Journal of Chromatography, 387 (1987) 528–531
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 141

Note

Stability-indicating high-performance liquid chromatographic analysis of trimethoprim in pharmaceuticals

J. J. BERGH* and J. C. BREYTENBACH
Department of Pharmaceutical Chemistry, Potchefstroom University for Christian Higher Education, Potchefstroom 2520 (South Africa)
(Received October 6th, 1986)

Although trimethoprim is a relatively stable drug it is susceptible to degradation after prolonged storage or after being subjected to severe conditions of heat or sunlight.

A number of papers regarding the analysis of trimethoprim have been published. The methods included spectrophotometry[1,2], polarography[3], NMR spectroscopy[4,5], thin-layer chromatography[6], gas–liquid chromatography[7,8] and high-performance liquid chromatography (HPLC)[9–11]. None of these, however, addressed the problem of stability, although one HPLC method[12] determined trimethoprim in the presence of two of its degradation products.

We now describe a rapid and uncomplicated procedure to determine trimethoprim in the presence of five of its degradation products. There is no interference from sulphamethoxazole (SMX), methyl *p*-hydroxybenzoate (MHB) and *n*-propyl *p*-hydroxybenzoate (PHB) which are frequently present in trimethoprim formulations.

EXPERIMENTAL

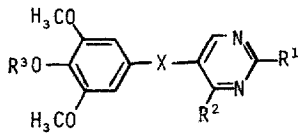
Degradation of trimethoprim

The degradation of trimethoprim (I) was achieved by heating the raw material under reflux in acidic media or by exposing suspensions of trimethoprim in various buffer solutions to direct sunlight. The products II–VI were identified mainly by NMR and mass spectrometry. (Pure samples of compounds II and V were supplied by the Wellcome Foundation, Dartford, U.K.)

Chromatographic system

A FR-30 dual-piston HPLC pump (Knauer, Bad Homburg, F.R.G.) and a Knauer variable-wavelength monitor Type 87 set at 271 nm were used. Sample injection was accomplished by means of a Rheodyne loop injector, Type 7010, using a 20-μl loop. A Supergrater-3A (Columbia Scientific Industries) was used to integrate the chromatographic peaks. The mobile phase was degassed acetonitrile–1-propanol–methanol–tetrahydrofuran–acetic acid–water (5:20:15:25:1:34), while separation was effected on a stainless-steel column (25 cm × 4.6 mm ID.) packed with Zorbax TMS 7 μm (DuPont) using a flow-rate of 2 ml/min.

0021-9673/87/$03.50



	R^1	R^2	R^3	X
I	NH_2	NH_2	CH_3	$-CH_2-$
II	OH	NH_2	CH_3	$-CH_2-$
III	NH_2	OH	H	$-CH_2-$
IV	NH_2	OH	CH_3	$-CH_2-$
V	NH_2	NH_2	CH_3	$-\overset{O}{\overset{\|}{C}}-$
VI	OH	OH	CH_3	$-CH_2-$

Chemicals

The methanol, 1-propanol, tetrahydrofuran and acetic acid were analytical grade (Merck Chemicals, South Africa) and the acetonitrile was HPLC grade (Saarchem, South Africa). Trimethoprim and SMX were supplied by Wellcome, South Africa while MHB and PHB were BDH GPR-grade reagents.

Preparation of chromatographic solutions

Standard solutions containing accurately weighed amounts of about 0.16, 0.18, 0.20, 0.22 and 0.24 mg of trimethoprim per ml ethanol (95%) were prepared. The samples were two brands of commercial tablets each containing 80 mg of trimethoprim and 400 mg of SMX per tablet, as well as a commercially available suspension containing the same amount of active ingredients as the above per 10 ml of suspension. Twenty tablets of each sample were crushed and powdered. An amount of the powder equivalent to 40 mg of trimethoprim was accurately weighed, dissolved, filtered and diluted in ethanol to 0.20 mg of trimethoprim per ml. A 5-ml volume of the suspension was diluted to 100 ml in ethanol and filtered. Simulated tablet powders containing 80 mg of trimethoprim and 400 mg of SMX as well as realistic amounts of excipients were prepared and treated in the some way as the powdered tablets.

RESULTS AND DISCUSSION

Linearity

The linear regression line for the peak area *vs.* concentration (mg/ml) of the standard solutions was determined for trimethoprim. The correlation coefficient was 0.998, the slope was 18.12 and the y-intercept was -80.11 integration units. The y-intercept deviated from the origin by 0.6% (y-intercept $\cdot$ 100/y at 100% level).

TABLE I

PERCENTAGE OF TRIMETHOPRIM FOUND

	Simulated tablets	*Tablet 1*	*Tablet 2*	*Suspension*
	101.5	94.6	102.4	99.6
	100.1	94.6	101.6	100.1
	97.8	96.5	100.9	100.9
	100.7	96.4	102.1	96.4
	99.8	97.1	98.3	99.7
	101.2	95.2	100.7	100.2
Mean ± S.D.	100.2 ± 1.33	95.7 ± 1.07	101.0 ± 1.47	99.5 ± 1.58

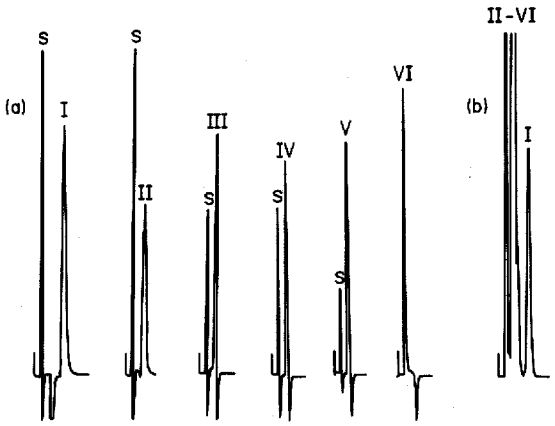


Fig. 1. (a) Chromatograms of trimethoprim (I) and its degradation products (II–VI). (b) Chromatogram of a mixture of compounds I–VI. The concentrations were 0.2 mg sample per ml ethanol, but the attenuation was varied. S = Solvent peak.

TABLE II

RETENTION TIMES OF TRIMETHOPRIM, ITS DEGRADATION PRODUCTS AND SMX, MHB AND PHB

Compound	*Retention time (min)*	*Compound*	*Retention time (min)*
I (Trimethoprim)	4.37	VI	1.09
II	2.49 and 2.71	SMX	1.21
III	2.25	MHB	1.40
IV	2.22	PHB	1.52
V	2.16		

Reproducibility

The quantity of trimethoprim in the tablets, simulated tablets and suspension was calculated using the slope and *y*-intercept of the regression line (Table I). The reproducibility of the method is demonstrated by the results obtained from the simulated tablets.

The method is stability-indicating as none of the degradation products interfered with the trimethoprim peak (Fig. 1). The retention times of these compounds are listed in Table II. SMX, PHB and MHB were eluted well in front of trimethoprim and presented no problem during the analysis (Table II).

ACKNOWLEDGEMENT

The authors thank The Wellcome Foundation, Dartford, U.K. for samples of some of the degradation products.

REFERENCES

1 A. K. Sanyal and D. Laha, *J. Assoc. Off. Anal. Chem.*, 66 (1983) 1447.
2 A. Ghanem, M. Meshali and A. Foda, *J. Pharm. Pharmacol.*, 31 (1979) 122.
3 L. G. Chatten, B. Stanley Pons and P. McLeod, *Analyst (London)*, 107 (1982) 1026.
4 M. R. Rodriguez, M. T. Pizzorno and S. M. Albonica, *J. Pharm. Sci.*, 66 (1977) 121.
5 J. W. Turczan, *Anal. Chim. Acta*, 92 (1977) 123.
6 S. A. Tammilehto, *J. Chromatogr.*, 323 (1985) 456.
7 R. Röder and W. Stuthe, *Dtsch. Apoth-Ztg.*, 115 (1975) 1461.
8 A. P. Lötter and A. P. Goossens, *S. Afr. Pharm. J.*, 44 (1977) 282.
9 P. Helboe and M. Thomsen, *Arch. Pharm. Chem. Sci. Ed.*, 5 (1977) 453.
10 L. Nordholm and L. Dalgaard, *J. Chromatogr.*, 233 (1982) 427.
11 O. Spreux-Varoquaux, J. P. Chapalain, P. Cordonnier, C. Advenier, M. Pays and L. Lamine, *J. Chromatogr.*, 274 (1983) 187.
12 P. O. Singletary, Jr. and F. D. Sancilio, *J. Pharm. Sci.*, 69 (1980) 144.