



Application of sulphanilamides disazo dyes with Tropaeolin O for simple, rapid and sensitive spectrophotometric assay of medicines

Maria Boiko^{a,b,*}, Teodoziya Vrublevska^a, Olha Korkuna^a, Grigory Teslyar^b

^a Ivan Franko Lviv National University, Chemistry Faculty, Analytical Chemistry Department, Kyrylo & Mefodiy Str., 6, 79005 Lviv, Ukraine

^b State Scientific Research Control Institute of Veterinary Preparations and Fodder Additives, Donetska Str., 11, 79019 Lviv, Ukraine

ARTICLE INFO

Article history:

Received 8 November 2010

Received in revised form 10 February 2011

Accepted 16 February 2011

Keywords:

Sulphanilamides
Azo dyes
Tropaeolin O
Azocoupling
Disazo dyes
Spectrophotometry

ABSTRACT

A rapid, simple and sensitive spectrophotometric method for the determination of some sulphanilamides is described. The method is based on the formation of blue coloured disazo dyes by the diazotization of sulphonamides viz. sulphanilamide (SA), sulphamerazine (SMR), sulphamethazine (SMZ), sulphadimethoxine (SDM), sulphamethoxazole (SMX), sulphadiazine (SDA), sulfathiazole (STZ), sulphaguanidine (SGN), sulphamonomethoxine (SMM), sulphamethoxy pyridazine (SMP) in 0.5 M hydrochloric acid media at ice bath followed by the azocoupling reaction with acid monoazo dye Tropaeolin O (TrO) at pH = 10.5. Formed products are stable for 10 h at room temperature. Effective molar absorptivities at absorbance maxima 595 nm for disazo dyes were $\sim 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. Stoichiometric ratios of the components of disazo dyes were determined by means of mole ratio and continuous variations methods. Linear ranges for sulphanilamides determination were 0.4–14.0 $\mu\text{g ml}^{-1}$. The methods were successfully approved at sulphanilamides determination in model solutions and commercial pharmaceutical preparations.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Sulphanilamides (SA) are synthetic chemotherapeutical medicaments, derivatives of sulphanilic acid, which are able to depress the development of Gram-positive and Gram-negative bacteria, some protozoons and pathogenic fungi. In spite of the fact, that sulphanilamides appeared in medical practice as far back as the beginning of XX century, this group of medications is widely used up today for the treatment of infectious pathology and realized at the pharmaceutical market on a large scale. Over 15 thousand derivatives of sulphanilic acid, about 40 of which are introduced in medical practice as anti-infectives medicinal, were synthesized for today.

Sulphanilamides are the analogues of *p*-aminobenzoic acid which is the constituent of structure of tetrahydrofolic acid – a cofactor of a few enzymes, which take part in the biosynthesis of purine and pyrimidine bases, entering into the structure of nucleic acids. Sulphanilamides' action on microbial cells is related with the disturbance of folic and tetrahydrofolic acid synthesis, which are necessary, in turn, for the biosynthesis of nucleic acids, the end-point of what is the microorganism's death. Combined preparations

of sulphanilamides with trimethoprim and antibiotics of different groups are more effective [1].

The harmless and effective use of medications needs a multilevel control of their quality, in particular, the control of homogeneity of the dosing units of medicines [2]. Utilizing few different bioactive substances in sulphanilamides composition usually requires the application of sensitive and selective methods for their determination both in the prepared drugs dosage forms and their rests in physiological liquids.

For quantitative sulphanilamides assay a high-performance liquid chromatography [3–6], immunoenzyme analysis [7–9] and voltammetry [10,11] are often used. However, these methods are expensive in the use, require the reagents of very high purity, though their use is reasonable at the analysis of sulphanilamides rests in animal food, background objects, biological liquids. For the routine laboratory analysis of sulphanilamides substances and their preparations nitritometry [12] is more frequently utilized, which is not very sensitive and requires plenty of the probed samples, and also it is not selective, because substances which contain primary or secondary amino groups as well as hydrazides form similar reaction products.

All spectrophotometric methods, suggested for sulphanilamides determination in preparations, can be divided into two groups. The first group of methods is based on SA determination due to own absorbance in UV spectrum range at $\lambda = 245\text{--}270 \text{ nm}$, however, they are nonselective, because most organic compounds absorb in the same spectrum range, so these methods are suitable only

* Corresponding author at: Ivan Franko Lviv National University, Chemistry Faculty, Analytical Chemistry Department, Kyrylo & Mefodiy Str., 6, 79005 Lviv, Ukraine. Tel.: +380 322394048.

E-mail address: boiko.maria@ukr.net (M. Boiko).

for the analysis of clean substances [13]. The second group of spectrophotometric methods is based on absorbance measuring of products of coloured reaction of SA with different organic reagents, in particular, aromatic aldehydes, phenols, imines, dinitrobenzene-furoxanes [14–24]. However, described methods require using of organic media and they are multistage as well as nonselective and insensitive. Among the dyes for the spectrophotometric determination of sulphanilamides only alizarin derivatives are offered, which form complex compounds with charge transfer [25], however, methods which are based on such interaction are low-sensitive and low-contrast.

As primary aromatic amino group is entered to the sulphanilamides structure, it can be diazotized and azocoupled with aromatic compounds. Monoazo dyes are the perspective reagents for the azocoupling with diazotized sulphanilamides for the obtaining colour products. Therefore we studied diazotized sulphanilamides interaction with acid monoazo dye Tropaeolin O (sodium salt of 2',4'-azophenolsulfonic acid, Acid Yellow, Acid Orange 6, CAS № 547-57-9, C.I. 14270) with the purpose to elaborate the spectrophotometric method of SA determination. Spectrophotometric utilization of Tropaeolin O is very limited, it is known only about its use for the determination of palladium(II) [26,27], osmium (IV) [28] and proteins of albumin and casein [29].

2. Experimental

2.1. Reagents and apparatus

All aqueous solutions were prepared using distilled water.

Sulphanilamide, sulphamerazine, sulphamethazine, sulphadimethoxine, sulphamethoxazole, sulphadiazine, sulphathiazole, sulphaguanidine, sulphamonomethoxine, sulphamethoxy-pyridazine were purchased from Sigma (USA). Solutions of sulphanilamides were prepared by dissolving appropriate amounts of the reagents of pharmacopoeia grade in 0.1 M sodium hydroxide solution because of their slight solubility in water [30].

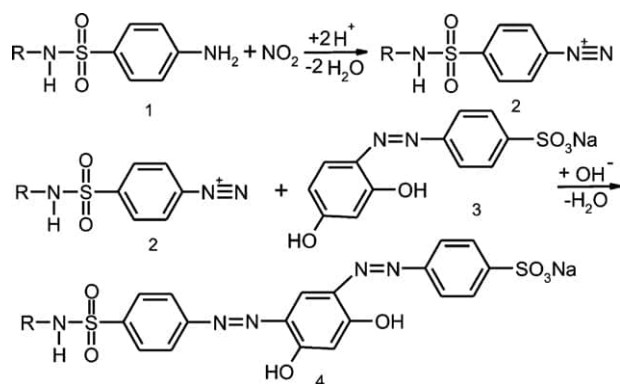
Solutions of Tropaeolin O (Merck, Germany) were prepared by dissolving appropriate amounts of the reagent of analytical grade $\geq 97\%$ purity in distilled water.

The solutions of hydrochloric acid, sodium hydroxide and sodium nitrite were prepared from the chemicals of the analytical grade.

UV-vis measurements were performed with UV-Vis scanning spectrophotometer CARY.WIN-UV-Vis-50 (Varian, USA) using 1 cm cuvettes. All absorbance measurements were performed at $\sim 20^\circ\text{C}$. pH measurements were carried out with a pH-meter, model pH 150M (Gomelsky Plant of Measuring Devices, Belarus), equipped with a glass electrode. pH of each solution was adjusted using diluted HCl and NaOH solutions.

2.2. Procedure of sulphanilamides determination

5.0 ml of 0.5 M hydrochloric acid solution was placed into a 25 ml volumetric flask. Then a sample of solution containing $0.4\text{--}14.0\ \mu\text{g ml}^{-1}$ of sulphanilamide in final volume was added. Next 5.0 ml of 1.25×10^{-2} M sodium nitrite solution was added into the flask. Obtained solution was stirred and cooled on an ice bath ($\sim 0^\circ\text{C}$) for 10 min. Then 1.0 ml of 1.25×10^{-3} M Tropaeolin O solution was added into the flask. Obtained mixture was neutralized by adding of 2.5 ml of 1 M sodium hydroxide solution and the pH value was adjusted to pH = 10.5 and distilled water was added to the full volume of 25 ml. Then the solution was mixed thoroughly and the absorbance measurements (at room temperature $\sim 20^\circ\text{C}$) were carried out against all corresponding reagents blank solution



Scheme 1. Reaction sequence for the formation of blue disazo dye under sulphanilamide interaction with Tropaeolin O.

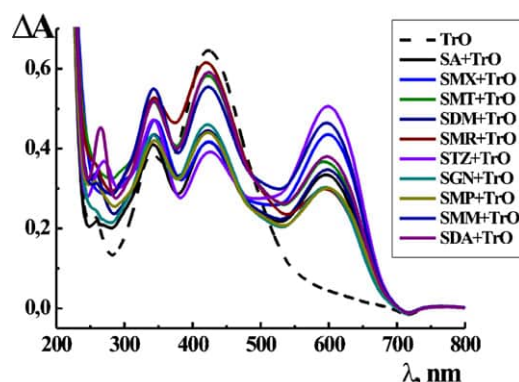


Fig. 1. Absorption spectra of Tropaeolin O and disazo dyes aqueous solutions; $C(\text{HCl}) = 0.5\ \text{M}$; $C(\text{TrO}) = 2.5 \times 10^{-5}\ \text{M}$; $C(\text{NaNO}_2) = 2.5 \times 10^{-4}\ \text{M}$; $C(\text{SA}) = 1.5 \times 10^{-5}\ \text{M}$; pH = 10.5; $l = 1\ \text{cm}$.

at 595 nm in 1.0 cm cuvettes. Sulphanilamide concentration was calculated using the methods of calibration curve and single-point standardization.

3. Results and discussion

The interaction of Tropaeolin O (TrO) with ten sulphanilamides (Table 1), which are more frequently used in medical and veterinary practice, has been investigated.

Quantitative determination of SA is based on the obtaining of water soluble coloured products of diazotized sulphanilamides with Tropaeolin O (Scheme 1). Nitrite-ions diazotize primary aromatic amino group of SA (1) in acid media with the formation of diazotized SA (2), which interact with Tropaeolin O in alkaline media forming blue coloured disazo dyes (4).

3.1. Absorption spectra

Absorption spectra of disazo dyes (products of diazotized sulphanilamides coupling with Tropaeolin O) are shown in Fig. 1.

Colour transition region for Tropaeolin O is pH = 11.1–12.7 from yellow to reddish-brown; $pK_a = 11.8$. At pH = 11.1 absorption maxima of the dye is in the range of waves lengths 420–440 nm ($\epsilon = 3.3 \times 10^4\ \text{M}^{-1}\ \text{cm}^{-1}$), and at pH = 12.7 – at 475–495 nm ($\epsilon = 2.2 \times 10^4\ \text{M}^{-1}\ \text{cm}^{-1}$) [31].

As can be seen from absorption spectra (Fig. 1), maximum absorbance of Tropaeolin is at 420 nm, while the absorbance maximum of disazo dyes for all tested sulphanilamides are in the range of waves lengths 589–604 nm, where TrO absorbance is negligible. More detailed absorbance maxima of all obtained disazo dyes

Table 1
Molecular structure of investigated sulphanilamides.

Sulphanilamide (SA), streptocidum <i>p</i> -aminobenzenesulphonamide, CAS ¹ 63-74-1	
Sulphamerazine (SMR) 2-(<i>p</i> -aminobenzenesulphonamido)-4-methylpyrimidine, CAS ¹ 127-79-7	
Sulphamethazine (SMZ), sulphadimezine 2-(<i>p</i> -aminobenzenesulphonamido)-4,6-dimethylpyrimidine, CAS ¹ 57-68-1	
Sulphadimethoxine (SDM) 6-(<i>p</i> -aminobenzenesulphonamido)-2,4-dimethoxypyrimidine, CAS ¹ 122-11-2	
Sulphamethoxazole (SMX) 3-(<i>p</i> -aminobenzenesulphonamido)-5-methyloxazole, CAS ¹ 723-46-6	
Sulphadiazine (SDA), 2-(<i>p</i> -aminobenzenesulphonamido)-pyrimidine, CAS ¹ 68-35-9	
Sulfathiazole (STZ), 2-(<i>p</i> -aminobenzenesulphonamido)-1,3-thiazol, CAS ¹ 72-14-0	
Sulphaguanidine (SGN), N-(<i>p</i> -aminobenzenesulphonamido)-aminoiminomethyl, CAS ¹ 57-67-0	
Sulphamonomethoxine (SMM), 4-(<i>p</i> -aminobenzenesulphonamido)-6-methoxypyrimidine, CAS ¹ 1220-83-3	
Sulphamethoxypyridazine (SMP), 6-(<i>p</i> -aminobenzenesulphonamido)-3-methoxypyridazine, CAS ¹ 000080-35-3	

Table 2Spectroscopic characteristics of the formed disazo dyes and validation results of sulphanilamides spectrophotometric determination with Tropaeolin O; C(HCl)=0.5 M; C(NaNO₂)=2.5 × 10⁻⁴ M; C(TrO)=2.5 × 10⁻⁵ M; pH=10.5; l=1 cm.

Parameters/characteristics	SA	SMR	SMZ	SDM	SMX	SDA	STZ	SGN	SMM	SMP
Colour						Blue				
$\Delta\lambda_{\max}$, nm	595–597	596–599	589–595	591–596	595–599	594–601	595–601	593–604	594–600	593–598
Stability, h						10				
Optimum photometric linear rang, $\mu\text{g ml}^{-1}$	0.4–7.0	0.4–12.0	0.4–10.0	0.4–10.0	0.4–9.0	0.4–9.0	0.4–8.0	0.4–13.0	0.4–12.0	0.6–14.0
Limit of quantification, $\mu\text{g ml}^{-1}$	0.19	0.14	0.14	0.16	0.14	0.25	0.09	0.22	0.24	0.22
Limit of quantification $\times 10^7$, M	11	5.3	5.0	5.2	5.5	10	3.5	10	8.6	7.8
Molar absorptivity, $\epsilon_{595} \times 10^{-4}$, $\text{M}^{-1} \text{cm}^{-1}$	2.7	2.0	3.1	3.1	3.0	2.9	3.4	2.1	2.3	2.1
Regression equation (A) ^a slope (b)	0.146	0.66	0.098	0.087	0.109	0.106	0.124	0.077	0.086	0.075
Intercept (a)	0.001	0.043	0.009	0.010	0.015	0.019	0.011	0.015	0.015	0.015
Correlation coefficient (R)	0.9995	0.9994	0.9998	0.9994	0.9997	0.9993	0.9998	0.9995	0.9994	0.9995

^a $A = bc + a$, where c is the concentration of sulphanilamide in $\mu\text{g ml}^{-1}$.

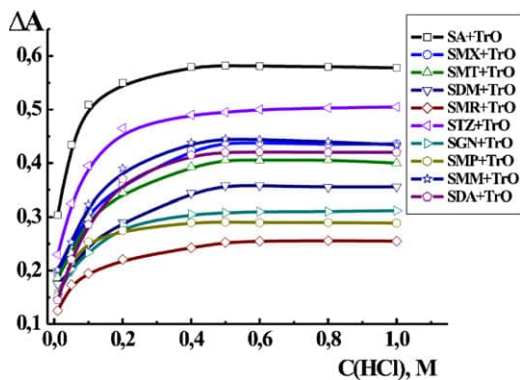


Fig. 2. Effect of hydrochloric acid concentration during SA diazotization on disazo dyes formation; $C(\text{TrO}) = 2.5 \times 10^{-5} \text{ M}$; $C(\text{NaNO}_2) = 2.5 \times 10^{-4} \text{ M}$; $C(\text{SA}) = 4 \mu\text{g ml}^{-1}$; $\text{pH} = 10.5$; $l = 1 \text{ cm}$; $\lambda = 595 \text{ nm}$.

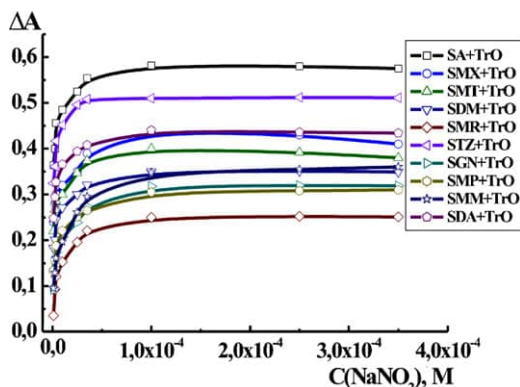


Fig. 3. Effect of sodium nitrite concentration during SA diazotization on disazo dyes formation; $C(\text{HCl}) = 0.5 \text{ M}$; $C(\text{TrO}) = 2.5 \times 10^{-5} \text{ M}$; $C(\text{SA}) = 4 \mu\text{g ml}^{-1}$; $\text{pH} = 10.5$; $l = 1 \text{ cm}$; $\lambda = 595 \text{ nm}$.

are shown in Table 2. Therefore, further investigations have been carried out at 595 nm.

3.2. Conditions of sulphanilamides diazotization

3.2.1. Effect of hydrochloric acid concentration

In order to establish optimal reaction conditions it was important to study the effect of hydrochloric acid concentration on the yield of diazotized sulphanilamides with the following formation of disazo dyes. The results are presented in Fig. 2.

As follows from Fig. 2, the maximal yield of azocoupling products of all examined SA with TrO are observed at diazotization in 0.4–1.0 M hydrochloric acid. When the hydrochloric acid with concentration $\geq 1.0 \text{ M}$ was used for this purpose the yield of azocoupling products has been decreased. Therefore we used 0.5 M HCl for SA diazotization in all following experiments.

3.2.2. Effect of sodium nitrite concentration

We also investigated the influence of sodium nitrite concentration as diazotizing reagent on the yield of sulphanilamides diazonium salts as well as disazo dyes accordingly. As it is shown in Fig. 3, $1 \times 10^{-4} \text{ M}$ sodium nitrite solution is optimal for SA diazotization. High nitrite concentrations, viz. $>0.1 \text{ M}$, caused the decomposition of azo reagent.

3.2.3. Effect of temperature

A temperature has significant influence on the diazotization of organic compounds, whereas it is known that reaction of diazonium salt formation is more effective at low temperatures. Therefore, the

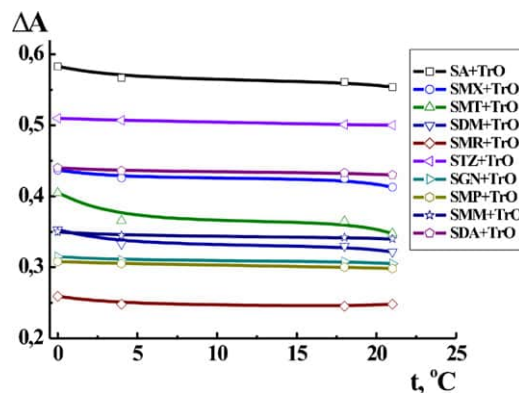


Fig. 4. Effect of temperature during SA diazotization on disazo dyes formation; $C(\text{HCl}) = 0.5 \text{ M}$; $C(\text{TrO}) = 2.5 \times 10^{-5} \text{ M}$; $C(\text{NaNO}_2) = 2.5 \times 10^{-4} \text{ M}$; $C(\text{SDM}) = 4 \mu\text{g ml}^{-1}$; $\text{pH} = 10.5$; $l = 1 \text{ cm}$; $\lambda = 595 \text{ nm}$.

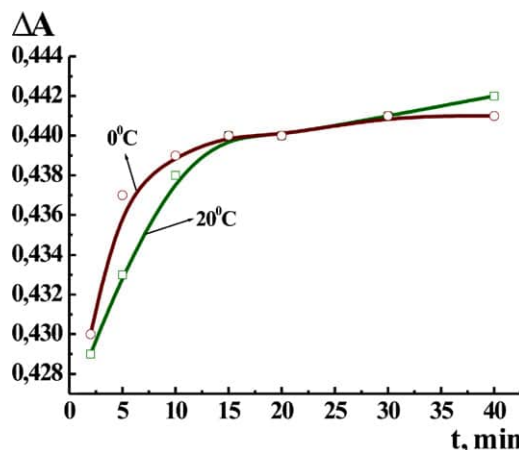


Fig. 5. Effect of time of sulphanilamide diazotization on the disazo dye yield; $C(\text{HCl}) = 0.5 \text{ M}$; $C(\text{NaNO}_2) = 2.5 \times 10^{-4} \text{ M}$; $C(\text{TrO}) = 2.5 \times 10^{-5} \text{ M}$; $C(\text{SA}) = 4 \mu\text{g ml}^{-1}$; $\text{pH} = 10.5$; $l = 1 \text{ cm}$; $\lambda = 595 \text{ nm}$.

temperature effect of SA diazotization on the yield of disazo dyes was investigated (Fig. 4). As follows from experimental data, the temperature effect is not considerable, however, the yield of diazotized sulphanilamides at $\sim 0^\circ\text{C}$ (solutions were cooled using an ice bath) increased insignificantly. Moreover, the results, obtained on cooling during diazotization, were more reproducible, because relative error of sulphanilamides determination ($n = 5$, $P = 0.95$) at 20°C was 4.5%, while at 0°C – 1.5%.

3.2.4. Time effect of SA diazotization on disazo dyes formation

According to the experimental results (Fig. 5), SDM diazonium salt is formed immediately after reagents mixing, however, its maximal yield as well as azocoupling products respectively has been observed in 15 min at room temperature and in 10 min at diazotization on an ice bath, that allows to shorten the analysis duration. The further increase of diazotization time did not reflect in disazo dyes yield. Time effect of SA diazotization on disazo dyes formation for all tested sulphanilamides is the same.

3.3. Azocoupling conditions of Tropaeolin O with diazotized sulphanilamides

3.3.1. Effect of pH

In order to establish optimal reaction conditions, the investigations of pH effect on the analytical signal value of diazotized SA with TrO were carried out. As follows from Fig. 6, the maximal yield of disazo dyes is observed in the range of $\text{pH} = 10\text{--}11$, that is inherent

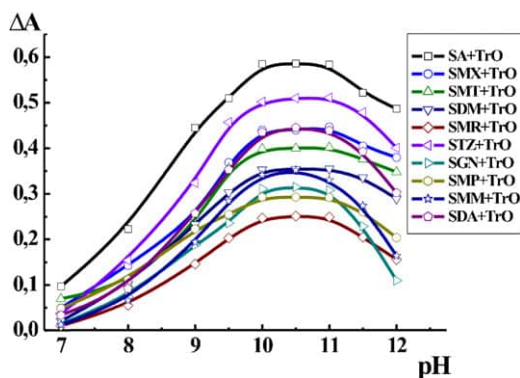


Fig. 6. Effect of pH value on Tropaeolin O azocoupling with diazotized sulphanilamides; $C(\text{NaNO}_2) = 2.5 \times 10^{-4} \text{ M}$; $C(\text{HCl}) = 0.5 \text{ M}$; $C(\text{TrO}) = 2.5 \times 10^{-5} \text{ M}$; $C(\text{SA}) = 4 \mu\text{g ml}^{-1}$; $l = 1 \text{ cm}$; $\lambda = 595 \text{ nm}$.

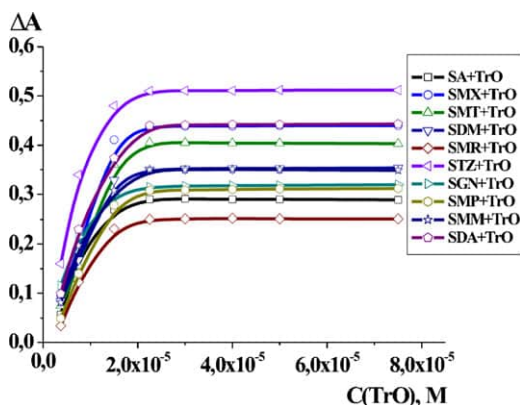


Fig. 7. The mole ratio curve for disazo dyes of diazotized sulphanilamides and Tropaeolin O; $C(\text{NaNO}_2) = 2.5 \times 10^{-4} \text{ M}$; $C(\text{HCl}) = 0.5 \text{ M}$; $C(\text{SA}) = 1.5 \times 10^{-5} \text{ M}$; $\text{pH} = 10.5$; $l = 1 \text{ cm}$; $\lambda = 595 \text{ nm}$.

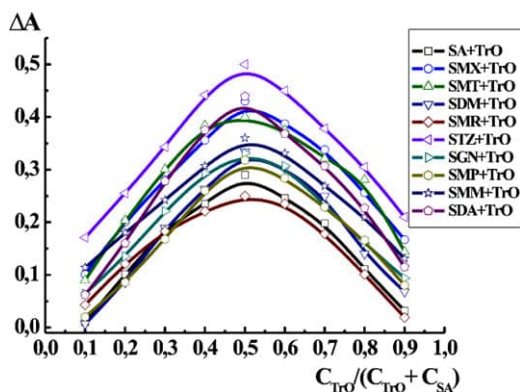


Fig. 8. The continuous variations curve for disazo dyes of diazotized sulphanilamides and Tropaeolin O; $C(\text{HCl}) = 0.5 \text{ M}$; $C(\text{NaNO}_2) = 2.5 \times 10^{-4} \text{ M}$; $C(\text{SA} + \text{TrO}) = 3 \times 10^{-5} \text{ M}$; $\text{pH} = 10.5$; $l = 1 \text{ cm}$; $\lambda = 595 \text{ nm}$.

for azocoupling of diazonium salts with the cresol group of second component [32]. All further investigations were carried out at $\text{pH} = 10.5$.

3.3.2. Effect of Tropaeolin O concentration

According to the experimental results (Fig. 7), maximal value of disazo dyes absorbance is observed at 1.5-fold reagent excess.

To establish the molar ratio of the components in the compounds of sulphanilamides with TrO, we used mole ratio method (Fig. 7) and method of continuous variations (Fig. 8). The stoichiometric SA:TrO ratios, estimated by both these methods,

were consistent and equaled to 1:1 for all tested sulphanilamides.

Effective molar absorptivities for all obtained disazo dyes were calculated using the results obtained in the method of continuous variations, which are presented in Table 2.

3.3.3. Stability of azocoupling products of diazotized sulphanilamides with Tropaeolin O

To study the stability of obtained disazo dyes in time we have measured the absorbance value during one week. As follows from obtained data, absorbance value of disazo dyes of all examined SA did not change for 10 h, but in 5 days diminished on 20%.

3.4. Sensitivity and linear range of sulphanilamides spectrophotometric determination with TrO

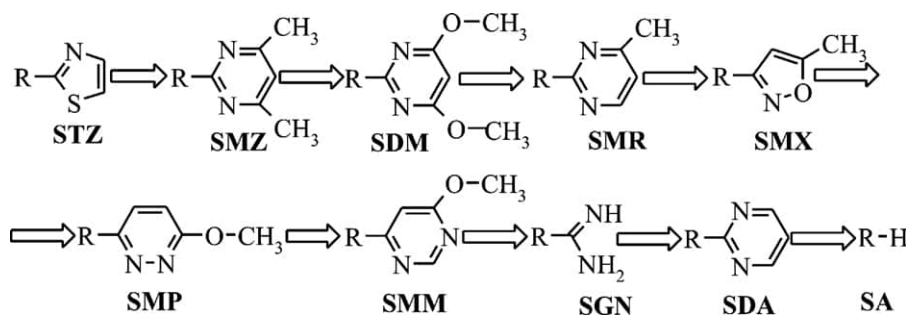
We have investigated that the absorbance of coloured products linearly depends on SA concentration in the solution. Validation results of spectrophotometric determination of five sulphanilamides by means of TrO are presented in Table 2.

Thus the elaborated methods for the spectrophotometric determination of all tested sulphanilamides with TrO possess wide linear ranges (almost one and a half orders of concentration); they are simple, rapid and sensitive. Sensitiveness of sulphathiazole determination was the highest, for all remainder SA it was practically the same. All SA according to the decrease for their determination sensitivity can be placed in the row, which is shown in Scheme 2.

As follows from Table 2 and Scheme 2 the absorbance value of products of SA interaction with Tropaeolin O depends on the presence of the diverse substituents in the sulphanilamide molecule. Heterocycles in sulphanilamide molecules are electron donor groups which influence on the absorbance value of azocoupling products and have a different effect depending on their heteroatom. In the row of heteroatoms $\text{S} > \text{O} > \text{N}$ electron donor influence of heterocyclic substituents diminishes [33]. The obtained results confirm this fact, as most sensitive absorbance band at 595 nm belongs to azocoupling product of diazotized sulphanilamide which contains sulphur atom in a heterocycle (STZ). The disazo dye of non substituted sulphanilamide streptocide has the least intensive band. Besides that the substituents presence in heterocycles influences on electronegativity of whole cycle, that is why a sensitivity for SMR, SDM, SMZ is higher than for SMX because they have substituents in heterocycles.

The nature of heterocycle substituent as well as substituents number influences on the sensitivity of SA determination, because they possess the different electronegativity and the different value and sign of inductive effect accordingly. So a methyl group is less electronegative than the methoxyl one. From the obtained results one can see, that the more substituents are in a heterocyclic ring the higher is the sensitivity of SA determination, hereby the presence of group with less electron acceptor ability (methyl) increases a sensitivity. For heterocycles with two heteroatoms both the nature of heteroatoms and their location as well as the position in which a sulphanilamide group is present in heterocycle have an influence. So SMP has the methoxy-pyridazine substituent (contains two nitrogen atoms in ortho-position one for one), and SMM has the methoxy-pyrimidine substituent (contains two nitrogen atoms in meta-position one for one), however we cannot compare the efficiency of substituents influence on the sensitivity value of SA determination because sulphanilamide group is bonded to heterocycle in different positions towards methoxyl group.

In another hand different maxima value on disazo dyes absorbance spectra are related to the mesomer effect caused by the presence of different heteroatomic constituents. The appearance of the additional peak at 589–604 is related to the absorbance of new azo group formed at azocoupling, what is characteristically for



Scheme 2. Series of sensitivity decrease for sulphanilamides determination at azocoupling reaction with Tropaeolin O.

Table 3

Accuracy of spectrophotometric determination of sulphanilamides in model solutions using Tropaeolin O; $P=0.95$; $n=5$; $C(\text{HCl})=0.5\text{ M}$; $C(\text{NaNO}_2)=2.5 \times 10^{-4}\text{ M}$; $C(\text{TrO})=2.5 \times 10^{-5}\text{ M}$; $\text{pH}=10.5$; $l=1\text{ cm}$; $\lambda=595\text{ nm}$.

Compound	Added, μg	Found $\bar{x} \pm S \cdot t_{\alpha}/\sqrt{n}$, μg	R.S.D., %
Sulphanilamide		100.0 ± 1.4	1.1
Sulphamerazine		100.0 ± 1.2	1.0
Sulphamethazine		100.2 ± 0.9	0.7
Sulphadimethoxine		99.9 ± 1.7	1.4
Sulphamethoxazole		100.1 ± 1.0	0.8
Sulphadiazine	100	100.4 ± 1.6	1.3
Sulphamonomethoxine		99.7 ± 1.5	1.3
Sulphathiazole		100.2 ± 1.2	1.0
Sulphamethoxypyridazine		100.4 ± 1.5	1.2
Sulphaguanidine		99.8 ± 1.3	1.1

Table 4

Effect of excipients on the sulphanilamides assays by Tropaeolin O; $P=0.95$; $n=5$.

Excipient (Exp)	m(SA):m(Exp) ^a	m(SA):m(Exp) ^b	% Recovery of SA, $\bar{x} \pm S \cdot t_{\alpha}/\sqrt{n}$		
			Sulphanilamide	Sulphamethazine	Sulphadimethoxine
Starch	1:0.1	1:25	101.8 ± 1.5	98.6 ± 1.7	99.5 ± 1.8
Gelatine	1:0.1	1:10	98.8 ± 1.5	97.2 ± 1.8	99.5 ± 1.1
Aerosil	1:0.1	1:5	95.3 ± 2.1	100.3 ± 1.3	97.4 ± 1.5
Calcium stearate	1:0.01	1:1	95.4 ± 1.9	96.0 ± 2.2	97.6 ± 1.7

^a Mass ratios of SA and excipient, which are present in tested drugs [2].

^b Maxima mass ratios of SA and excipient, which were examined.

molecules with the planar location of the conjugate chromophore system [32]. Presence of two other peaks at 345 and 420 nm is specified for Tropaeolin O, however they have diverse absorbance value. This phenomenon can be explained by the conjugation effect of π -electron clouds in the double bond of chromophore groups.

3.5. Application to samples

The accuracy of spectrophotometric determination of sulphanilamides with Tropaeolin O was tested on model solutions using the method “added–found”. SA amounts were calculated using method of single-point standardization (Table 3).

The obtained results correlate well with the amount of SA added into the model solutions, and the values of R.S.D. are within the range of ones for spectrophotometry. Thus the elaborated methods of sulphanilamides determination by means of TrO can be effectively applied for the analyses of SA substances.

3.5.1. Approbation of the methods during the analysis of drugs

Elaborated method of sulphanilamides determination by mean of TrO was approved at testing of sulphadimezine, sulphadimethoxine, sulphanilamide quantity in tablets “Streptocidum”, “Sulfadimesinum”, “Sulfadimethoxine” accordingly. Amounts of bioactive substances in these drugs vary in the range 0.475–0.525 g according to formal quality control method [12].

The common excipients such as starch, calcium stearate, gelatine, aerosil are present in examined pharmaceutical preparations. Therefore, the effect of common tablet excipient and additives on the procedure of SA determination by means of Tropaeolin O was investigated. An error of 5.0% in the absorbance readings was considered tolerable. The actual SA to excipient ratio is nearly 10- to 100-fold with respect to the excipient in pharmaceutical preparations. But as follows from Table 4 the conditions under which the experiments were carried out, viz., the ratio of SA to excipient is nearly equal to 500–10,000 with respect to excipient. That means, the excipients were taken in very large excess for the experiment. Thus the tested common excipients, which often accompany the pharmaceutical preparations do not interfere in the present method.

3.5.2. Procedure of tablet preparation

Ten tablets were weighed and finely powdered in porcelain mortar. The accurate amount of powder, equal to $\sim 500\text{ mg}$ of SA, was placed into a 100 ml volumetric flask and dissolved in 50 ml of 0.1 M NaOH for obtaining of SA extraction. Then solution was mixed for 10 min and 0.1 M NaOH was added to complete the volume to 100 ml. Obtained solution was mixed again and filtered through the fold filter of medium porosity. The filtrate was 50-fold diluted. Thereto 1.0 ml of filtrate was placed into a 50 ml volumetric flask and diluted by 0.1 M sodium hydroxide solution to the full volume of 50 ml. Nominal SA content in solution obtained in such way was

Table 5
Determination of sulphanilamides in pharmaceuticals; $P=0.95$; $n=5$.

Tablets	Label claim, g	Amount of drug found, mg			
		Proposed method		Pharmaceutical method (nitritometry) [12]	
		$\bar{x} \pm S \cdot t_{\alpha}/\sqrt{n}$	R.S.D., %	$\bar{x} \pm S \cdot t_{\alpha}/\sqrt{n}$	R.S.D., %
Streptocidum (SA) ^a	0.5	501 ± 6	1.0	500 ± 8	1.3
Sulfadimesinum (SMZ) ^b	0.5	501 ± 5	0.8	501 ± 7	1.1
Sulfadimethoxinum (SDM) ^a	0.5	499 ± 8	1.3	501 ± 8	1.3

^a Marketed by VAT "Monfarm", Ukraine.

^b SIC "Borshchahivskiy Chemical-Pharmaceutical Plant" CJSC (BCPP), Kyiv, Ukraine.

100 µg ml⁻¹. For assay solution an aliquot of 1 ml was pick out and then treated as described above in the recommended procedure for the sulphanilamides determination.

The assay results are presented in Table 5.

According to Table 5, obtained data were comparable to those obtained using the official methods of nitritometry for each of the studied drugs.

4. Conclusions

At first the formation of disazo dyes by the interaction of diazotized sulphanilamides with acid monoazo dye Tropaeolin O has been established. Azocoupling occurs due to the cresol group of second component (TrO), which has compatibly oriented substituents. Optimum conditions for sulphanilamide, sulphamerazine, sulphamethazine, sulphadimethoxine, sulphamethoxazole sulphadiazine, sulphathiazole, sulphaguanidine, sulphamonomethoxine, sulphamethoxyypyridazine interaction with Tropaeolin O on the stages of diazotization as well as azocoupling have been investigated. Spectroscopic and validation characteristics of sulphanilamides determination with Tropaeolin O have been established. The components ratio in the disazo dyes are SA:TrO = 1:1. The effective molar absorptivity ϵ_{595} is $\sim 10^4$ M⁻¹ cm⁻¹. The proposed method is found to be simple, rapid, and economical, allows to determine wide range of SA concentrations (0.4–14.0 µg ml⁻¹) and competes with most of the spectrophotometric methods available in literature. The method is advantageous over many spectrophotometric methods with special reference to stability and sensitivity. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. Elaborated method has been approved during analysis of model solutions and commercial pharmaceutical preparations. Obtained data were well correlated to those obtained using the official methods of nitritometry for each of the studied pills. So the recommended procedure is well-suited for the assay and evaluation of drugs in pharmaceutical preparations to assure high standard of quality control.

References

- [1] British Pharmacopoeia, US Pharmacopoeia, XXVI, 2006.
[2] State Pharmacopoeia of Ukraine Add.2, Kharkiv, 2008.

- [3] Y. Ito, H. Oka, Y. Ikai, H. Matsumoto, Y. Miyazaki, H. Nagase, J. Chromatogr. A 898 (2000) 95–102.
[4] V. Gamba, C. Terzano, L. Fioroni, S. Moretti, G. Dusia, R. Galarinic, *Analyt. Chim. Acta* 637 (2009) 18–23.
[5] A.F. Forti, G. Scortichini, *Analyt. Chim. Acta* 637 (2009) 214–219.
[6] C. Hartig, T. Storm, M. Jekel, J. Chromatogr. A 854 (1999) 163–173.
[7] Z. Wang, S. Zhang, I.S. Nesterenko, S.A. Eremin, J. Shen, J. Agric. Food Chem. 55 (2007) 6871–6878.
[8] S. Zhang, Z. Wang, I.S. Nesterenko, S.A. Eremin, J. Shen, *Int. J. Food Sci. Technol.* 42 (2007) 36–44.
[9] I.S. Nesterenko, M.A. Nokel', S.A. Yeriomin, *Zhurn. Analit. Khim.* 64 (2009) 1–20.
[10] T.A.M. Msagati, J.C. Ngila, *Talanta* 58 (2002) 605–610.
[11] A. Wang, F. Gong, H. Li, Y. Fang, *Analyt. Chim. Acta* 386 (1999) 265–269.
[12] State Pharmacopoeia of Ukraine Add.1, Kharkiv, 2004.
[13] P. Nagaraja, K.R. Sunitha, H.S. Yathirajan, R.A. Vasantha, *Ind. J. Pharm. Sci.* 65 (2003) 82–84.
[14] E.V. Klokovala, S.G. Dmitrienko, *Vestn. Mosk. Un-ta. Ser. 2. Khimiya* 49 (2008) 339–343.
[15] M.I. Eugeniev, S.Yu. Garmonov, L.Sh. Shakorova, F.S. Levinson, *Zhurn. Analit. Khim.* 55 (2000) 888–895.
[16] P. Nagaraja, H.S. Yathirajan, C.R. Raju, R.A. Vasantha, P. Nagendra, M.S.H. Kumar, *Il Farmaco* 58 (2003) 1295–1300.
[17] P. Nagaraja, K.R. Sunitha, R.A. Vasantha, H.S. Yathirajan, *Ind. Drugs* 38 (2001) 489–490.
[18] P. Nagaraja, K.R. Sunitha, R.A. Vasantha, H.S. Yathirajan, *Eur. J. Pharm. Biopharm.* 53 (2002) 187–192.
[19] P. Nagaraja, H.S. Yathirajan, K.R. Sunitha, R.A. Vasantha, *J. Assoc. Off. Anal. Chem.* 85 (2002) 869–874.
[20] N.D. Dinesh, P. Nagaraja, K.S. Rangappa, *Proc. Natl. Acad. Sci. India* 72A (2002) 231–235.
[21] P. Nagaraja, H.S. Yathirajan, K.R. Sunitha, R.A. Vasantha, *Anal. Lett.* 35 (2002) 1531–1540.
[22] P. Nagaraja, K.R. Sunitha, R.A. Vasantha, H.S. Yathirajan, *Ind. J. Pharm. Sci.* 64 (2002) 391–393.
[23] N. Yongnian, Q. Zhengbao, S. Kokot, *Chemometr. Intell. Lab. Sys.* 82 (2006) 241–247.
[24] F.H.M. Vaid, M. Aminuddin, K. Mehmood, *Pak. J. Pharm. Sci.* 17 (2004) 77–84.
[25] A.S. Amin, G.O. El-Sayed, Y. Missa, *Microchem. J.* 51 (1995) 367–373.
[26] K.K. Saxena, A.K. Dey, *Anal. Chem.* 40 (1968) 1280–1285.
[27] R.L. Seth, A.K. Dey, *J. Indian Chem. Soc.* 40 (1963) 794–796.
[28] O.S. Bonishko, M.V. Polko, O.Ya. Korkuna, T.Ya. Vrublevska, *Visn. Kharkiv Univ. Ser. Khim.* 15 (38, 770) (2007) 70–75.
[29] M. Rydchuk, M. Boiko, T. Vrublevska, O. Korkuna, O. Tropaeolin, The aspects of the spectrophotometric application, in: Twelfth Scientific Conference Lviv Chemical Readings–2009, Lviv, Ukraine, 2009, p. A16.
[30] European Pharmacopoeia (Eur. Ph.), fourth ed., Council of Europe, Strasbourg, 2002.
[31] K. Venkataraman, *The Chemistry of Synthetic Dyes*, vol. III, Goskhimizdat, Leningrad, 1974.
[32] B.I. Stepanov, *Introduction to the Chemistry and Technology of Organic Dyes*, Khimiya, Moscow, 1977.
[33] H. Zollinger, *Chemistry of Azo Dyes*, Goskhimizdat, Leningrad, 1960 (Russian translation).