1.9530
RSD%					1.7829
Accuracy, %					99.9997±1.9530
Recovery, %					99.9997±1.7829

1 – sample, g * 10 * 1000 / 100; 2 – (maximum absorbance of sample + 0.0186) / 0.1329; 3 – sample concentration calculated by equation * 100 / sample theoretical concentration.

The above calibration curve could be used for simple and fast evaluation of 2-MBT concentration in the sample after appropriate dilution in water-methanol (1:1).

Slope, intercept and correlation coefficient were determined from the regression analysis calculations (Table 2).

The limit of detection (LOD) was found to be 0.84 cg of 2-MBT in sample (8.4 mg of ointment), while limit of quantification (LOQ) was 2.53 cg of 2-MBT in sample (2.5 cg of ointment).

The ruggedness of the method was studied by performing the same method by different researchers during different days to check the reproducibility. Also, the results were found to be highly reproducible during the day – RSD was 0.87%, but during the week it decreased to 1.32% (Table 3).

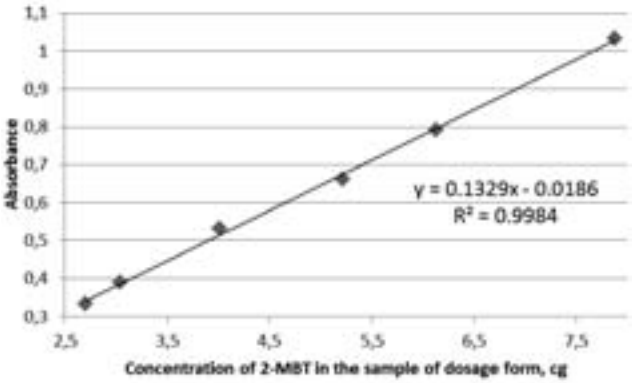


Fig. 3. Calibration curve of 2-mercaptobenzothiazol in 0.002-0.006% ointment solutions in water-methanol (1:1) based on maximum absorbance at the 324 nm

Table 2
Linearity data, accuracy and precision of 2-MBT in water – methanol (1:1) solution

Parameters	2-MBT data
Slope	0.1329
Intercept	0.0186
Linearity (mg/sample)	2.5–7.9
Regression equation	y = 0.1329x-0.0186
r^2	0.9984
SE of intercept	0.0137
SD of intercept	0.0337
LOD, cg of 2-MBT / sample	0.8358
LQD cg of 2-MBT / sample	2.5329
LOD, g of sample	0.0084
LQD g of sample	0.0253

Table 3
Intra-day and inter-day precision data of 2-MBT in ointment sample of 0.0502 g in water-methanol solution (1:1)

#	Absorbance	
	Intra-day precision	Inter-day precision
1	0.6738	0.6738
2	0.6765	0.6602
3	0.6747	0.6656
4	0.6622	0.6534
5	0.6647	0.6506
6	0.6638	0.6497
Mean	0.6693	0.6589
SD	0.0064	0.0095
%RSD	0.8692	1.3178

To determine the robustness of the method, experimental conditions like room temperature, stirring and different methanol series were investigated. It was found, that usage of the cuvette without glass cap, due to methanol evaporation, negatively influenced even the daily results. Hence, common physical stirring for 20 min. and temperature of 18–22 °C were the most optimal factors to obtain accurate data.

Hence, to detect the amount of 2-MBT in the investigated pharmaceutical dosage form, namely, ointment the next methodic is proposed.

Quantitatively place 0.0400–0.0700 g of ointment in the 50.00 mL flask, dissolve with water – methanol (1:1) solution. Stir for 20 min. Quantitatively transfer 1.00 mL of obtained suspension into the 25.00 mL flask, add water – methanol (1:1) solution. Stir for 10 min. Determine the amount of 2-MBT by employing UV maximum absorption at the wavelength of 324 nm in comparison to water – methanol (1:1) solution in 3 mL cuvette with 1 cm layer.

Calculate the concentration in accordance to the next equations:

Concentration of 2-MBT in the dosage form:

C, % or g/100.0 = (maximum absorbance of sample * concentration of standard (0.0005%) * flasks volumes (50*25.00 mL)) / (maximum absorbance

of standard at 324 nm (0.6787)* pipette volume (1.00 mL) * sample mass (g) * cuvette layer (1 cm)) [24];

or based at the found equation:

C, % or g/100.0 = (maximum absorbance of sample + 0.0186) * 100 / (0.1329 * 1000 * sample mass in g).

When comparing results of the above formulas, it was detected, that absorption of 8-Q overlapping with 2-MTB, has made a little impact on its final concentration results, so, if calculated by the first formula, the correlation coefficient 0.835 should be applied, namely:

C, % or g/100.0 = (maximum absorbance of sample * concentration of standard (0.0005%) * flasks volumes (50*25.00 mL) * 0.835) / (maximum absorbance of standard (0.6787)* pipette volume (1.00 mL) * sample mass in gram) * cuvette layer (1 cm)).

Quinoline-8-ol sulphate determination

The found earlier peak at the 241 nm in the UV spectrum of 8-Q, presented in the Fig. 2, was not appropriate for quantity determination, because of the partial overlap with 2-MBT absorbance at the 232 nm. So, it was decided to conduct selective coloration reaction to obtain maximum absorbance dealing only with 8-Q.

Several years ago, reaction with Fast Red GL Salt (CAS No. 85223-03-6, 4-methyl-2-nitrobenzenediazonium naphthalene-1,5-disulphonate (1:1)) was developed to detect 8-Q at the 505 nm by Vasyuk S. [30]. So, this method was used. Thus, in the result of various ointment concentrations studies, the 0.016% one was found to be among the optimal ones (Fig. 3).

As it is seen in the Fig. 4, there was no absorbance of 2-MBT in the same concentration and at the same wavelength.

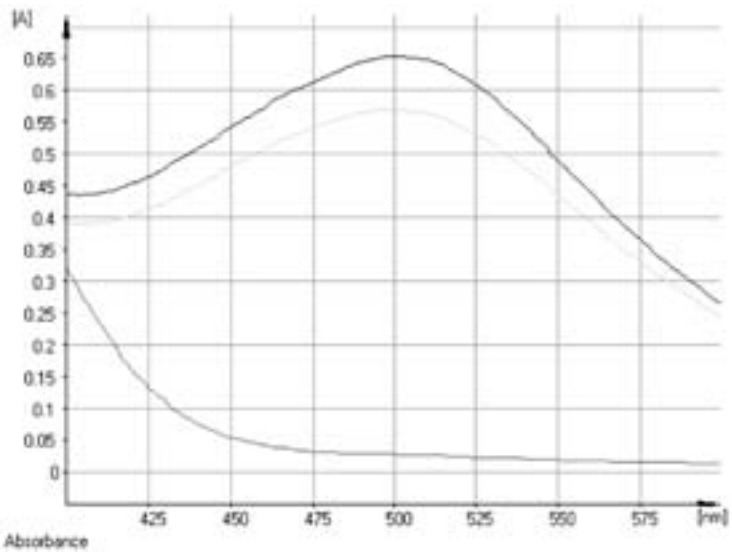


Fig. 4. UV spectra of substances with 0.1% water solution of Fast Red GL Salt: the first graph – 0.016% solution of ointment in ethanol-water (1:5), the second one – 0.0004% solution of 8-Q in ethanol-water (1:5), the third one – 0.0004% solution of 2-MBT in ethanol-water (1:5)

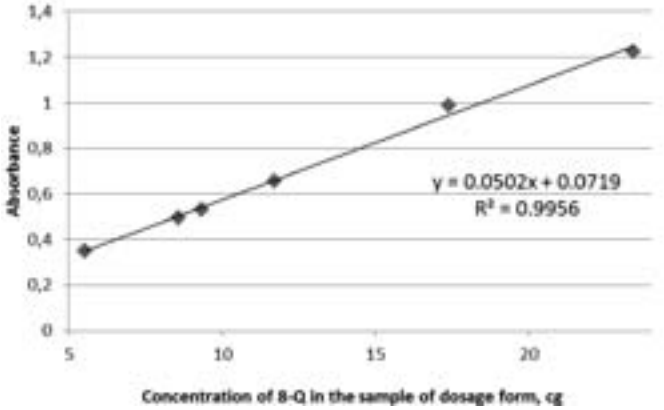


Fig. 5. Calibration curve of quinoline-8-ol sulphate in 0.008–0.032% ointment solutions in ethanol-water (1:5) based on its maximum absorbance at the 505 nm

Validation of the methods was prepared as the mentioned earlier [29].

The six working standard solutions were made dissolving 0.2000–0.8000 g of ointment to obtain 0.008–0.032% final ethanol-water (1:5) solutions. And their absorbancies were measured at the 505 nm after addition of 0.1% water solution of Fast Red GL Salt.

The correlation coefficient was 0.9956 for the range of 5-20 mg of 8-Q in the ointment sample (Fig. 5). The below curve could be used for fast 8-Q determination in the dosage form.

The high accuracy and recovery results (99.87±2.56% and 99.87±2.33%) are given in the Table 4.

1 = sample, g * 10 * 1000 / 100; 2 = (maximum absorbance of sample – 0.0719) / 0.0502; 3 = sample concentration calculated by equation * 100 / sample theoretical concentration.

Table 4
Concentration of working 8-Q solutions in ethanol – water (1:5) solution after addition of Fast Red GL Salt, their absorbancies, recovery and accuracy data

#	Sample of dosage form, g	ΔA	[1]Theoretical concentration, mg in sample	[2]Calculated concentration, mg in sample	[3]Recovery
1	0.1835	0.3488	5.5050	5.5159	100.1987
2	0.2851	0.4957	8.5530	8.4422	98.7049
3	0.3105	0.5307	9.3150	9.1394	98.1153
4	0.3901	0.6555	11.7030	11.6255	99.3378
5	0.5801	0.9875	17.4030	18.2390	104.8040
6	0.7805	1.2242	23.4150	22.9542	98.0320
Mean (n=6)					99.8654
SD					2.5515
RSD%					2.3323
Accuracy, %					99.8654±2.5515
Recovery, %					99.8654±2.3323

Table 5
Linearity data, accuracy and precision of 8-Q in ethanol – water (1:5) solution with addition of Fast Red GL Salt

Parameters	8-Q data
Slope	0.0502
Intercept	-0.0719
Linearity (mg/sample)	5.0-20.0
Regression equation	y = 0.0502x-0.0719
r^2	0.9956
SE of intercept	0.0233
SD of intercept	0.0571
LOD, mg of 8-Q / sample	3.7566
LQD mg of 8-Q / sample	11.3836
LOD, g of sample	0.1252
LQD g of sample	0.3795

The LOD appeared to be 3.76 mg of 8-Q per sample (12.5 cg of ointment) and the LQD was 11.38 mg of 8-Q per sample (38.0 cg of ointment) (Table 5).

The calculated precision during day and week was a little bit worse, than of 2-MBT, still with good data - intra-day RSD was 2.97% and inter-day RSD was 4.22% (Table 6).

Hence, the next method of 8-Q determination in the studied ointment is proposed.

Quantitatively place 0.3000-0.6000 g of ointment in the 100.0 mL flask, dissolve it with 10.00 mL of 95% ethanol and add distilled water to the flask line. Stir for 20 min. Quantitatively transfer 1.00 mL of obtained solution into the 25.00 mL flask, add 10.00 mL of ethanol 95%, 5.00 mL of freshly prepared 0.1% water solution of Fast Red GL Salt (CAS No. 85223-03-6, 4-methyl-2-nitrobenzenediazonium naphthalene-1,5-disulphonate (1:1)), 0.50 mL of 1% water solution of K_2CO_3, and add distilled water to the flask line. Stir for 10 min. To make 0.0004% standard solution place 0.0100 g of 8-Q into the 100.0 mL flask, dissolve it with 10.00 mL of 95% ethanol and add

Table 6
Intra-day and inter-day precision data of 8-Q for sample of 0.4016 g of ointment in ethanol–water (1:5) solution of investigated pharmaceutical dosage form

#	Absorbance	
	Intra-day precision	Inter-day precision
1	0.7154	0.7154
2	0.7095	0.6680
3	0.6811	0.6580
4	0.6797	0.6451
5	0.6646	0.6389
6	0.6626	0.6307
Mean	0.6855	0.6594
SD	0.0223	0.0305
%RSD	2.9684	4.2269

distilled water to the flask line. Stir for 20 min. Quantitatively transfer 1.00 mL of obtained solution in the 25.00 mL flask and add all the mentioned above reagents and distilled water. In parallel, place all reagents without 8-Q in the 25.00 mL flask, add distilled water to the flask line to make control solution. Determine the UV maximum absorption at the wavelength of 505 nm for experimental and standard solutions of 8-Q in comparison to control one in 3 mL cuvette with 1 cm layer.

Calculate the concentration in accordance the next equation:

Concentration of 8-Q in pharmaceutical dosage form:

C, % or g/100.0 = (maximum absorbance of sample * concentration of standard (0.0004%) * flasks volumes (100*25.00 mL)) / (maximum absorbance of standard (0.5770)* pipette volume (1.00 mL) * sample mass in g) * cuvette layer (1 cm);

or according to the calculated equation:

C, % or g/100.0 = (maximum absorbance of sample – 0.0719) * 100 / (0.0502 * 1000 * sample mass in g).

■ CONCLUSIONS

The simple, sensitive and low cost UV-vis spectrophotometric methods have been developed and validated for the quantification of 2-mercaptobenzothiazole and quinolin-8-ol sulfate in simultaneous presence in the antifungal ointment. The valid maximum absorbancies were recorded at the 324 nm for 2-mercaptobenzothiazole and at the 505 nm for quinolin-8-ol sulfate after addition of Fast Red GL. The correlation coefficients were 0.9984 and 0.9956 correspondingly. The proposed techniques could be used to accurately detect 2-MBT and 8-Q in the investigated pharmaceutical dosage form, namely, ointment, using the proposed equations in the common laboratories by the means of UV-vis spectrometer.

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