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Note

A rapid method for the simultaneous determination of the major metabolites of sulphasalazine in plasma

P.N. SHAW, A.L. SIVNER, L. AARONS* and J.B. HOUSTON

Department of Pharmacy, University of Manchester, Manchester M13 9PL (Great Britain)

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Sulphasalazine (SASP) is widely used in the treatment of Crohn's disease and ulcerative colitis [1,2]. After an oral dose 10—30% of the drug is absorbed unchanged while the remainder is cleaved by bacterial azo reductases in the large intestine to 5-aminosalicylate (AS) and sulphapyridine (SP) which are subsequently absorbed [3]. It is currently believed that AS is the active therapeutic species whereas most of the toxic side effects are due to SP and consequently SASP therapy is generally followed by monitoring plasma concentrations of SP [4].

AS in plasma has been assayed fluorimetrically [5] whilst SP in plasma has been determined using a spectrophotometric method [6]. More recently the pharmacokinetics of SASP and its metabolites have been studied by Klotz and co-workers using assay techniques based on high-performance liquid chromatography (HPLC) [7—10]. Overbach et al. [11] have compared the HPLC assay of SP with the spectrophotometric assay and found them comparable. However, the acetylated metabolite of SP has to be measured by difference (after hydrolysis) in the spectrophotometric assay whereas it is determined directly in the HPLC assay. Hansen [12] has recently described an improved HPLC assay for the determination of AS and its acetylated metabolite.

In the course of recent studies on the disposition of SASP in patients with large bowel disease we have developed a rapid, sensitive HPLC method for the determination of AS, SP and their acetylated metabolites (AcAS and AcSP). The advantage of the present assay over previous HPLC assays is that it measures all the metabolites simultaneously and consequently is more rapid.

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EXPERIMENTAL

Materials

All chemicals were of analytical grade. AcAS and AcSP were prepared by reacting AS and SP respectively with acetic anhydride followed by recrystallization.

Apparatus

Separations were performed on a 200 mm × 4.6 mm I.D. column packed with short alkyl chain bonded silica (Hypersil-SAS, 5 μm). The mobile phase was pumped through the column by a Waters Assoc. Model 6000A pump. Detection was achieved by coupling a UV monitor (Pye Unicam LC3, wavelength 290 nm) to a fluorimetric detector (Schoeffel LC fluorimeter FS970, excitation wavelength 320 nm; emission cut-off 389 nm) in series.

Chromatographic conditions

The mobile phase consisted of a mixture of methanol (22.5%) and 0.05 *M* phosphate buffer, pH 7.4, containing 0.1% tetrabutylammonium hydrogen sulphate and was pumped through the column at a flow-rate of 1 ml min^{-1} with a resulting back pressure of 150 bar. All assays were performed at ambient temperature.

Sample preparation

A 500-μl volume of methanol containing salicylic acid (SA, 30 mg l^{-1}) was added to either 500 μl of plasma obtained from a patient or a standard prepared in blank plasma. After vortexing, the solution was centrifuged at 650 *g* for 2 min. A 10-μl aliquot of the clear supernatant was then injected directly onto the column. Calibration plots of peak height ratio against concentration were then prepared.

RESULTS AND DISCUSSION

The concentrations of AS and AcAS observed after SASP administration are much less than those of SP and AcSP [8]. Consequently, it is necessary to use fluorimetry in order to detect AS and AcAS. However since AS and AcAS only fluoresce in their ionized form, to get them to run on a reversed-phase column a pairing ion [tetrabutylammonium (TBA)] had to be added.

The differential effect of the TBA concentration on the capacity factors of AS and SP is shown in Fig. 1. A TBA concentration of 1 g l^{-1} gave sufficient resolution of all components. SP and AcSP were determined on the UV detector and AS and AcAS on the fluorimetric detector. AcSP fluoresces but does not interfere with the determination of AS or AcAS.

The internal standard in this assay must be fluorescent as well as chromatographing in the right region. Salicylic acid proved to be an ideal internal standard but unfortunately there is a danger that patients may be taking aspirin which would invalidate the use of salicylic acid as an internal standard. Consequently plasma samples were run without the addition of internal standard to check for the presence of salicylate.

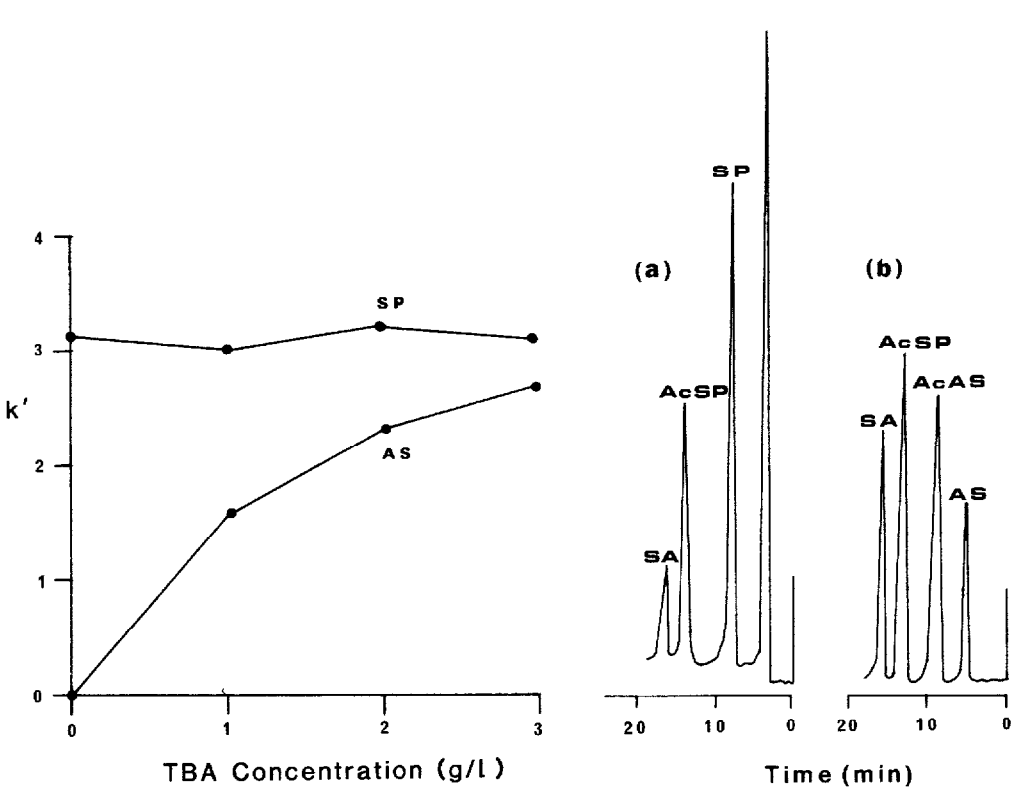

Fig. 1. The effect of counter-ion concentration (TBA) on the capacity factor (k') of sulphapyridine (SP) and 5-aminosalicylate (AS).

Fig. 2. Typical chromatograms of a standard solution prepared in plasma containing SP (20 mg l^{-1}), AcSP (20 mg l^{-1}), AS (5 mg l^{-1}), AcAS (5 mg l^{-1}) and SA (15 mg l^{-1}). (a) Recording from the UV monitor; (b) recording from fluorescence monitor. Apart from concentration, chromatograms obtained from patient samples were very similar to the ones shown above.

TABLE I

INTRA-DAY AND INTER-DAY VARIABILITY OF HPLC ANALYSIS OF SULPHASALAZINE METABOLITES

Intra-day values are the mean of five replicates. Inter-day values are the mean of assays carried out on five separate days.

	Intra-day				Inter-day			
	SP	AcSP	AS	AcAS	SP	AcSP	AS	AcAS
Actual concentration (mg l^{-1})	20	20	5	5	20	20	5	5
Estimated concentration (mg l^{-1})	20.4	19.7	4.8	5.2	20.8	20.7	5.0	5.2
Coefficient of variation (%)	3.9	3.6	3.1	1.9	4.8	5.3	3.0	2.9

A typical chromatogram is shown in Fig. 2. The retention times of AS, SP, AcAS and SA were 5.5, 7.5, 9.0, 13.5 and 16.0 min, respectively. Calibration plots were linear over the range 0.5—50 mg l^{-1} for SP and AcSP and 0.5—10 mg l^{-1} for AS and AcAS. Using the present assay it is easily possible to measure concentrations of each metabolite down to 0.5 mg l^{-1}. At concentrations of 20 mg l^{-1} of SP and AcSP and 5 mg l^{-1} of AS and AcAS the coefficient of variation of the assay was generally less than 5% (Table I).

The SASP metabolite time profiles in a subject suffering from Crohn's disease are shown in Fig. 3. The subject was receiving SASP 1 g every 12 h and the profile shown was measured on the 8th day of therapy.

Although the assay involves direct injection of diluted plasma, providing the column was thoroughly washed out with methanol—water (50 : 50) overnight, the column remained efficient for a period in excess of six months.

In summary the assay described in this report requires little sample preparation, is rapid and is sufficiently sensitive to monitor sulphasalazine therapy in patients with large bowel disease.

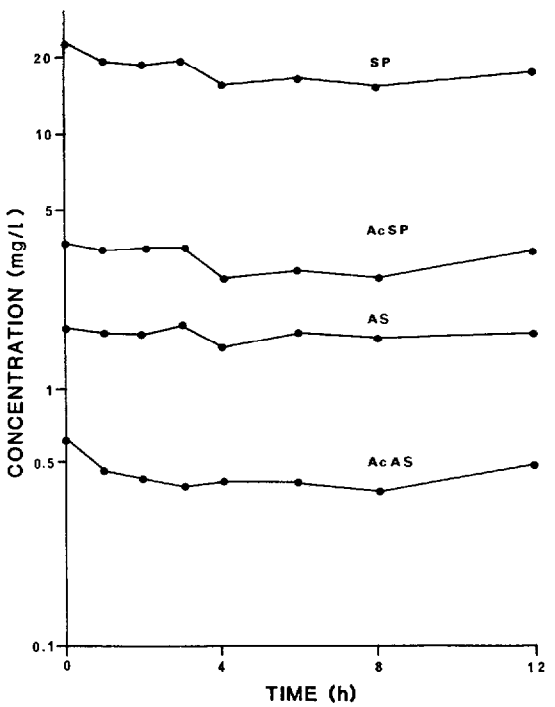

Fig. 3. Sulphasalazine metabolite time profiles in a subject taking 1 g of sulphasalazine every 12 h.

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REFERENCES

1 R.W. Summers, O.M. Switz, J.T. Sessions, J.M. Beckel, W.R. Best, F. Kern and J.W. Singleton, Gastroenterology, 77 (1979) 847.
2 A.Z. Azad Khan, D.T. Howes, J. Pris and S.C. Truelove, Gut, 21 (1980) 232.
3 H. Schröder and D.E.S. Campbell, Clin. Pharmacol. Ther., 13 (1972) 539.
4 K.M. Das and R. Dubin, Clin. Pharmacokin., 1 (1976) 406.
5 K.A. Hannson and M. Sandberg, Acta Pharm. Suecica, 10 (1973) 153.
6 K.A. Hannson and M. Sandberg, Acta Pharm. Suecica, 10 (1973) 87.
7 C. Fischer and U. Klotz, J. Chromatogr., 146 (1978) 157.
8 C. Fischer and U. Klotz, J. Chromatogr., 162 (1979) 237.
9 C. Fischer, K. Maier and U. Klotz, J. Chromatogr., 225 (1981) 498.
10 U. Klotz, K. Maier, C. Fischer and K. Heinkel, New Engl. J. Med., 303 (1980) 1499.
11 J. Overbach, N.F. Johnson, T.R. Bates, H.J. Pieniaszek and W.J. Jusko, J. Pharm. Sci., 67 (1978) 1250.
12 S.H. Hansen, J. Chromatogr., 226 (1981) 504.