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ARTICLES

SORPTION-SPECTROSCOPIC DETERMINATION OF DIMEDROL IN THE FORM OF IONIC ASSOCIATES WITH ANIONIC AZO DYES

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Abstract. The sorption of ionic associates of acidic azo dyes – sulfonazo and Congo red with diphenhydramine on polyurethane foam is studied depending on pH, phase contact time, and component ratio. A method for determining diphenhydramine in medicinal products is developed based on its sorption in the form of ion associates with dyes and subsequent detection on the sorbent surface using diffuse reflectance spectroscopy.

Keywords: *diffuse reflectance spectroscopy, ion associates, polyurethane foam, azo dyes, sorption, diphenhydramine*

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A significant increase in the production and consumption of medicines requires special quality control and determination of their content in various biological fluids, wastewater from pharmaceutical enterprises and other facilities.

Diphenhydramine (β-dimethylaminoethyl ether of benzydrol hydrochloride, **DM**) is widely used in medicine as an antihistamine, local anesthetic and antispasmodic agent. Chromatographic [1-15], electrochemical [16], and spectrophotometric methods [17-19] are used to determine diphenhydramine, many of which require the use of complex equipment and take a long time.

A simple and rapid extraction-spectrophotometric method for the determination of diphenhydramine in the form of an ionic associate with azo dyes is known [20]. However, liquid extraction is inferior to sorption, a more technologically advanced method of separation and concentration that does not require the use of toxic organic solvents.

It is of interest to study the possibility of determining diphenhydramine as an ionic associate with azo dyes by diffuse reflection spectroscopy after extraction onto a sorbent. Polyurethane foam was chosen as the sorbent, which is widely used for the sorption of various organic and inorganic compounds, including ionic associates [21-23].

The aim of the work was to study the formation and sorption of ionic associates of acidic azo dyes sulfonazo (**SFN**) and congo red (**Cr**) with diphenhydramine on polyurethane foam (**PUF**).

METHODS AND MATERIALS

Reagents and equipment. Aqueous solutions of dyes were used – sulfonazole AR grade (Lenrective, Russia), congo red AR grade (Lenrective, Russia) and the organic base of diphenhydramine of pharmacopoeial purity. The acidity of the solutions was controlled by an Expert 001 pH meter (Econix-Expert, Russia).

The polyurethane foam sorbent based on esters was used in the form of tablets with a thickness of 1 cm, a diameter of 2 cm and a weight of 0.04–0.05 g, which were obtained using a metal punch from an industrial polymer sheet. An LS-220 mixing device was used to mix the solution with the sorbent. Diffuse reflection of sorbates was measured using UV-3600 (Shimadzu, Japan) with a diffuse reflection prefix. The optical density of the solutions was measured on a Specord 210 Plus spectrophotometer (Analytik Jena, Germany).

Experimental methodology. Into volumetric flasks. Solutions of dye and diphenhydramine, hydrochloric acid or sodium hydroxide were added to 25 ml to create the required pH value of the solutions and transferred to glass vials where a PU tablet was placed. To ensure the penetration of the solution into the sorbent volume, it was pressed with a rubber piston. The solutions were shaken for the time required to establish sorption equilibrium. The tablets were removed, placed between sheets of filter paper, and dried for further analysis by diffuse reflection spectroscopy. The distribution was controlled by determining the equilibrium concentration of the dye in the aqueous phase by the spectrophotometric method.

DISCUSSION OF RESULTS

The photometric method for the determination of diphenhydramine is based on the difference in the extraction conditions of the dye and its ionic associate with diphenhydramine in heterogeneous systems, depending on the pH of the solution.

Dependence of sorption of dyes and their ionic associates with diphenhydramine on the acidity of the solution. SFN and Cr were used as azo dye reagents for the formation of ionic associates with diphenhydramine, which are characterized by the presence of several ionizing substituents in their molecules, including sulfogroups capable of providing solubility of compounds in water. Sulfonazo and congo red are octobasic and dibasic acids, respectively, which, depending on pH, can be in solution in various ionized forms, so the acidity of the aqueous phase can significantly affect their sorption on polyurethane foam. Diagrams of the distribution of azo dye forms calculated using the ACD Labs program are shown in Fig. 1.

The dependence of the dye extraction efficiency on the pH of the foam solution in the static mode was studied (Fig. 2). As can be seen, the degree of cure of SFN on PU foam is maximal in the highly acidic region, since sorption of this dye can occur due to the electrostatic interaction of anionic particles with the main groups of PU foam protonated in an acidic medium. A decrease in the degree of SFN extraction with an increase in the pH of the solution is associated with a decrease in the degree of protonation of nitrogenous groups of PUF. The maximum extraction of Cr is observed in the pH range of 2-5, probably in this case, the sorption of the dye is carried out due to both electrostatic and hydrophobic interactions with the sorbent surface, since the hydrophobicity factor of Cr (Table. 1) far exceeds the hydrophobicity factor of SFN. In the pH < 2 region, the appearance of a third phase is observed, which is due to the high hydrophobicity of the molecular form of Cr, which prevails according to the distribution diagram of the dye forms in acidic media.

The sorption pattern of dyes in the presence of DM changes dramatically, which may be due to the formation of ionic associates, the extraction of which is strongly influenced by the hydrophobicity of the compounds included in their composition [24].

Diphenhydramine has the properties of a base (pH = 8.76) due to the presence of a tertiary nitrogen atom. In this regard, the formation of stable hydrophobic associates with the anionic forms of SPH and Cr is possible. The structural formulas of the molecules and the values of the hydrophobicity factors of azo dyes and diphenhydramine are given in Table 1.

For the formation and extraction of ionic associates, as well as to exclude sorption of pure azo dyes, it is necessary to create an appropriate acidity in the solution, ensuring the formation of a reactive form of the azo dye and a base cation reactive to it.

The pH value of 8 was chosen as the optimal level of acidity of the solution for the extraction of ionic associates of SFN–DM and Cr–DM (Fig. 2), at which azo dyes are practically not extracted (the degree of extraction of pure azo dye is no more than 4%).

Spectral characteristics. It was found that the light absorption maxima of the SPH and Cr azo dyes lie at 560 and 500 nm, with the introduction of DM, the maxima in the absorption spectra practically do not shift and are at 570 and 500 nm. The minima of light reflection in the diffuse reflection spectra of SFN and Cc are at 560 and 510 nm, and with the introduction of DM – at 570 and 520 nm, respectively. The absence of spectroscopic effects in the absorption spectra is associated with the formation of ionic associates in the sulfone group of azo dyes having an isolated π-system.

Time to sorption equilibrium. It was found that 30 minutes is sufficient to establish equilibrium in the case of Cc, while 60 minutes is sufficient for an associate with SPH.

Effect of ionic strength. The effect of the ionic strength of the solution on the extraction of azo dyes and their

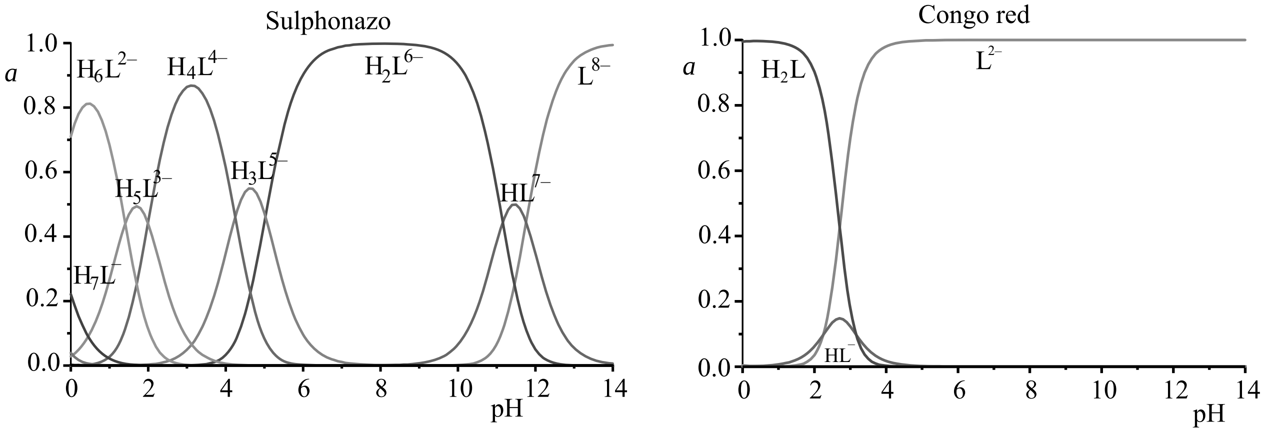
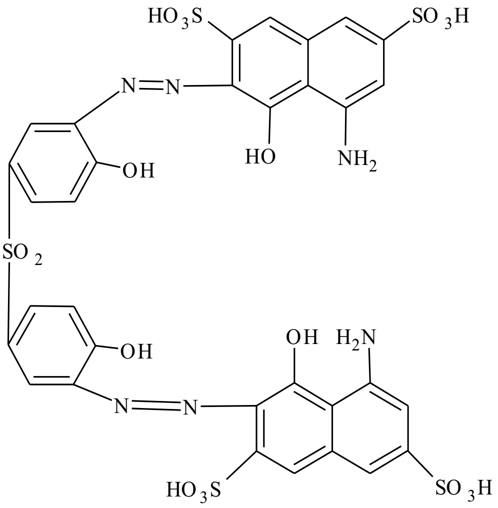
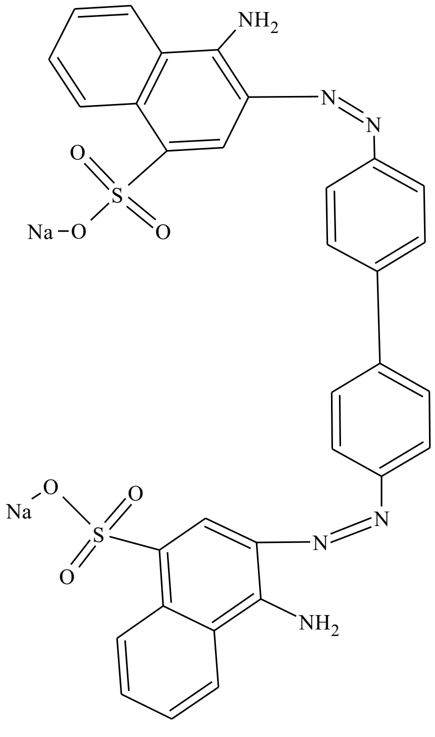
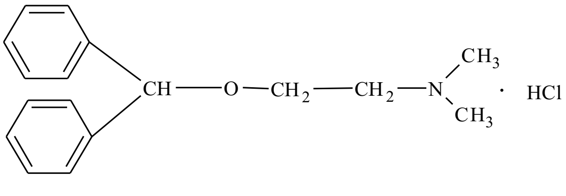


Fig. 1. Diagrams of the distribution of forms of azo dyes (*a* is the proportion of the ionized form of the dye).

Table 1. Structural formulas and values of the hydrophobicity factor log*P* (in the octanol–water system) for anionic azo dyes and diphenhydramine

Name of the substance	Structural formula	log*P**
Sulfonazo		-5.83 ± 1.75
Congo red		4.22 ± 0.52
Diphenhydramine		3.66 ± 0.37

Note: *Calculated using the ACD Labs program.

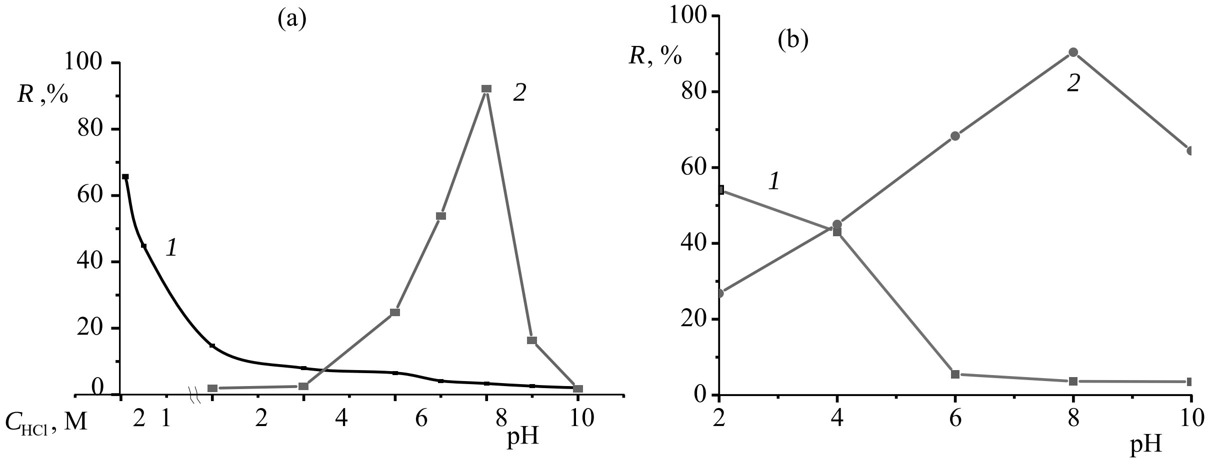


Fig. 2. Effect of the acidity of the aqueous phase on sorption: (a) sulfonazo (*1*) and sulfonazo in the presence of diphenhydramine (*2*), $c_{SFN} = 0.6 \times 10^{-4}$ M, $c_{DM} = 13.7 \times 10^{-4}$ M; (b) congo red (*1*) and congo red in the presence of diphenhydramine (*2*), $c_{Cr} = 0.16 \times 10^{-4}$ M, $c_{DM} = 5.48 \times 10^{-5}$ M.

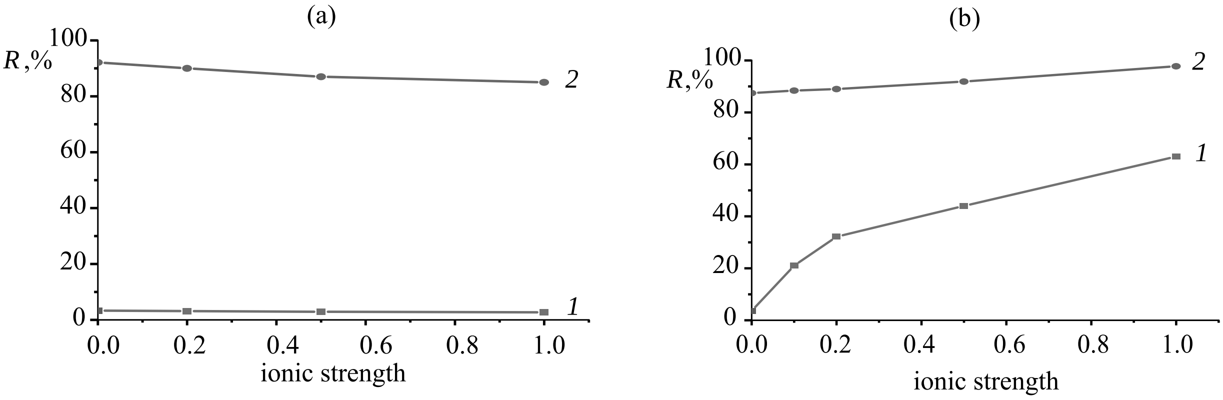


Fig. 3. Dependence of the degree of sorption on the ionic strength of a solution of (a) sulfonazo (*1*) and ion associate SFN–DM (*2*), pH 8, $V = 25$ ml, $m_{sorbent} = 0.040 \pm 0.001$ g; (b) congo red (*1*) and ion associate Cr–DM (*2*), pH 8 ,$V = 25$ ml, $m_{sorbent} = 0.040 \pm 0.001$ g.

ionic associates with DM was studied with an increase in the concentration of sodium chloride from 0 to 1 M. As can be seen from Fig. 3a, the degree of extraction of the SFN azo dye is practically independent of the ionic strength of the solution, and its ionic associate with DM decreases slightly.

In the case of Cr (Fig. 3b), the degree of extraction of the Cr–DM ion associate increases by 10% with an increase in the concentration of sodium chloride from 0 to 1 M, which may be due to the “salt effect" – the effect of the electrolyte on the solubility of the compound. To an even greater extent, the ionic strength of the solution affects the sorption of the dye itself, the degree of extraction of which increases from 4 to 60%. Thus, the sorption of the less hydrophobic SFN dye and its ionic associate with DM is more resistant to changes in the ionic strength of the solution, unlike Cc.

Dependence of the completeness of the formation of ionic associates on the concentration of diphenhydramine. The color intensity of the ionic associates of DM dyes is determined by the spectral characteristics of the dyes, the concentrations of which were selected in such a way that the magnitude of the analytical signal was in the operating range of the equipment used for subsequent detection by both spectrophotometry and diffuse reflection spectroscopy. Fig. 4a shows the dependence of the *F* function of the SFN dye obtained by sorption of the dye in the presence of different concentrations of DM at pH 8. As can be seen from the figure, in the concentration range from 0.68×10^{-4} M to 10.96×10^{-4} M, the dependence of the analytical signal on the concentration of DM is linear, the inflection point of the saturation curve corresponds to the ratio of the concentration of SFN reagents: DM = 1 : 18.

A similar relationship was constructed for the azo dye Cr (Fig. 4b). It was found that in this case, the inflection point of the saturation curve is observed with a ratio of components Cr : DM = 1 : 3.4, and in the concentration range from 0.137×10^{-5} to 5.48×10^{-5} M, the dependence

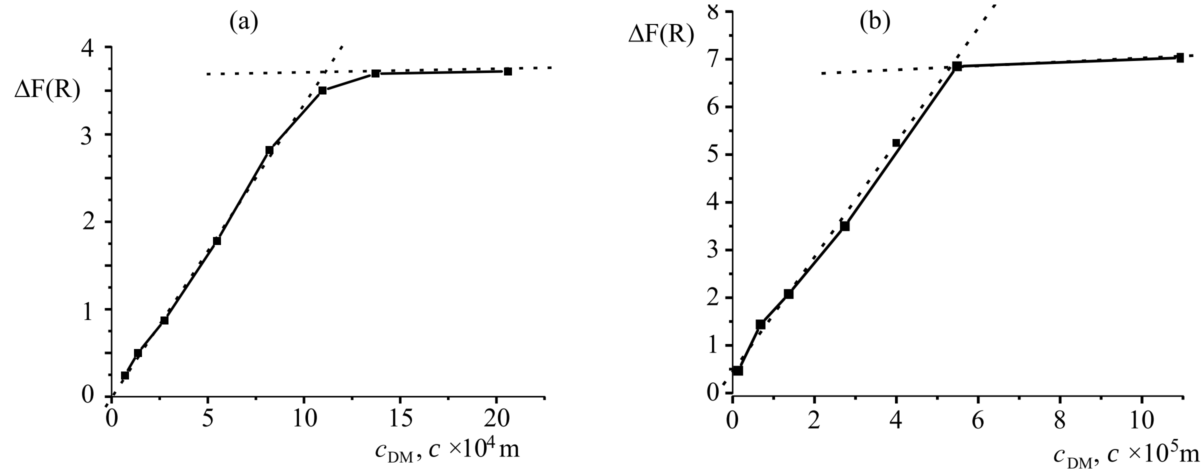


Fig. 4. Dependence of the Gurevich–Kubelka–Munk function of colored test forms on the concentration of diphenhydramine. (a) PU foam [SFN–DM], $c_{SFN} = 0.6 \times 10^{-4}$ M, λ = 570 nm; (b) PU foam [Cr–DM], $c_{Cr} = 0.16 \times 10^{-4}$ M, λ = 520 nm.

of the analytical signal on the concentration of DM is linear.

Taking into account the value of pH (DM) = 8.76, it can be assumed that at pH 8, not all diphenhydramine is in a protonated form capable of forming an ionic associate. For this reason, it is more correct to use the dye: protonated DM ratio to evaluate the stoichiometry of the interaction. Based on the value of pK_a (DM), the dye: protonated DM ratio was calculated; it was 1 : 2.8 for Cc and 1 : 15 for SFN. Based on the structure of the Cr dye, which has two sulfogroups in its structure, it can be concluded that a strong associate of the dye with DM is formed, for which a three-fold excess of DM is sufficient. In the case of SPH – octobasic acid, an almost fifteen-fold excess of DM is required.

Under the selected conditions, calibration graphs of the dependence of the function *F* on the concentration of DM in solution were constructed (Fig. 5). In a row of measuring flasks of capacity. 25 ml was added 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 ml of DM solution with a concentration of 1 mg/ml and 10 ml of 1 × 10^{-4} M SPH solution, or 0.1, 0.5, 1.0, 2.0, 4.0 ml of DM solution with a concentration of 0.1 mg/ml and 4 ml of 1 × solution 10^{-4} M Cr, the optimal pH value of the solution was set to 8, and adjusted to the mark with distilled water. The resulting solutions were then transferred to shaking jars, where the prepared PU foam tablets were placed and shaken on a mixing device for an hour.

The analytical characteristics of the determination of diphenhydramine by diffuse reflection spectroscopy were evaluated. The metrological characteristics of the developed methods are presented in Table 2. The data obtained indicate a significant influence of the nature of the dye on the properties of ionic associates and the conditions of analysis. The sensitivity coefficient of the calibration dependence in the case of Cr is 36 times higher, and the detection limit is an order of magnitude lower compared with the use of SFN.

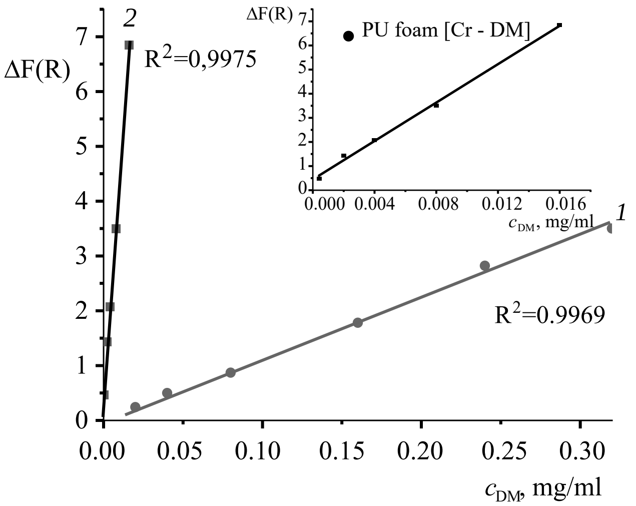


Fig. 5. Calibration graphs of the dependence of the function *F* on the concentration of diphenhydramine in solution when determined as ionic associates with sulfonase (*1*) (c_{SFN}= 0.6 × 10^{-4} M, λ = 570 nm) and congo red (*2*) (c_{Cr} = 0.16 × $10^{-}4$ M, λ = 520 nm).

Determination of diphenhydramine in medicinal products. The developed method was used to determine DM in some medicines (Table 3). To determine DM in tablets, they were ground, weighed and dissolved in 30 ml of distilled water. To separate the formative insoluble inclusions (talc, starch, calcium stearate), the solution was filtered into a volumetric flask. 50 ml and adjusted to the mark with distilled water. The aliquot part was selected from the obtained filtrate and analyzed according to the method of constructing calibration graphs for SFN–DM and Cr–DM associates.

It was previously established that the removal of starch and calcium stearate, which form poorly soluble inclusions, by filtration, as well as the presence of

Table 2. Characteristics of linear regression equations for the determination of diphenhydramine by sorption spectroscopic method using sulfonazo ($c_{SFN} = 0.6\times10^{-4}$ M, $\lambda = 570$) and congo red ($c_{Cr} = 0.16\times10^{-4}$ M, $\lambda = 520$ nm) on polyurethane foam ($m_{sorbent} = 0.04 \pm 0.01$ g, $V = 25.0$ ml)

Ionic associate	**Equations of calibration graphs $\Delta F = bc + a$; c, mg/ml**	**Ratio correlations**	**Area linearity, mg/ml**	**Detection limit, c_{min}, µg/ml**
SFN–DM (pH 8)	$y = 11.08x - 0.030$	$r = 0.9985$	0.02–0.32	0.623
Cr–DM (pH 8)	y = 398.14x + 0.445	r = 0.9984	0.0004–0.016	0.017

Table 3. Results of determination of diphenhydramine in dosage forms ($n = 5$, $P = 0.95$)

Diphenhydramine content according to the manufacturer	**System**	**Metrological characteristics**	
		$X_{mean} \pm \Delta$	s_r
Diphenhydramine tablets (JSC Biosynthesis, Russia)*			
0.050 g	Cr–DM	0.049 ± 0.002	0.033
	SFN–DM	0.050 ± 0.002	0.033
1% diphenhydramine solution (JSC Dalkhimpharm, Russia)**			
0.010 g/1 ml	Cr–DM	0.0098 ± 0.0011	0.012
	SFN–DM	0.0099 ± 0.0012	0.012

Note: *Excipients: lactose monohydrate (milk sugar) – 0.075 g, potato starch – 0.0205 g, talc – 0.003 g, calcium stearate – 0.0015 g.
**Excipients: water for injection, conforms to FS.2.2.0019.18.

lactose monohydrate at a ratio of 1 : 2, did not affect the determination of DM.

To determine DM in the injection solution, the contents of the ampoule (1 ml) were transferred to a 50 ml flask and adjusted to the mark with distilled water. Then, an aliquot of the resulting solution was selected and analyzed according to the method of constructing calibration graphs for ion associates SFN–DM and Cr–DM.

As can be seen from Table 3, the proposed methods for determining DM in medicinal products are characterized by good reproducibility and accuracy of the results: the data obtained are comparable with the manufacturer's data. The methods are suitable for analytical purposes and can be recommended for evaluating the quality of tablets and solution for injection based on diphenhydramine.

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CONFLICT OF INTERESTS

The authors of this paper declare that they have no conflict of interest.

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