

Simultaneous TLC Determination of Co-trimoxazole and Impurities of Sulfanilamide and Sulfanilic Acid in Pharmaceuticals

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Abstract

A simple and reliable thin-layer chromatographic method for the determination of sulfamethoxazole, trimethoprim, and impurities of sulfanilamide and sulfanilic acid is developed and validated. The effect of the polarity and the composition of eluents on the separation of this mixture is investigated. A chloroform-*n*-heptane-ethanol (3:3:3, v/v/v) solvent system is used for a quantitative evaluation of chromatograms. The chromatographic zones, corresponding to the spots of sulfamethoxazole, trimethoprim, sulfanilamide, and sulfanilic acid on the silica gel plates, are scanned in the reflectance/absorbance mode at 260 nm. The method is found to be reproducible and convenient for the quantitative analysis and purity control of co-trimoxazole in its raw form and in its dosage forms.

Introduction

Sulfonamide-trimethoprim combinations of drugs are extensively used as antibacterial and therapeutic agents for urinary tract infections (1). Co-trimoxazole represents a mixture of five parts of sulfamethoxazole and one part of trimethoprim.

The optimization of solvent systems using thin-layer chromatographic (TLC) separation and the influence of ion-pair reagents (2) or different modifiers (3,4) on the retention behavior of numerous sulfonamides in normal or reversed-phase TLC, were investigated.

TLC-densitometric methods were used for the determination of other sulfonamides, such as a sulfametrol-trimethoprim combination (5) and sulfanilamide or sulfafurazole in different dosage forms (6). TLC separation of sulfamethoxazole and trimethoprim with subsequent densitometric measurements was described using the single standard method, but data were missing for densitometric linearity and precision of the method (7-10). Using different scanning modes (11) or a different calibration function (12), investigators performed a TLC-high-performance TLC (HPTLC) densitometric determination for a

quantitative assay of the active drugs sulfamethoxazole and trimethoprim in pharmaceutical dosage forms.

There are no reports of the simultaneous TLC assay of co-trimoxazole and impurities of sulfanilamide or sulfanilic acid in the literature. The major impurities in sulfamethoxazole drug substances and dosage forms are sulfanilamide and sulfanilic acid. The current United States Pharmacopeia 23rd revision (USP XXIII) specifications allow 0.2% (w/w) sulfanilamide and sulfanilic acid in sulfamethoxazole drug substances and 0.5% or 0.3% sulfanilamide and sulfanilic acid in dosage forms, respectively (13).

Therefore, we describe a simple, accurate, and quick TLC technique for the simultaneous determination of co-trimoxazole and its impurities in sulfamethoxazole drug substances and in its dosage forms.

Experimental

Reagents

Sulfamethoxazole and trimethoprim were obtained from Roche (Herts, U.K.). Sulfanilamide and sulfanilic acid were obtained from Fluka (Buchs, Switzerland). Trimosul tablets, each containing 400 mg sulfamethoxazole and 80 mg trimethoprim, were obtained from Panfarma (Belgrade). All other chemicals and solvents were of analytical grade.

Instruments

Chromatoplates (20 × 10, precoated with 0.2-mm silica gel 60F₂₅₄) were purchased from Merck (Darmstadt, Germany). A Nanomat III was used as the application device (Camag; Muttenz, Switzerland). A TLC Scanner II with a computer system and CATS software were provided by Camag.

Standard and sample solutions

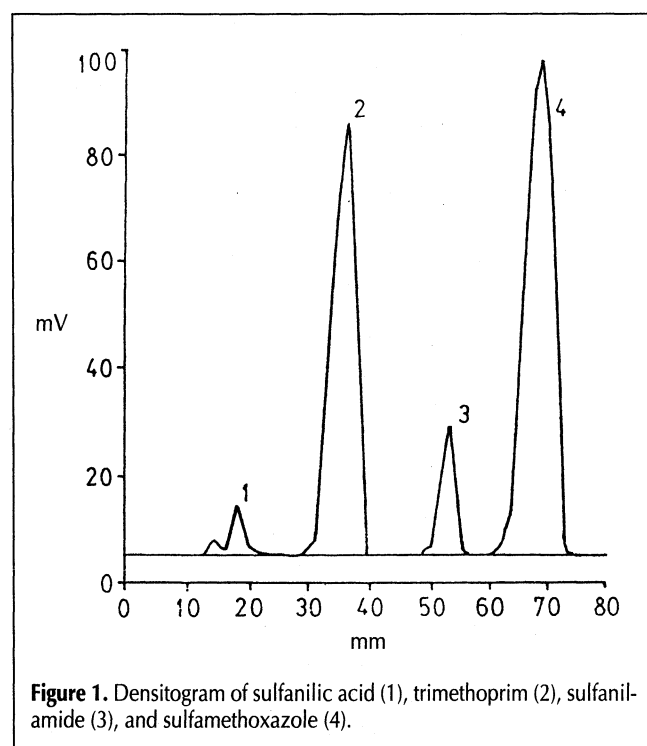
A stock solution containing 40 mg/mL of sulfamethoxazole and 8 mg/mL of trimethoprim was prepared in a mixture of chloroform-methanol (1:1, v/v). For an assay of sulfamethoxazole and trimethoprim, calibration curves were prepared by

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Table I. Linearity Constants for the Equations

Substance	36–42% Alcohol fraction + 4% strong ammonia solution in 1, 2-dichloroethane						30–58% Fraction of alcohol + 16% glacial acetic acid in diisopropyl ether–methyl ethyl ketone		
	A*			B*			C*		
	Constant	-m	r	Constant	-m	r	Constant	-m	r
Sulfamethoxazole	6.7	4.6	0.997	0.2	3.5	0.999	0.2	1.5	0.993
Sulfanilamide	3.2	2.2	0.998	0.06	1.7	0.999	1.6	3.0	0.994
Sulfanilic acid	10.5	5.9	0.965	2.1	4.6	0.990	11.5	15.8	0.840

* A, $R_M = \text{constant} - m \log C (\%)$; B, $R_M = \text{constant} - m \log P$ (alcoholic fraction); C, $R_M = \text{constant} - m \log P$ (mobile phase); m is equal to the absolute values of the slope.



diluting the stock solution so that solutions were furnished with final concentrations of 2, 1.5, 1, 0.8, and 0.5 mg/mL sulfamethoxazole and 0.4, 0.3, 0.2, 0.16, and 0.1 mg/mL trimethoprim. These levels represented 62.5–250% of the labeled amounts of sulfamethoxazole and trimethoprim in the drug substances and tablets.

A stock solution containing 1 mg/mL of sulfanilamide and 0.1 mg/mL of sulfanilic acid was prepared in a mixture of chloroform–methanol (1:1, v/v), and 0.15 mL of a strong (25%) ammonia solution was added. For an assay of sulfanilamide and sulfanilic acid, calibration curves were prepared by diluting the stock solution so that solutions were furnished with final concentrations of 0.5, 0.3, 0.1, 0.05, and 0.025 $\mu\text{g}/\mu\text{L}$ sulfanilamide and sulfanilic acid, and 1 μL of the diluted solutions was applied to the TLC plates. The 25–500 ng per spot value represented 31.25–250% of both USP limits of 0.2% (w/w) of an impurity in a sulfamethoxazole drug substance and 0.3% or 0.5% (w/w) of a sulfanilic acid or sulfanilamide impurity, respectively, in the co-trimoxazole tablets.

For an actual assay of sulfanilic acid and sulfanilamide in a sulfamethoxazole drug substance and in co-trimoxazole dosage forms, a solution containing approximately 40 mg/mL of a sulfamethoxazole drug substance in a mixture of chloroform–methanol (1:1, v/v) was prepared, and 1 μL was applied to the TLC plate for determination of the sulfanilic acid and sulfanilamide content. The 40-mg/mL solutions were further diluted to approximately 80 $\mu\text{g}/\text{mL}$, and 1 μL was applied to the plate for determination of the sulfamethoxazole–trimethoprim content.

Chromatography

TLC was performed on 20- × 10-mm plates coated with a 250- μm layer of silica gel 60 (Merck). Samples and standards were applied to the plates by means of a Nanomat III (Camag). The plates were developed with chloroform–*n*-heptane–ethanol (9:9:9, v/v/v) to a distance of 90 mm. Each plate could accommodate 14 sample spots and 5 standards. Scanning densitometry was performed with a Scanner II densitometer (Camag) coupled to an IBM PC with CATS software (V3.15) using absorbance/reflectance mode. The scanning wavelength was 260 nm.

Results and Discussion

Chromatographic separation

Separation of sulfamethoxazole, sulfanilamide, sulfanilic acid, and trimethoprim was accomplished by using neutral, acidic, or basic mobile phases; it was necessary that the mobile phases contained alcohol(s) as modifiers. The dependence of the concentration of alcohols and the polarity of the alcoholic fraction in the mobile phase and the polarity of the mobile phases on retention of sulfamethoxazole, sulfanilamide, and sulfanilic acid was investigated.

The R_f values obtained were converted into R_M values using the equation $R_M = \log(1 - R_f)/R_f$. The relationship between R_M and the mobile phase composition was expressed by the equation $R_M = R_M^\circ - m \log C (\%)$, where R_M° is equal to constant extrapolated values obtained at $C = 1\%$ (alcohol) and m is the slope of the linear plot. The polarity of the alcohol fraction in the mobile phase was calculated using the expression $P = P_n \times f_n$, where P_n is the solvent strength parameter and f_n is part of the component.

A correlation was observed between R_M values and the con-

centration (36–42%) or polarity (0.17–0.25) of an alcoholic fraction in mobile phases containing binary mixtures (*n*-butanol–methanol, *n*-pentanol–methanol), ternary mixtures (*n*-pentanol–*n*-butanol–*n*-methanol), or 4% of a strong ammonium solution in 1,2-dichloroethane as a diluent (Table I). The slopes of plots $\log C$ (% alcohol) or $\log P$ (alcohol fraction) versus R_M were found to correlate strongly with the pK_a of sul-

famethoxazole (5.6), sulfanilamide (10.5), and sulfanilic acid (3.5); the correlation coefficients were 0.997 and 0.996, respectively.

The R_M values of sulfa compounds with lower pK_a values were strongly influenced by the content and polarity of the alcoholic fraction of the mobile phase. Sulfanilic acid exhibited no mobility in the eluent containing less than 30% of the alcoholic fraction.

The mobile phase containing 15–18% formic acid or glacial acetic acid and a 30–60% fraction of alcohol (ethanol, *n*-butanol, *t*-butanol, or *n*-pentanol) in diisopropyl ether or methyl ethyl ketone as a diluent accomplished the separation of all components of the mixture. The R_M values of sulfamethoxazole, sulfanilamide, and sulfanilic acid were more affected by the polarity of the mobile phase than by the polarity of the alcoholic fraction or its concentrations. R_M values versus P of the mobile phase correlated; the obtained correlation coefficients for sulfamethoxazole and sulfanilamide ($r = 0.993$ and 0.994 , respectively) were somewhat higher than for sulfanilic acid ($r = 0.830$) (Table I).

Quantitative assay

During the development of the quantitative assay, two solvent mixtures were evaluated as mobile phases: chloroform–*n*-heptane–ethanol (3:3:3, v/v/v) and *n*-butanol–methyl ethyl ketone–water–acetic acid (5:1:1:1.5, v/v/v/v). Both selected mixtures gave an excellent separation with minimum tailing. Migration distances of sulfamethoxazole, sulfanilamide, trimethoprim, and sulfanilic acid were $67.05 \text{ mm} \pm 0.08$, $52.5 \text{ mm} \pm 0.16$, $33.6 \text{ mm} \pm 0.13$, and $18.12 \text{ mm} \pm 0.08$, respectively, in the chloroform–*n*-heptane–ethanol mixture and $83 \text{ mm} \pm 0.1$, $79.1 \text{ mm} \pm 0.15$, $52.7 \text{ mm} \pm 0.16$, and $42.7 \text{ mm} \pm 0.17$, respectively, in the *n*-butanol–methyl ethyl ketone–water–acetic acid mixture.

A typical TLC chromatogram of the separation of sulfamethoxazole, trimethoprim, and impurities of sulfanilamide and sulfanilic acid is shown in Figure 1. For accurate densitometric reading, the migration distances of separated substances are required to be between 30 and 70 mm. Even though sulfanilic acid has a lower migration distance in the chloroform–*n*-heptane–ethanol mixture, the results of its comparable quantitative assay using both mixtures greatly agreed. Therefore, the chloroform–*n*-heptane–ethanol mixture has been further used for the simultaneous determination of active drugs and impurities.

The content of the active drugs sulfamethoxazole and trimethoprim in dosage

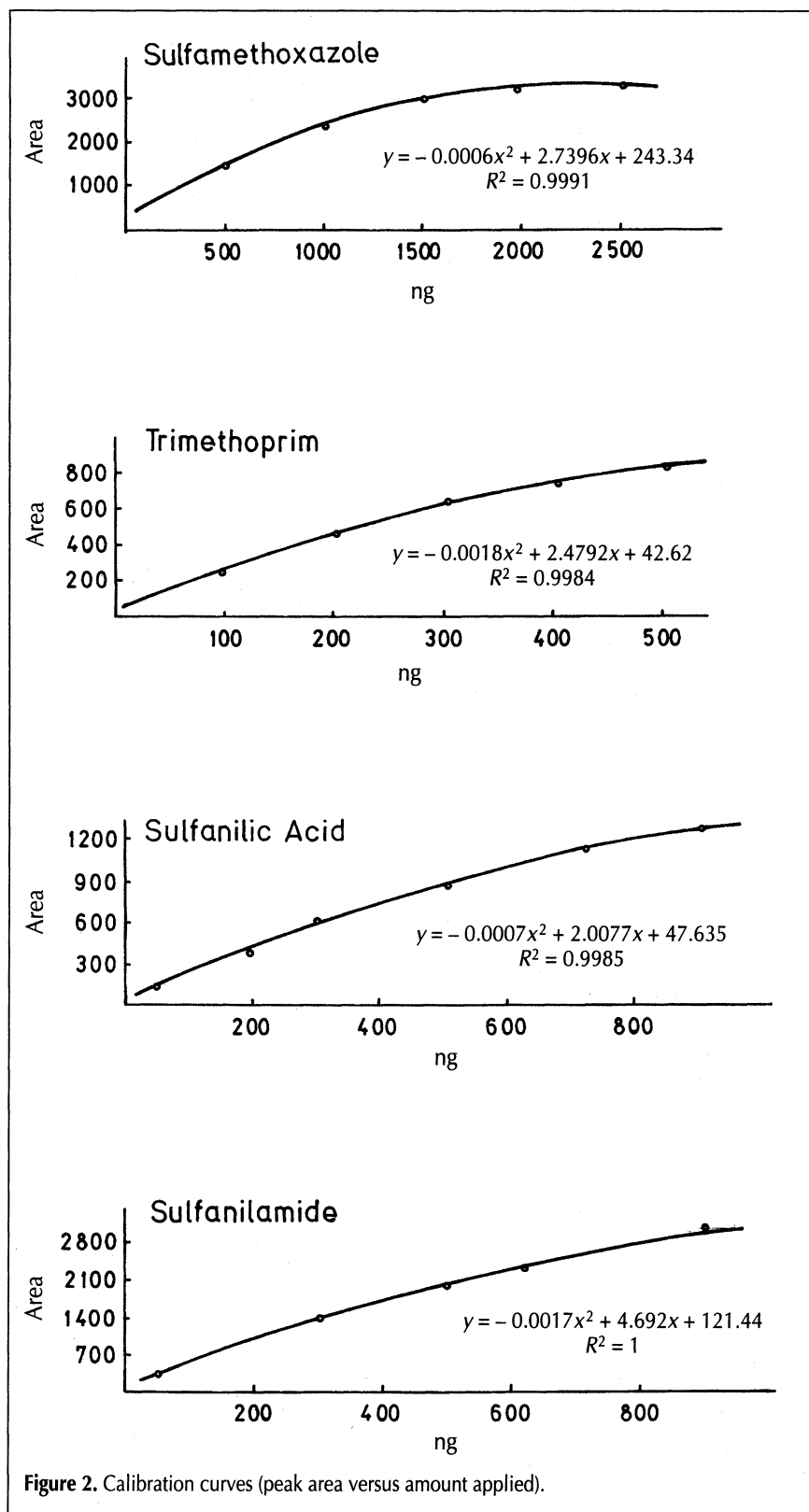


Figure 2. Calibration curves (peak area versus amount applied).

Table II. Assay of Co-trimisol, Sulfanilamide, and Sulfanilic Acid

	Sulfamethoxazole		Trimethoprim		Sulfanilamide		Sulfanilic acid	
	Taken (mg)	Found (mg \pm SD*)	Taken (mg)	Found (mg \pm SD)	%	RSD*	%	RSD
Raw material	400	412.5 \pm 6.1	80	80.6 \pm 1.4	0.06	1.8	–	–
Trimisol tablet	400	411.8 \pm 7.1	80	80.2 \pm 1.7	0.05	2.1	–	–

* Abbreviations: SD, standard deviation; RSD, relative standard deviation.

forms was previously analyzed by using the single standard method (7–10), linear regression (11), and log/log linear regression (12). Because the amount of possible degradation products, sulfanilamide, and sulfanilic acid in drug substances and dosage forms is usually unpredictable, the calibration function has to be constructed for a wider range of concentrations; sulfanilamide and sulfanilic acid were tested over the range 50–900 ng per spot. The best fit for the calibration lines was found when the calibration data were analyzed using a second-degree polynomial regression. The nonlinear polynomial function described by the equation $R = A_0 + A_1C + A_2C^2$ can be used in quantitative TLC without disturbing the error distribution (14).

The calibration functions of sulfamethoxazole and trimethoprim were constructed for the concentration ratios 500–2500 and 100–500 ng per spot, respectively. The calibration curves (peak area versus the amount of substance applied) are shown in Figure 2. The standard deviations of calibration curves for sulfamethoxazole, trimethoprim, sulfanilamide, and sulfanilic acid were 1.7%, 2.1%, 2.3%, and 1.2%, respectively.

Because they used HPTLC, Tammilehto (12) and Datta and Das (11) obtained significantly lower concentrations of sulfamethoxazole and trimethoprim; log/log linear regression and linear regression were used for applied amounts of 20–160 and 250–1000 ng per spot, respectively, for both substances.

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined by fitting the interday, back-calculated standard deviations of each calibration standard. The y -intercept was then equal to SD_0 (the estimated standard deviation at a concentration of zero). The LOD was defined as $3SD_0$, and the LOQ was defined as $10SD_0$. The LODs for sulfanilamide and sulfanilic acid were found to be 4.5 and 4.1 ng/ μ L, respectively (equivalent to impurity levels of 0.01%), and the LOQs were 13.5 and 12.4 ng/ μ L, respectively (equivalent to impurity levels of 0.03%). The LOQs for sulfamethoxazole and trimethoprim were found to be 19 and 16 ng/ μ L, respectively.

The repeatability of the analytical system was determined by using two samples of co-trimisol found to contain 0.22 and 0.53% of sulfanilamide and 0.19 and 0.28% of sulfanilic acid; five replicate applications of each sample gave relative standard deviations (RSDs) of 0.62 and 1.5% for sulfanilamide and 2.2 and 1.8% for sulfanilic acid, respectively. The repeatability of the sulfamethoxazole–trimethoprim assay, using concentrations of 800 and 120 ng/ μ L, is shown by RSDs of 1.82 and 1.79%, respectively.

The accuracy of the densitometric method was proved by a determination of sulfamethoxazole and trimethoprim from the laboratory-made tablet excipient mixture spiked with 400 mg

sulfamethoxazole and 80 mg trimethoprim; recoveries were 102.8 and 100.24%, respectively.

A solution containing Co-trimisol with a previously determined amount of sulfanilamide and sulfanilic acid was spiked with aliquots of the impurity solution at two concentrations of sulfanilamide, 0.08 and 0.2 mg/mL (equivalent to 0.2 and 0.5%), and at two concentrations of sulfanilic acid, 0.08 and 0.12 mg/mL (equivalent to 0.2 and 0.3%). Recoveries obtained were 105.5 and 103.5% for sulfanilamide, respectively, and 97.12 and 98.08% for sulfanilic acid, respectively.

The method was used to screen the raw material and dosage forms for impurities (Table II). The results obtained for sulfanilamide (0.06% for the raw material and 0.056% for dosage forms) meet the requirements of the USP XXIII, which are not to exceed 0.2%. There was no trace of impurities of sulfanilic acid detected in the raw material and dosage forms. With regard to the label declaration, the recovery values for sulfamethoxazole and trimethoprim in the raw material and dosage forms were very high. The values of the RSDs obtained for the raw material (1.5–1.7%) and dosage forms (1.8–2.2%) confirm the accuracy and reproducibility of the method.

Conclusion

The described quantitative densitometric method is simple, rapid, and sufficiently accurate to determine trace impurities in pharmaceutical preparations.

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