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Note

High-performance thin-layer chromatographic determination of trimethoprim and sulphamethoxazole in pharmaceutical dosage forms

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Trimethoprim (5-[(3,4,5-trimethoxyphenyl)methyl]pyrimidine-2,4-diamine) in combination with sulphamethoxazole [4-amino-N-(5-methyl-3-isoxazolyl)benzenesulphonamide] is an effective antimicrobial agent, used, for example, in the treatment of urinary tract infections. Various analytical techniques are available for the simultaneous determination of the compounds in pharmaceutical preparations and biological fluids. The most widely used method is high-performance liquid chromatography with reversed-phase[1–4] or normal-phase[5] techniques. The disadvantage of gas chromatography is the need for derivatisation of sulphamethoxazole before the analysis[6,7]. Nuclear magnetic resonance spectrometry provides an attractive tool for the determination of mixtures of trimethoprim and sulphamethoxazole because of the simple sample preparation[8]. The methods of stepwise potentiometric titration[9] and spectrometric measurement in the ultra-violet[10] or visible region after formation of coloured products[11] lack selectivity and sensitivity, but are, nevertheless, suitable for the quantitation of trimethoprim and sulphamethoxazole combinations in pharmaceutical dosage forms.

Thin-layer chromatographic (TLC) separation of trimethoprim and sulphamethoxazole with subsequent densitometric measurement has been described for conventional TLC plates[12,13]. High-performance thin-layer chromatography (HPTLC) offers some advantages over conventional TLC, *e.g.*, better separation properties, increase in the number of samples on one plate, and reduction of analysis time and solvent consumption. The aim of the present study was to develop a specific, stability-indicating HPTLC method for the routine analysis of trimethoprim and sulphamethoxazole in pharmaceutical formulations.

EXPERIMENTAL

Materials

Trimethoprim, sulphamethoxazole and sulphanilamide were kindly supplied by Orion Oy (Espoo, Finland). The identity and purity of the compounds were verified by TLC, melting-point (Gallenkamp MF 370) and UV (Unicam SP 500 spectrometer) and IR (Unicam SP 1000 infrared spectrometer) spectrometry. All the solvents used were of analytical grade (E. Merck, Darmstadt, F.R.G.).

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HPTLC conditions

HPTLC separations were carried out on 10 × 10 cm precoated HPTLC silica gel $60F_{254}$ plates (E. Merck). Before the analysis, the plates were developed with chloroform–ethanol (9:1) and thereafter with methanol to assure a sufficiently low background for photometric measurements. Plates were dried at room temperature and used immediately. Solutions (200 nl) were applied with a variable-volume nano-applicator (Camag, Muttenz, Switzerland) along two opposite sides of the plates, at a distance of 5 mm apart. The plates were eluted in a HPTLC linear developing chamber (Camag) with the solvent system chloroform–ethanol (9:1), the migration distance being 40 mm.

Photometric measurement

Photometric measurements were performed in the reflectance mode with a Zeiss PMQ II chromatogram spectrometer connected to a Servogor 310 recorder (BBC, Goerz, Austria). Spots were scanned in the direction of chromatography with a slit width of 0.7 mm and a slit length of 3.5 mm. The wavelength used was 284 nm. The scanning speed was 50 mm/min and the recorder speed 60 mm/min.

Calibration graphs

A stock solution containing 1 mg/ml of trimethoprim and sulphamethoxazole and an internal-standard solution containing 3 mg/ml of sulphanilamide were prepared in acetone–chloroform (7:3). To obtain the calibration graphs, 0.5–4.0 ml of the stock solution and 0.5 ml of the sulphanilamide solution were diluted to exactly 5 ml in acetone–chloroform. Aliquots (200 nl) of each solution were applied in triplicate to the plate and chromatographed as described above. The calibration graphs were constructed by plotting peak-height ratios of trimethoprim or sulphamethoxazole to the internal standard *versus* concentration of the compound. The calibration data were analysed using the log–log regression equation

$$\log y = A \log x + B$$

where y = peak height ratio and x = concentration.

Sample preparation

Tablets. One tablet was finely powdered, and an accurately weighed amount of the tablet mass (equivalent to *ca.* 5 mg of trimethoprim and 25 mg of sulphamethoxazole) was suspended in 15 ml of chloroform and sonicated for 5 min. Thereafter, 5.0 ml of the internal standard solution was added and the mixture was diluted to 50.0 ml with acetone (= sample solution). A standard solution containing 5 mg of trimethoprim, 25 mg of sulphamethoxazole and 15 mg of sulphanilamide in 50.0 ml of acetone–chloroform (7:3) was prepared in a similar way. For the HPTLC analysis, aliquots (200 nl) of the sample and standard solutions were applied to the plate and chromatographed as described above.

The amounts of the individual compounds were evaluated by comparing the peak-height ratios (trimethoprim or sulphamethoxazole to the internal standard) of the sample spots to those of the standard spots.

Oral suspension. To an aliquot of 0.5 ml of the oral suspension (equivalent to

4 mg of trimethoprim and 20 mg of sulphamethoxazole) in a 50-ml calibrated flask was added 25 ml of distilled water. The mixture was warmed for 15 min in a water bath (80°C) with occasional shaking. After cooling, 5.0 ml of the internal-standard solution was added and the solution was adjusted to volume with acetone. The chromatography was performed as described above.

RESULTS AND DISCUSSION

Trimethoprim and sulphamethoxazole are well separated from each other with several solvent systems (Table I). When sulphanilamide was used as internal standard, a mixture of chloroform–ethanol proved to be the most suitable eluent (Figs. 1 and 2). With this solvent, the interference of *p*-hydroxybenzoic acid esters ("Parabens"), used as preservatives in oral suspensions, was also avoided.

A low background absorption of the plate-coating material is an essential requirement to obtain reproducible quantitative results by spectrodensitometry. To remove impurities from the layer and to improve the uniformity of the baseline, the plates were developed before the analysis with chloroform–ethanol, and thereafter with methanol. The repeatability of the photometry was estimated by scanning the same lane of spots seven times under identical conditions. The relative standard deviations of the peak heights were 0.34, 0.42 and 0.7% for sulphamethoxazole, sulphanilamide and trimethoprim, respectively. The wide variation in the peak heights of trimethoprim is probably attributable to the small concentration of this compound compared with that of the sulphonamides.

Spectrodensitometric measurements of both compounds were carried out at 284 nm, the maximum wavelength of the reflectance spectrum of trimethoprim. The maximum absorption of sulphamethoxazole is at 270 nm, but the large concentration and intense ultra-violet absorption of the compound allow it to be accurately determined also at 284 nm.

The quantitation of trimethoprim and sulphamethoxazole was performed by using an internal standard in order to improve the reproducibility of sample appli-

TABLE I

R_F VALUES OF SULPHAMETHOXAZOLE (SMO), SULPHANILAMIDE (SA) AND TRIMETHOPRIM (TMP) WITH DIFFERENT SOLVENT SYSTEMS

Solvent system	*R_F values*		
	SMO	*SA*	*TMP*
Chloroform–ethanol (9:1)	0.51	0.27	0.15
Chloroform–ethanol (8:2)	0.71	0.48	0.21
Chloroform–methanol (8:2)	0.72	0.57	0.36
Chloroform–acetone-ethanol (8:2:2)	0.72	0.69	0.17
Toluene–ethanol–water (10:10:1)	0.55	0.51	0.25

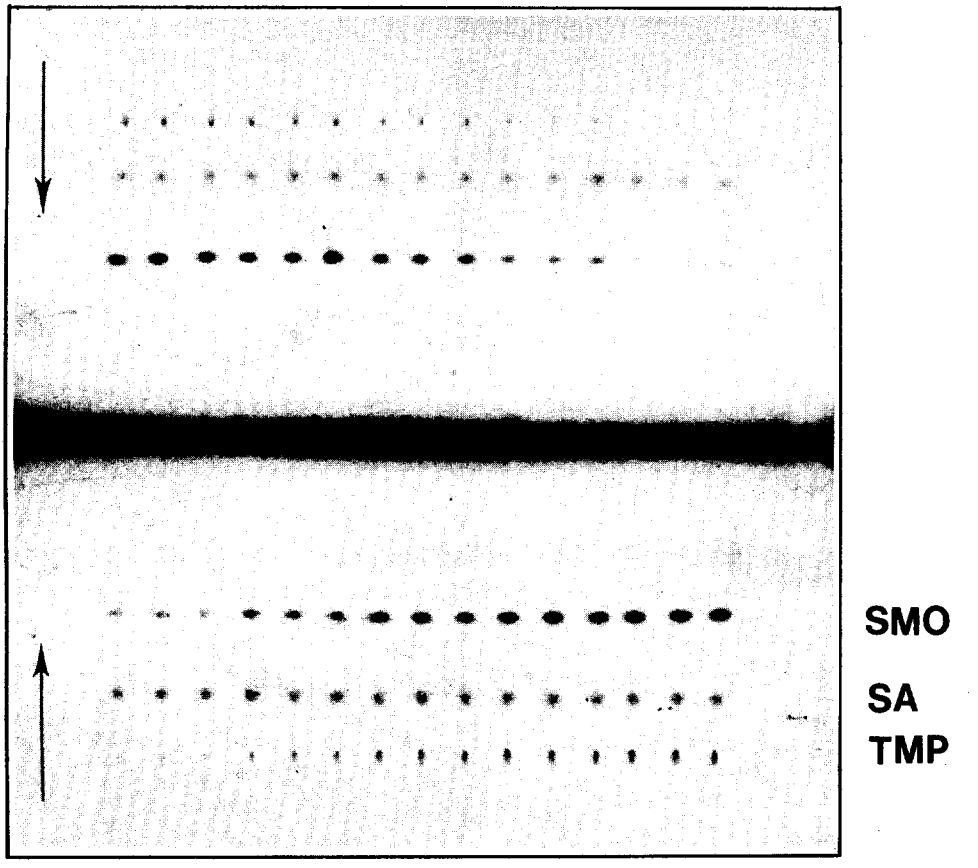

Fig. 1. High-performance thin-layer chromatographic run for the calibration graphs of trimethoprim (TMP) and sulphamethoxazole (SMO) with sulphanilamide (SA) as internal standard. Solvent system: chloroform–ethanol (9:1).

cation and chromatographic run. When 30 samples of 200 nl of a sulphamethoxazole solution containing the internal standard were chromatographed on the same plate, the relative standard deviation was 1.6% calculated from the peak-height ratios of the compound to the internal standard, and 2.7% calculated from the peak heights of sulphamethoxazole.

The calibration graphs were linear over the concentration range of 20–160 ng per spot for both substances. The best fit for the calibration lines was found when the calibration data were analysed using the log/log linear regression[14]. The mean fit varied between 0.9 and 3.2% and the standard deviation between 0.4 and 1.6%.

The determination of trimethoprim and sulphamethoxazole in pharmaceutical dosage forms was carried out without isolation of the compounds before the analysis. No interference of excipients present in tablets and oral suspensions was observed. The results collected in Table II show that the accuracy and precision of the method are satisfactory for both components and in good agreement with the analysis data obtained by colorimetry[11]. The use of only one standard concentration close to that

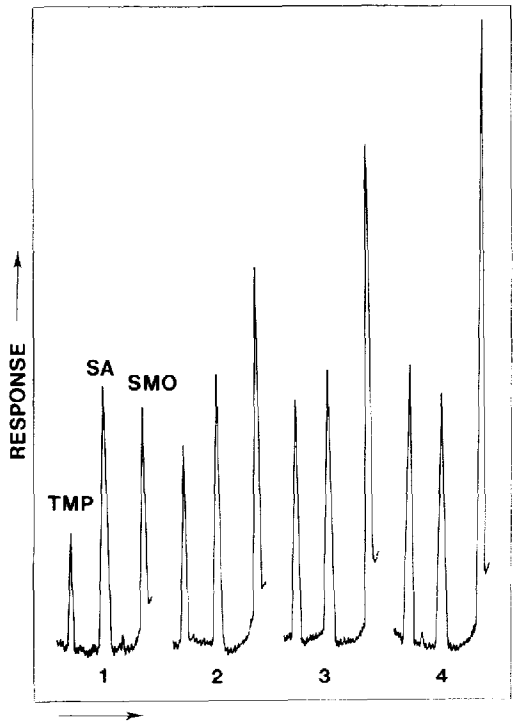

Fig. 2. Densitograms of different concentrations of the compounds. Symbols as in Fig. 1. Sulphanilamide concentration: 60 ng per spot. Trimethoprim and sulphamethoxazole concentrations: (1) 20 ng; (2) 40 ng; (3) 60 ng; (4) 80 ng per spot.

of the sample solution is sufficient for the accurate quantitation. The reproducibility of the assay procedure was not significantly improved when the calculations were carried out with calibration graphs constructed separately for each plate.

In conclusion, the present results show that HPTLC can provide a valuable alternative technique for the determination of mixtures of trimethoprim and sulpha-

TABLE II

DETERMINATION OF SULPHAMETHOXAZOLE (SMO) AND TRIMETHOPRIM (TMP) IN PHARMACEUTICAL DOSAGE FORMS

Dosage form	*Found, % of labelled amount*	
	HPTLC	*Colorimetry*[11]
Tablet		
SMO 400 mg per tablet	99.6 ± 1.64*	98.7 ± 1.64*
TMP 80 mg per tablet	101.3 ± 2.32	100.6 ± 1.25
Suspension		
SMO 40 mg per ml	100.1 ± 1.30	
TMP 8 mg per ml	100.3 ± 2.32	100.8 ± 2.06

* Average of eight determinations ± relative standard deviation.

methoxazole. The main advantages of the proposed method are simple sample preparation and large sample capacity, both reducing the analysis time considerably. Preliminary experiments on irradiated sulphamethoxazole solutions indicated further that the method is applicable to stability studies on sulphonamides.

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