SIMULTANEOUS HPLC DETERMINATION OF TRIMETHOPRIM, SULFAMETHOXAZOLE, AND METHYL- AND PROPYLPARABEN IN SUSPENSIONS OF THE CO-TRIMOXAZOLE TYPE

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 36, No. 12, pp. 37 – 41, December, 2002.

Original article submitted September 25, 2002.

Medicinal suspensions containing trimethoprim and sulfamethoxazole are usually analyzed by three principal methods: spectrophotometry, HPLC, and TLC. According to the British Pharmacopoeia, trimethoprim and sulfamethoxazole in suspensions of the co-trimoxazole type are determined by spectrophotometry after extraction and derivatization with N-(1-naphthyl)ethylenediamine hydrochloride [1]. The US Pharmacopoeia [2] proposes HPLC analysis for the same purpose. However, neither of the two Pharmacopoeias stipulates determining preservatives in these drugs.

According to the existing Russian normative documentation, trimethoprim and sulfamethoxazole in suspensions of the biseptol type are determined spectrophotometrically, and the content of methyl- and propylparabens is additionally evaluated by TLC [3]. At the same time, septrin suspensions are analyzed simultaneously for trimethoprim, sulfamethoxazole, methylparaben, and sodium benzoate by HPLC [4], but this is performed using two different chromatographic systems neither of which is suited for the determination of propylparaben (whose retention time significantly differs from those of the other components).

The purpose of our study was to develop an HPLC procedure capable of simultaneously determining trimethoprim, sulfamethoxazole, methylparaben, and propylparaben in suspensions of the co-trimoxazole type. The new technique is described below in accordance with the general approach to the validation of methods proposed for determining parent substances and preservatives in drug suspensions.

EXPERIMENTAL PART

Equipment. The analyses were performed with a Waters chromatographic system (Alliance 2690) equipped with a Waters Model 996 photodiode array detector. This detector was also used for measuring the optical absorption spectra in the chromatographic peaks of analyzed substances for optimization of the analytical wavelength and for evaluation of the "spectral homogeneity" of the HPLC peaks (i.e., purity of the components). The experimental data acquisition and the processing of chromatograms and absorption spectra were performed on a computer using the Waters MILLENNIUM program package.

Preparation of Solutions. Calculation of the Content of Main Components and Preservatives

Solvent. The drug samples were dissolved in a 1 : 3 (v/v) mixture of acetonitrile and diluted (1 : 100) acetic acid.

Working Standard (WS) solution of methylparaben (solution No. 1). An exactly weighed amount (about 12.5 mg) of the WS of methylparaben was placed in a 25-ml measuring flask and dissolved in ~15 ml of the solvent. Then the flask was filled with the same solvent to the mark and the solution was thoroughly stirred.

WS solution of propylparaben (solution No. 2). An exactly weighed amount (about 10 mg) of the WS of propylparaben was placed in a 100-ml measuring flask and dissolved in ~75 ml of the solvent. Then the flask was filled with the same solvent to the mark and the solution was thoroughly stirred.

Solution of a WS mixture of trimethoprim, sulfamethoxazole, methylparaben, and propylparaben. To exactly weighed amounts of sulfamethoxazole WS (about 100 mg) and trimethoprim WS (about 20 mg) in a 50-ml measuring flask were added 5-ml portions of solutions Nos. 1 and 2 and 25 ml of the solvent. The mixture was treated with ultrasound to complete dissolution (~10 min) and cooled to room temperature. Then the flask was filled to the mark and the solution was thoroughly stirred.

Test solution for HPLC analysis of co-trimoxazole type suspensions. To an exactly weighed sample of suspen-

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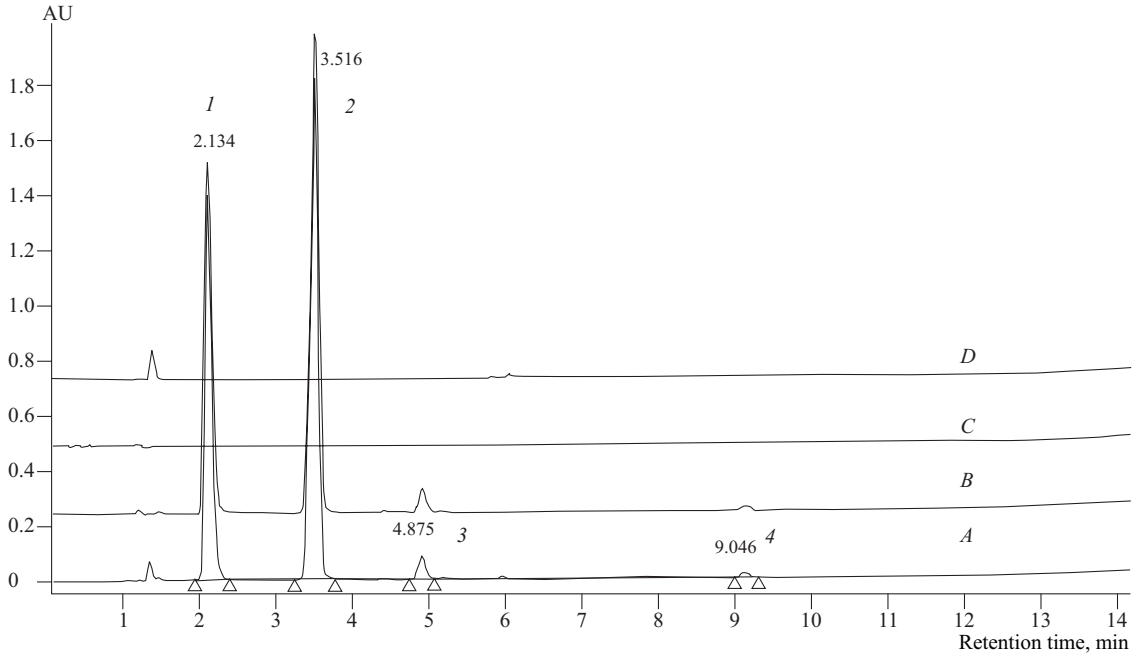

Fig. 1. Typical chromatograms of model solutions (*D*) measured with the detector tuned to 235 nm (for trimethoprim and sulfamethoxazole detection): (*A*) co-trimoxazole type suspension; (*B*) a model solutions of (*1*) trimethoprim, (*2*) sulfamethoxazole, (*3*) methylparaben, and (*4*); (*C*) solvent; (*D*) placebo solution.

sion (about 5 ml) in a 100-ml measuring flask (exactly weighed) was added 20 ml of the solvent (followed by 5-min ultrasonic treatment) and 25 ml of methanol (followed by 5-min ultrasonic treatment). To this mixture was added 0.1 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) via funnel (washed with 10 ml of the solvent). The mixture was treated for 20 min in a shaker (700 cpm) and allowed to stand until the foam vanished. Then the flask was filled to the mark and the solution was thoroughly stirred and allowed to stand for about 15 min until sedimentation of the suspended matter. Finally, the solution was filtered through Millipore Millex-LCR 0.45 μm filter (the first 2-ml portion of the filtrate is rejected).

Analytical calculations. The content (X) of sulfamethoxazole (trimethoprim, methylparaben, or propylparaben) in grams per 5-ml aliquot of the test suspension is calculated by the formula

$$X = \frac{Sa_0(P/100)5d_4^{20}}{S_0Da} = \frac{Sa_0Pd_4^{20}}{20S_0Da},$$

where S is the average area under the peak (AUP) of the corresponding component in the chromatogram, S_0 is the average AUP of the same component in the mixed WS solution chromatogram, a_0 is the weight of the WS of the given component [g], P is the parent drug content in the WS of the given component [%], a is the suspension sample weight [g], d_4^{20} is the suspension density [g/ml], and D is the relative dilution of the WS and test solutions (D = 0.5 for sulfamethoxazole and trimethoprim, 2.5 for methylparaben, and 10 for propylparaben).

Preparation of Model Mixed Drug Solutions

Model WS solutions of methylparaben. Exactly weighed amounts of the WS of methylparaben were placed in 50-ml measuring flasks (about 25 mg for 100% nominal suspension, 30 mg for the 120% level, and 20 mg for the 80% level) and dissolved in ~25 ml of the solvent. Then the flasks were filled with the same solvent to the mark and the solutions were thoroughly stirred.

Model WS solutions of propylparaben. Exactly weighed amounts of the WS of methylparaben were placed in 100-ml measuring flasks (about 12 mg for 100% nominal

TABLE 1. The Results of Analytical Calculations for Chromatogram A (Fig. 1)

Peak No.	Compound	Retention time, min	Area under peak	Peak height	Coefficient of		Number of theoretical plates
					separation	asymmetry	
1	Trimethoprim	2.134	8595493	1406364	–	1.20	2785
2	Sulfamethoxazole	3.516	13078439	1839847	7.56	0.79	5185
3	Methylparaben	4.875	545034	84522	7.30	1.05	12659
4	Propylparaben	9.046	121895	16822	22.52	1.04	34311

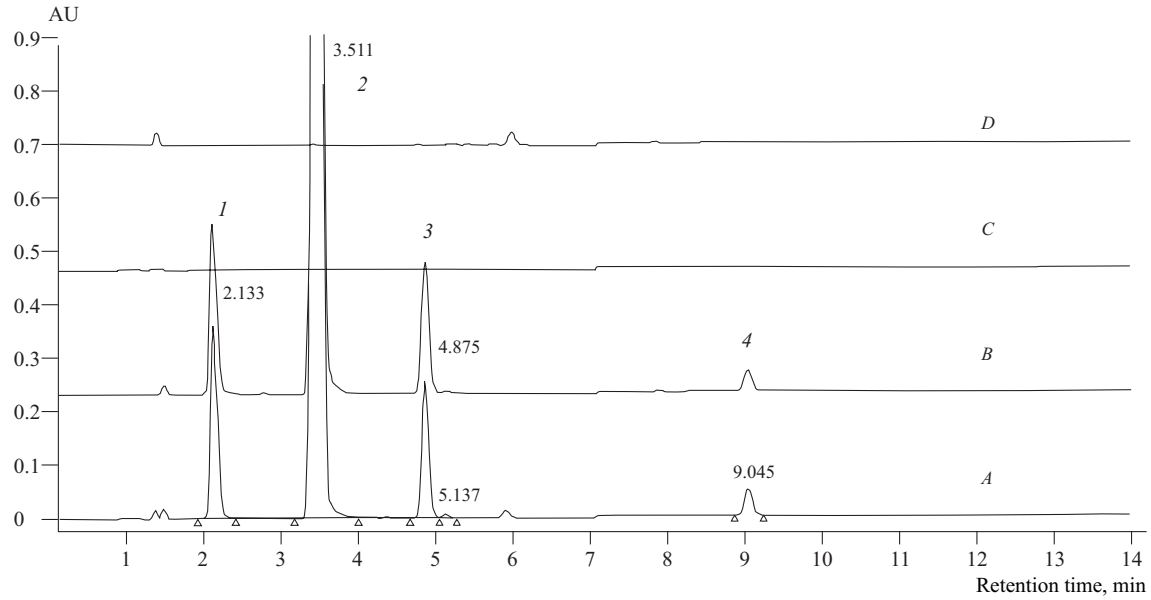

Fig. 2. Typical chromatograms of model solutions (*D*) measured with the detector tuned to 254 nm (for methyl- and propylparaben detection): (*A*) co-trimoxazole type suspension; (*B*) a model solutions of (*1*) trimethoprim, (*2*) sulfamethoxazole, (*3*) methylparaben, and (*4*); (*C*) solvent; (*D*) placebo solution.

suspension, 14.4 mg for the 120% level, and 8.8 mg for the 80% level) and dissolved in ~75 ml of the solvent. Then the flasks were filled with the same solvent to the mark and the solutions were thoroughly stirred.

Model suspensions of co-trimoxazole type. Exactly weighed amounts of sulfamethoxazole WS and trimethoprim WS (about 200 and 40 mg, respectively, for the 100% level; 240 and 48 mg for the 120% level, and 160 and 32 mg for the 80% level) were placed in 100-ml measuring flasks. To these mixtures were added 10-ml portions of the model solutions of methyl- and propylparaben of the corresponding levels, 5 ml of placebo (liquid), and 20 ml of the solvent. Then the mixtures were treated as described above for the test solution of co-trimoxazole.

Since the analyzed mixture components significantly differ with respect to the retention time, it was necessary to check for the possibility of their separation by means of HPLC in the gradient mode. The task was to separate the peaks of both analyzed components and the other detectable suspension components. The experiments were performed with several possible reverse-phase sorbents: Nova-Pak C18; Symmetry C18; XTerra RP18. Optimum results were obtained with XTerra RP18, a new sorbent available from Waters Company. The optimum HPLC conditions selected in these experiments are as follows:

(i) HPLC column: XTerra RP_{18} (5 μm), 150 × 4.6 mm, with the protective column XTerra RP_{18} (5 μm), 20 × 3.9 mm.

(ii) Gradient:

Time, min	Flow rate, ml/min	Phase A, %	Phase B, %	Curve
0	1.8	95	5	*
11	1.8	60	40	6
14	1.8	20	80	6
15	1.8	95	5	6
19	1.8	95	5	6

Phase A. A mixture of acetonitrile (150 ml), water (800 ml), and triethanolamine (1 ml) in a 1000-ml measuring flask was stirred and adjusted at pH 5.9 with diluted (1 : 100) glacial ascetic acid. Then the flask was filled with water to the mark and the solution was thoroughly stirred.

Phase B. Acetonitrile.

TABLE 2. The Results of Analytical Calculations for Chromatogram A (Fig. 2)

Peak No.	Compound	Retention time, min	Area under peak	Peak height	Coefficient of		Number of theoretical plates
					separation	asymmetry	
1	Trimethoprim	2.133	2134882	349263	–	1.19	2778
2	Sulfamethoxazole	3.511	26525026	3338557	7.27	0.82	4382
3	Methylparaben	4.875	1590271	245884	7.06	1.06	12620
4	Propylparaben	9.045	361440	49566	17.67	1.06	34049

TABLE 3. The Results of Analyses of Solutions Containing Trimethoprim, Sulfamethoxazole, Methylparaben, and Propylparaben Modeling Co-Trimoxazole Suspensions (see the text for explanations)

No.	Content, mg		Average area S*	Recovery $R = m_{in}100/m_f$, %	Content, mg		Average area S*	Recovery $R = m_{in}100/m_f$, %
	m_{in}	m_f			m_{in}	m_f		
	Trimethoprim				Sulfamethoxazole			
1	33.00	33.03	1072.40	100.09	164.08	163.78	1033.68	99.82
2	32.81	32.76	1063.98	99.85	158.92	160.04	1010.10	100.70
3	40.40	40.46	1313.21	100.15	208.10	208.44	1315.58	100.16
4	37.78	37.82	1227.92	100.11	207.34	207.94	1312.39	100.29
5	48.09	48.58	1570.02	101.02	244.18	246.98	1570.02	101.15
6	47.78	47.94	1557.55	100.33	241.97	244.95	1557.55	101.23
Average				**100.26**				**100.56**
Standard single deviation				0.4041				0.5654
Standard average deviation				0.1650				0.2308
Student *t*-criterion				2.57				2.57
Confidence interval				1.04				1.45
Average confidence interval				**0.42**				**0.59**
Relative error of single determination				1.04				1.45
Relative error of average				0.42				0.59
Relative standard deviation				0.40				0.56
	Methylparaben				Propylparaben			
1	20.69	20.61	130.76	99.61	10.33	10.53	29.46	101.94
2	21.78	21.66	138.27	99.45	9.67	9.86	27.59	101.96
3	25.16	25.04	159.86	99.52	13.09	13.05	36.53	99.69
4	25.89	25.86	165.08	99.88	11.90	11.72	32.82	98.49
5	30.60	30.77	196.42	100.56	14.87	14.94	41.26	100.47
6	30.63	30.62	195.47	99.97	14.69	14.90	41.14	101.43
Average				**99.83**				**100.66**
Standard single deviation				0.4085				1.3865
Standard average deviation				0.1668				0.5660
Student *t*-criterion				2.57				2.57
Confidence interval				1.05				3.56
Average confidence interval				**0.43**				**1.45**
Relative error of single determination				1.05				3.54
Relative error of average				0.43				1.45
Relative standard deviation				0.41				1.38

* *S* values determined by dividing the area under the peak by 10^4 and taking the first four digits.

(iii) Sample volume: 10 μl; temperature, 40°C; detection wavelength: trimethoprim and sulfamethoxazole, 235 nm; methylparaben and propylparaben, 254 nm.

(iv) Under these conditions, the retention times are as follows: trimethoprim, ~2.1 min; sulfamethoxazole, ~3.5 min; methylparaben, ~4.9 min; and propylparaben, ~9.0 min.

TABLE 4. Regression Equations $S = a_1 + b_1 m_{in}$ Determined by Least Squares

Compound	Equation	Correlation coefficient
Trimethoprim	$S = -17.66 + 32.98 m_{in}$	0.9999
Sulfamethoxazole	$S = -51.69 + 6.622 m_{in}$	0.9994
Methylparaben	$S = -4.722 + 6.554 m_{in}$	0.9999
Propylparaben	$S = 1.711 + 2.663 m_{in}$	0.9985

RESULTS AND DISCUSSION

Selectivity. Figures 1 and 2 show typical chromatograms of a co-trimoxazole type suspension (*A*), a model solutions of trimethoprim, sulfamethoxazole, methylparaben, and propylparaben WS mixture (*B*), solvent (*C*), and placebo solution (*D*) measured with the detector tuned to 235 nm (trimethoprim and sulfamethoxazole detection) and 254 nm (methylparaben and propylparaben detection). The results of calculations for the chromatograms in Figs. 1 and 2, are presented in Tables 1 and 2, respectively. As can be seen, the peaks of placebo, trimethoprim, sulfamethoxazole, methylparaben, and propylparaben do not overlap. The tail of the peak of methylparaben reveals an additional impurity, but even this peak is sufficiently well resolved (with a separation coefficient of 1.519 in Fig. 2).

TABLE 5. Regression Equations $m_f = a + bm_{in}$ Determined by Least Squares

Compound	a	b	S_a	S_b	t_a	t_b	r	S
Trimethoprim	– 0.794	1.024	0.336	8.30E-03	2.37	2.76	0.9999	1.26E-01
Sulfamethoxazole	– 4.516	1.028	2.43	1.17E-02	1.86	2.41	0.9997	9.61E-01
Methylparaben	– 0.533	1.019	0.220	8.45E-03	2.42	2.30	0.9999	7.97E-02
Propylparaben	0.194	0.990	0.453	3.60E-02	0.43	0.27	0.9974	1.75E-01

Notes: r is the correlation coefficient; S is the standard deviation of m_f values (see the text for explanations).

Reproducibility. This characteristic was evaluated from the reproducibility of the areas under the peaks of the analyzed substances. For three sequential sample injections, the relative standard deviation (RSD) of AUP did not exceed 0.4%.

The intralaboratory convergence of the results of quantitative determinations was evaluated by performing the analysis of six samples of the same suspension. The RSD was 1.1% for trimethoprim, 0.9% for sulfamethoxazole, 1.3% for methylparaben, and 1.9% for propylparaben, so that the overall RSD does not exceed 2%.

Linearity and correctness of analysis. Table 3 presents the results of analysis of several mixtures of trimethoprim, sulfamethoxazole, methylparaben, and propylparaben with placebo, modeling suspensions of the co-trimoxazole type with the relative content of components varied from ~80 to 120% with respect to nominal values. Note that the model mixtures were prepared using the substances employed as working standards. This excluded systematic errors related to the uncertainty of determination of the content of parent substances in WS solutions. Because of the relatively low content of preservatives as compared to that of the parent drugs, methyl- and propylparabens were added as solutions of exactly known concentrations to the mixtures of exactly weighed amounts of trimethoprim, sulfamethoxazole, and placebo.

The experiments showed that the complete extraction of trimethoprim and sulfamethoxazole requires using methanol, while the complete extraction of methyl- and propylparabens is provided by a mixture of acetonitrile with acetic acid. Unfortunately, the resulting suspension is poorly filtered because of the presence of sodium carboxymethylcellulose. Readily filtered solutions of suspensions were obtained using a special preparation method developed previously for the analysis of suspensions [5]. This technique increases the working life of filters and chromatographic columns.

Linearity of the analytical method with respect to the concentrations of suspension components was checked by measuring the dependences of the area under the peak S on the weight m_{in} of the corresponding component introduced into the mixture (i.e., the WS weight used for the model mixture preparation). Regression equations of the type $S = a_1 + b_1 m_{in}$ were determined by least squares. The results of this test are presented in Table 4. The high values of correlation coefficients (≥ 0.9985) indicate linearity of the analysis with respect to all components. This was confirmed by the arrangement of points in the experimental plots of S versus m_{in}.

Correctness of the method was initially evaluated by the recovery R of the suspension components, defined as $R = m_f \times 100/m_{in}$, where m_{in} and m_f are the component weights introduced and found in the mixture. As can be seen from Table 3, the recovery falls within $100.0 \pm 2\%$, amounting to $100.26 \pm 0.42\%$ for trimethoprim, $100.56 \pm 0.59\%$ for sulfamethoxazole, $99.83 \pm 0.43\%$ for methylparaben, and $100.66 \pm 1.45\%$ for propylparaben.

Since the error in recovery can be related to both random and systematic errors, we have also determined the systematic error (the main criterion of correctness) in terms of Student t-criterion [6]. For this purpose, $m_f = a + bm_{in}$ relations were determined by least squares, the parameters $t_a = |a|/S_a$ and $t_b = |1 - b|/S_b$ were calculated, and the results were compared to the cortical (tabulated) values of the Student criterion $t(P, f = N - 2)$, where S_a and S_b are standard deviations of the coefficients a and b, respectively; $P = 95\%$ is the confidence probability; and N is the number of model mixtures used for the analysis. The results are presented in Table 5. As can be seen, the t_a and t_b values are below the critical level of $t(95\%, f = 4) = 2.78$. From this we may conclude (to within 95%) that the results of analyses contain no significant constant or linearly varying systematic errors.

Thus, a reliable HPLC technique has been developed for simultaneously determining trimethoprim, sulfamethoxazole, methylparaben, and propylparaben in suspensions of the co-trimoxazole type. A thorough validation procedure confirms the selectivity, linearity, reproducibility, and correctness of the proposed method.

REFERENCES

1. *British Pharmacopoeia* (1998), Vol. 2, p. 1610.
2. *US Pharmacopoeia*, NF19 (2000), p. 1463.
3. ND 42–657–96. *Biseptol Suspension*.
4. ND 42–2046–00. *Septrin Suspension*.
5. N. A. Épshtein, *Khim.-Farm. Zh.*, **35**(12), 38 – 41 (2001)
5. C. Doerffel, *Statistics in Analytical Chemistry* [Russian translation], Mir, Moscow (1994), p. 177.