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Note

High-performance liquid chromatographic determination of phenazopyridine hydrochloride, tetracycline hydrochloride and sulphamethizole in combination

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Phenazopyridine hydrochloride, a very old drug, has been determined in dosage forms by column chromatography[1] and spectrophotometry[2,3]. Although spectrophotometry can be used when the drug is alone in a dopsage form, its combinations with other drugs present problems for assay in solution.

The separation of tetracyclines was recently described[4]. A number of high-performance liquid chromatographic (HPLC) methods for the determination of tetracyclines in biological material[5–10] can be used for the assay of tetracyclines in solution.

For single sulphonamides either UV spectrophotometry or colorimetry using the Bratton–Marshall method can be used for the assay in solution. A number of HPLC methods[11–14] for the assay of sulphonamides in biological material can also be adapted for the assay of sulphonamides in solution.

This report shows that sulphamethizole can also be determined with minor adaptations by the HPLC method of Knox and Jurand[4], which was developed for tetracyclines. A new method is also described for the determination of phenazopyridine hydrochloride without interference from tetracycline hydrochloride and sulphamethizole, simply by changing the mobile phase and the wavelength of detection.

EXPERIMENTAL

Apparatus

A Knauer FR-30 dual-piston HPLC pump (Bad Homburg, F.R.G.) was used. A Knauer variable-wavelength monitor type 87 was used for detection. A Rheodyne loop injector type 70-10 fitted with a 20-μl loop was used for sample injection.

Column

The stainless-steel column (25 cm × 4.6 mm I.D.) was packed with Zorbax TMS 7 μm (Du Pont).

Chemicals

All chemicals used were analytical grade supplied by Merck Chemicals, RSA.

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Methanol, dimethylformamide, 2-propanol and acetic acid were analytical grade, supplied by Merck Chemicals (South Africa). The acetonitrile was HPLC grade, supplied by Saarchem (South Africa). Water used was twice distilled in glass.

Chromatographic analysis

Capsules containing tetracycline hydrochloride (125 mg), sulphamethizole (250 mg) and phenazopyridine hydrochloride (50 mg) were dissolved in 1000 ml of 0.1 *M* hydrochloric acid. The highest concentrations possible were therefoire: tetracycline hydrochloride, 125 μg/ml; sulphamethizole, 250 μg/ml; and phenazopyridine hydrochloride, 50 μg/ml. The Rheodyne injector was used to inject 20 μl of standards in 0.1 *M* hydrochloric acid.

The mobile phase described in ref. 4 was used for the assay of tetracycline hydrochloride and sulphamethizole. It consisted of degassed dimethylformamide–water (10:90), containing 0.005 *M* sodium ethylenediaminetetraacetate, 0.1 *M* citric acid, 0.02 *M* sodium citrate and 0.05 *M* potassium nitrate. The flow-rate was 1.8 ml/min, the chart speed 20 cm /h, the wavelength of detection 270 nm and the detector sensitivity 0.5 a.u.f.s.

The mobile phase used for phenazopyridine hydrochloride was degassed acetonitrile–2-propanol–acetic acid–water (30:30:1:39). The flow-rate was 1.8 ml/min, the chart speed 20 cm/h, the wavelength of detection 405 nm and the detector sensitivity 1.0 a.u.f.s.

RESULTS AND DISCUSSION

Analysis of tetracycline hydrochloride and sulphamethizole

The separation of tetracycline hydrochloride and sulphamethizole is shown in Fig. 1. Phenazopyridine hydrochloride, sodium hexametaphosphate, magnesium stearate, lactose and gelatin, which were all also contained in the capsules, were each dissolved in 0.1 *M* hydrochloric acid, filtered and subjected to the first HPLC method. None of these ingredients eluted at the same time as either tetracycline hydrochloride or sulphamethizole, or after them. Tetracycline hydrochloride and sulphamethizole could thus be assayed in the presence of phenazopyridine and the other ingredients without interference. What is also clear is that the method developed for the separation of tetracyclines could also be used for sulphonamides.

The coefficient of variation was 5.7% and 5.3% for tetracycline hydrochloride and sulphamethizole, respectively. Tetracycline eluted after 203 sec, and sulphamethizole after 360 sec.

Analysis of phenazopyridine hydrochloride

The chromatogram for the assay of phenazopyridine hydrochloride in the presence of the other two drugs is shown in Fig. 2. Tetracycline hydrochloride, sulphamethizole and the other ingredients mentioned were each dissolved in 0.1 *M* hydrochloric acid, filtered and subjected to the second HPLC method. None of these ingredients eluted at the same time as phenazopyridine hydrochloride, so the method can be used for the assay of phenazopyridine hydrochloride in the presence of these other ingredients without interference. The coefficient of variation was 2.82% for phenazopyridine hydrochloride, and the retention time was 315 sec.

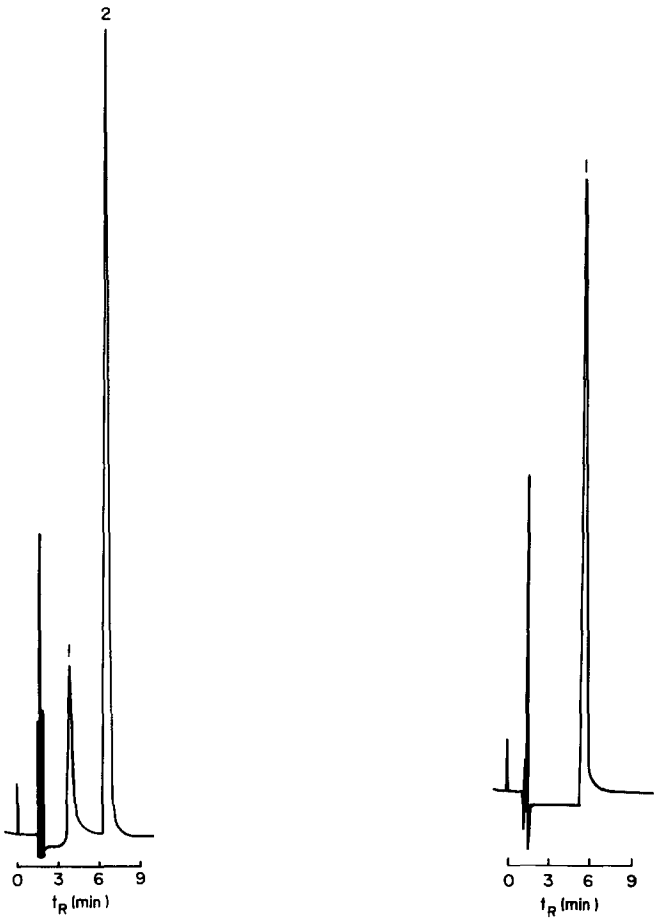

Fig. 1. Chromatogram of a dissolution sample showing peaks of tetracycline hydrochloride (1) and sulphamethizole (2).

Fig. 2. Chromatogram of a dissolution sample showing the phenazopyridine peak (1). The tetracycline and the sulphamethizole both elute on the solvent front.

The methods described had been used for determination of the content and content uniformity of the capsule after manufacture of a number of batches. The method is thus not only applicable to the assay in solution but is also a fast method for use during routine quality control.

The method for phenazopyridine hydrochloride can of course be adapted for the assay of other dosage forms, and is one of the first if not the first HPLC method described for the determination of phenazopyridine hydrochloride.

REFERENCES

1 B. R. Rader, *J. Pharm. Sci.*, 62 (1973) 1148.
2 M. N. Vora and K. Maheswaran, *Indian J. Pharm.*, 38 (1976) 98.

3 L. Szabolcs, *Acta Pharm. Hung.*, 48 (1978) 155.
4 J. H. Knox and J. Jurand, *J. Chromatogr.*, 186 (1979) 763.
5 E. R. White, M. A. Carroll, J. E. Zarembo and A. D. Bender, *J. Antibiot.*, 28 (1975) 205.
6 J. P. Sharma, E. G. Perkins and R. F. Bevill, *J. Chromatogr.*, 134 (1977) 441.
7 J. P. Sharma, G. D. Koritz, E. G. Perkins and R. F. Bevill, *J. Pharm. Sci.*, 66 (1977) 1319.
8 J. P. Sharma and R. F. Bevill, *J. Chromatogr.*, 166 (1978) 213.
9 I. Nilsson-Ehle, T. T. Yoshikawa, M. C. Schotz and L. B. Guze, *Antimicrob. Agents Chemother.*, 9 (1976) 754.
10 S. E. Magic, *J. Chromatogr.*, 129 (1976) 73.
11 M. C. Allfred and D. L. Damire, *J. Chromatogr. Sci.*, 16 (1978) 533.
12 J. Owerbach, N. F. Johnson, T. R. Bates, H. J. Pieniaszek and W. J. Jusko, *J. Pharm. Sci.*, 67 (1978) 1250.
13 G. W. Peng, M. A. F. Cadalla and W. L. Chiou, *Res. Commun. Chem. Pathol. Pharmacol.*, 18 (1977) 233.
14 J. P. Sharma, E. G. Perkins and R. F. Bevill, *J. Pharm. Sci.*, 65 (1976) 1606.