Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, No. 1, pp. 103–107, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00

Short Communication

A modified reversed-phase high-performance liquid chromatographic method for the determination of plasma sulphasalazine concentrations

C. ASTBURY

Clinical Pharmacology Unit, Royal Bath Hospital, Harrogate, North Yorkshire HG1 2PS, UK

Keywords: *Sulphasalazine; reversed-phase HPLC.*

Introduction

Sulphasalazine (SASP) has been used to treat inflammatory bowel disease since the late 1940s. However, since its "re-discovery" by rheumatologists in the late 1970s [1], it has become established as a second-line agent in the management of rheumatoid arthritis.

The most commonly used high-performance liquid chromatographic (HPLC) method for the determination of plasma concentrations of SASP was developed by Lanbeck and Lindstrom [2]. Unfortunately, a number of non-steroidal anti-inflammatory drugs (NSAIDs) are co-eluted with SASP; hence the assay cannot be used for patients receiving concomitant therapy with such drugs, as is usually the case in rheumatoid arthritis. Also, this method does not incorporate an internal standard and thus does not allow any compensation for loss of SASP during the extraction procedure. This imposes restrictions on the reproducibility and accuracy of the assay.

The present method represents a modification of the original assay [2] and is suitable for pharmacokinetic analyses and for the determination of steady-state SASP concentrations in patients with rheumatoid arthritis. Piroxicam is used as an internal standard.

Experimental

Materials

Ethyl acetate, acetonitrile, methanol, hydrochloric acid, disodium hydrogen phosphate and sodium dihydrogen phosphate (all AnalaR grade) were purchased from BDH (Poole, UK). SASP was a gift from Pharmacia Ltd and piroxicam was provided by Pfizer Ltd. All aqueous dilutions were carried out with double-distilled water.

Calibration procedure

Stock solutions of SASP (100 μg ml^{-1}) and piroxicam (1 mg ml^{-1}) in methanol were prepared and stored in darkness at 4°C. An aqueous dilution of piroxicam (50 μg ml^{-1}) was prepared daily. Six samples of blank plasma (500 μl) were spiked with increasing volumes of SASP to give concentrations in the range 1.0–20.0 μg ml^{-1}; these were diluted to 100 μl with methanol as necessary to ensure a constant addition volume. A 100-μl aliquot of aqueous piroxicam solution was added to each sample. The procedure outlined below was then followed.

Extraction procedure

Aqueous piroxicam solution (100 μl) and methanol (100 μl) were added to 500 μl of all unknown and quality control plasma samples. All samples were mixed with 1 ml of 1 M hydrochloric acid and extracted with 4 ml of ethyl acetate by vigorous shaking for 2 min. The organic and aqueous layers were separated by centrifugation at 550 g for 10 min at room temperature. The organic layer was transferred to a clean tube and evaporated to dryness at 50°C under oxygen-free nitrogen. The residue was dissolved in 500 μl of mobile phase [acetonitrile–0.05 M phosphate buffer, pH 7.9, (20 : 80, v/v)].

Chromatography

The HPLC system comprised a Constametric III dual piston pump, a Spectromonitor III dual cell UV detector, a 308 computing integrator and a flat bed recorder (Laboratory Data Control). A Rheodyne injection system with a 20-μl loop was used. The 150 × 3.2 mm i.d. analytical column was packed with 5-μm LiChrosorb RP 18 (HPLC Technology) and coupled with a 75 mm CO: Pell ODS guard column (Whatman). The system was operated at room temperature with a flow rate of 1.50 ml min^{-1} and a back-pressure of 2000 lb in^{-2} (13.7 MPa). Samples were injected automatically at 7-min intervals using a Series 8000 auto-sampler (Varian Associates Ltd).

Application of the assay

The assay has been used to determine plasma concentrations of SASP in patients with rheumatoid arthritis following single- and multiple-dose oral administration of SASP (2 g day^{-1}).

Results

Chromatograms of standard solutions of SASP and piroxicam indicated that both peaks were well resolved with retention times of 220 and 130 s, respectively. Injections of extracts of blank plasma showed that there was no interference from endogenous compounds. Typical chromatograms of blank, unknown and standard plasma extracts are illustrated in Fig. 1. Calibration curves for SASP of peak-height ratio versus concentration were linear and reproducible over the calibration range (1.0–20.0 μg ml^{-1}). The concentration of SASP in unknown samples was determined by unweighted linear least-squares regression analysis.

The precision of the assay was acceptable; the mean inter-run relative standard deviation was 7.4% and the mean accuracy was 102%, determined from results on standard solutions in 12 analytical runs (Table 1). Each analytical run was carried out on a separate day by the same analyst. The mean regression equation was: $y = 0.13x + 0.02$.

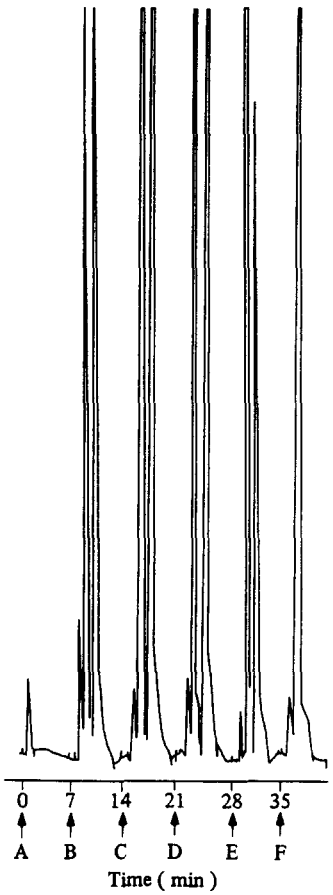


Figure 1
Typical chromatograms of standard and unknown plasma extracts. Retention times: Internal standard 130 s, SASP 220 s. A, Extract of blank plasma; B, Plasma from a patient 7 h after receiving SASP; C, Quality control sample containing 10 μg ml^{-1} SASP; D, Standard plasma extract containing 8 μg ml^{-1} SASP; E, Plasma from a patient receiving concomitant NSAID therapy 2 h after administration of SASP; F, Plasma from a patient receiving NSAID therapy, before administration of SASP. Samples B–F were spiked with internal standard before extraction.

Table 1
Results, correlation coefficients, gradients and intercepts obtained from 12 analytical runs on standard solutions of sulphasalazine

	Sulphasalazine concentration (μg ml^{-1})									
Batch No.	1.0	2.0	4.0	8.0	12.0	16.0	20.0	*r*	Gradient	Intercept
1	1.09	2.07	3.85	8.42	11.54	16.32	19.88	0.9993	0.1207	0.01
2	1.39	2.52	3.58	7.12	12.22	15.74	20.44	0.9974	0.1197	−0.05
3	1.43	1.67	3.95	8.02	11.63	16.88	19.54	0.9981	0.1625	0.03
4	1.10	2.05	4.09	7.86	11.24	15.84	20.61	0.9986	0.1546	−0.02
5	1.42	†NS	4.31	7.68	11.49	15.42	20.82	0.9976	0.1095	0.03
6	0.76	2.05	3.71	9.02	12.23	15.29	20.09	0.9977	0.1149	0.02
7	1.08	2.20	4.00	7.50	†NS	15.79	20.20	0.9995	0.1250	−0.01
8	1.25	2.24	3.78	7.83	12.28	15.95	19.95	0.9995	0.1353	0.07
9	0.93	1.86	3.79	7.95	12.96	15.79	19.67	0.9985	0.1276	0.07
10	0.94	2.02	4.26	7.21	12.89	15.92	19.79	0.9980	0.1144	0.01
11	1.21	1.89	4.11	7.46	11.89	15.84	20.38	0.9992	0.1446	−0.02
12	0.99	2.00	3.75	8.18	12.17	16.59	19.41	0.9989	0.1235	0.05
Mean	1.13	2.05	3.94	7.85	12.05	15.95	20.07	0.9985	0.1294	0.02
±SD	0.21	0.22	0.23	0.53	0.56	0.45	0.44			
Precision* (%)	18.6	10.7	5.9	6.7	4.6	2.8	2.2			
Accuracy (%)	113.0	102.5	98.5	98.1	100.4	99.7	100.4			

* Relative standard deviation.
† NS = No sample.

The intra-run relative standard deviation was 3.3%, calculated from six quality control samples containing 10 $\mu g\ ml^{-1}$ of SASP. The mean recoveries of SASP and piroxicam were 86 and 85%, respectively, and the detection limit for SASP was 200 $ng\ ml^{-1}$.

A typical single-dose pharmacokinetic profile for a patient with rheumatoid arthritis after oral administration of SASP (2 g) is illustrated in Fig. 2. *In vitro* addition of commonly prescribed NSAIDs to blank plasma demonstrated that aspirin, indomethacin, ibuprofen and diclofenac do not interfere with the assay.

Discussion

This modified assay provides a rapid, sensitive, specific and reproducible method for the quantitative analysis of SASP in plasma of patients with rheumatoid arthritis. Although the internal standard and SASP have different chemical structures (Fig. 3), both have UV absorption maxima at 360 nm and the percentage recoveries of each compound after extraction are very similar.

Peak-height and peak-area analyses were compared and found to give very similar results. However, owing to the short and consistent retention times of SASP and piroxicam and to the geometry of the peaks, peak-height analysis was preferred.

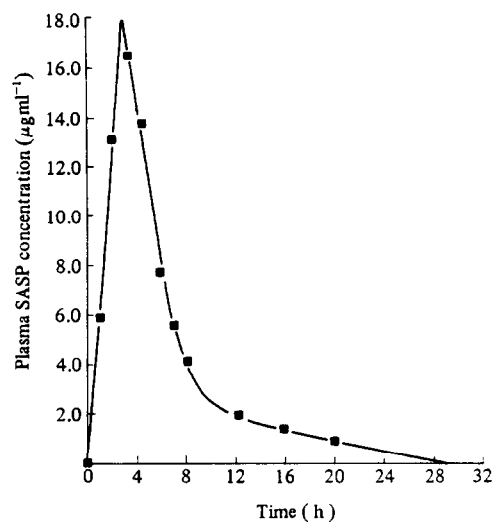
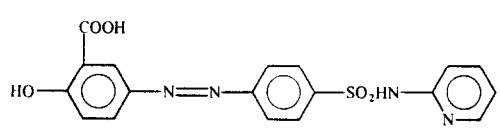
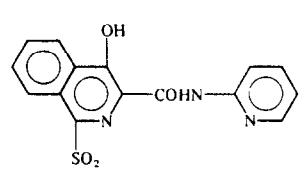


Figure 2
Typical single-dose pharmacokinetic profile of SASP (2 g orally) in patients with rheumatoid arthritis.



Sulphasalazine



Piroxicam

Figure 3

The concentration of SASP in plasma obtained from patients already receiving concomitant therapy with piroxicam may be determined by the external standards method from the peak-heights of SASP in the standard samples using linear least squares regression analysis.

The major metabolites of SASP (sulphapyridine, acetyl-sulphapyridine, 5-aminosalicylic acid and acetyl-5-aminosalicylic acid) were not detected on the chromatogram. Recoveries of sulphapyridine and acetylsulphapyridine are poor when extracted with hydrochloric acid and ethyl acetate and both have UV absorption maxima near 270 nm. 5-aminosalicylic acid and acetyl-5-aminosalicylic acid are not easily extracted with organic solvents and fluorescence detection has to be employed to measure physiological concentrations of these metabolites.

Acknowledgements: The author wishes to thank Drs J. S. Dixon and N. Roberts for helpful discussion and advice, and the Arthritis and Rheumatism Council for financial support. The Clinical Pharmacology Unit acknowledges the financial support of Roche Products Ltd.

References

[1] B. McConkey, R. S. Amos, E. P. Butler, R. A. Crockson, A. P. Crockson and L. Walsh, *Agents and Actions* **8**, 438–441 (1978).

[2] K. Lanbeck and B. Lindstrom, *J. Chromatogr.* **154**, 321–324 (1978).

[*Received for review 14 April 1987; revised manuscript received 23 June 1987*]