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Note

High-performance liquid chromatography of some sulphonamides

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The sulphonamides constitute a therapeutically important group of antimicrobial agents. Much work has been carried out on methods of separation and quantitative evaluation in such fields as pharmacology[1], therapeutics[2], and analytical chemistry[3]. The techniques used include paper chromatography[4], thin-layer chromatography (TLC)[5], gas–liquid chromatography (GLC)[6], ion-exchange chromatography[7], and high-pressure reversed-phase chromatography[8]. This communication describes a high-performance liquid chromatographic (HPLC) procedure for the separation of a range of sulphonamides utilising silica gel as the column packing material.

EXPERIMENTAL

Chromatographic separations were performed on a Cecil CE 210 liquid chromatograph, and elution was monitored at 260 nm using a CE 212 variable-wavelength spectrophotometer fitted with a 10-μl capacity flow cell (Cecil Instruments, Cambridge, Great Britain).

The stainless-steel column was 250 mm long with an internal diameter of 4 mm and was packed with Spherisorb S5W 5-μm-diameter spherical silica gel particles (Phase Separations, Queensferry, Clwyd, Great Britain) using the method of Kirkland[9].

All solvents used were of AnalaR® quality (BDH, Poole, Great Britain), and the mobile phase consisted of a mixture of 85.7% cyclohexane, 11.4% anhydrous ethanol, and 2.9% glacial acetic acid. The chromatograph was operated at ambient temperature and the mobile phase was driven at a rate of 2 ml/min by an isobaric application of 7 MN/m^2.

0.05% solutions of the sulphonamides were prepared in chloroform–methanol (1:1) and 1.0 μl samples were injected just below the surface of the column packing by a 701 N microlitre syringe through a PTFE-faced, silicone rubber septum (Hamilton Micromesure, The Hague, The Netherlands).

RESULTS AND DISCUSSION

A range of columns were prepared using irregular and spherical silica gel

packings of 5- and 10-μm particle diameter. Optimum resolution and analysis time was obtained with a 250-mm column packed with Spherisorb S5W spherical particles. Initial separations were obtained using cyclohexane–ethanol mixtures and it was found that increasing the ethanol content decreased the observed retention times. Addition of small amounts of acetic acid significantly increased column efficiency without altering resolution. An optimum balance between resolution, efficiency and analysis time was obtained with the described mobile phase. A specimen chromatogram is shown in Fig. 1 and typical retention times for all sulphonamides examined are given in Table I.

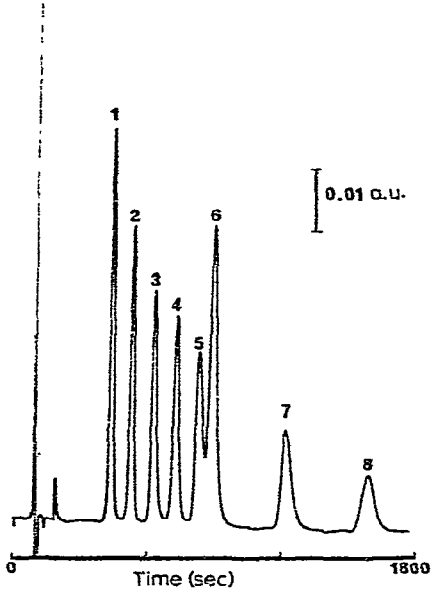


Fig. 1. Typical high-performance liquid chromatogram of a mixture of sulphonamides. 1 = Sulphapyridine; 2 = sulphamethoxazole; 3 = sulphisoxazole; 4 = sulphamethazine; 5 = sulphameter; 6 = sulphanilamide; 7 = sulphamoxole; 8 = succinyl sulphathiazole.

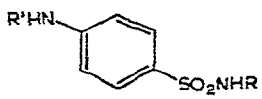
As a technique HPLC offers superior resolving power to TLC combined with a quantitative precision similar to that obtained with GLC. In this instance, however, GLC requires prior derivatisation of the sulphonamides with its attendant disadvantages.

This method may be applied to the identification or assay of single sulphonamides and sulphonamide mixtures, whilst the chromatographic data should enable a suitable internal standard to be selected.

TABLE I

RETENTION DATA FOR A RANGE OF SULPHONAMIDES

Chromatographic conditions as in Experimental.



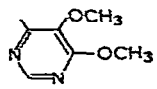
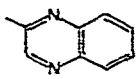
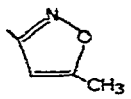
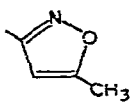
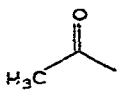
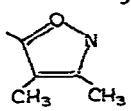
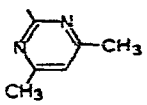
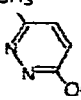
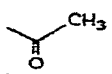
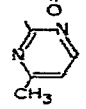
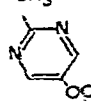
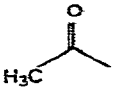
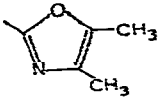
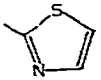
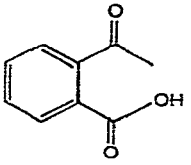
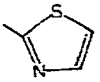
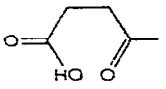
Compound name	*R*	*R'*	*Retention time (sec)*
Sulphachloropyridazine		H	371
Sulphapyridine		H	423
Sulphormethoxine (Sulphadoxine)		H	474
Sulphaquinoxaline		H	504
Sulphamethoxazole		H	509
N^4-Acetylsulphamethoxazole			515
Sulphisoxazole (Sulphafurazole)		H	607
Sulphamethazine (Sulphadimidine)		H	707
Sulphamethoxypyridazine		H	740
Sulphacetamide		H	759
Sulphamerazine (Sulphamethyldiazine)		H	795
Sulphameter (Sulphamethoxydiazine)		H	807
Sulphadiazine		H	845

TABLE I *(continued)*

Compound name	*R*	*R'*	*Retention time (sec)*
Sulphanilamide	H	H	865
N^4-Acetylsulphanilamide	H		926
Sulphamoxole		H	1189
Sulphathiazole		H	1254
Phthalyl sulphathiazole			1313
Succinyl sulphathiazole			1553

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REFERENCES

1 J. K. Sedgel and E. Krüger-Theiner, *Arzneim.-Forsch. Drug. Res.*, 14 (1964) 1294.
2 E. Krüger-Theiner, *Proc. 3rd Int. Pharm. Meet., São Paulo, 1966*, 7 (1967) 63.
3 A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, 128 (1939) 537.
4 K. Macek, *Česk. Farm.*, 12 (1963) 356.
5 G. Hanel, G. Heinisch and E. Luszczak, *Sci. Pharm.*, 42 (1974) 33.
6 E. Roeder and W. Struthe, *Z. Anal. Chem.*, 266 (1973) 358.
7 T. C. Kram, *J. Pharm. Sci.*, 61 (1972) 254.
8 F. M. Rabel, *Anal. Chem.*, 45 (1974) 957.
9 J. J. Kirkland, *J. Chromatogr. Sci.*, 10 (1972) 593.