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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC EVALUATION OF SALICYLOYL PYRIDIXOL AND SYSTEMIC METABOLITES IN BIOLOGICAL SAMPLES

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SUMMARY

An high-performance liquid chromatographic analytical method, which allows quantitative evaluation of both salicyloyl pyridixol and its metabolite salicylic acid, is reported. This method has demonstrated to possess the required specifications in terms of linearity, sensitivity, extraction recovery, reproducibility and specificity for pharmacokinetic investigations in both human subjects and experimental animals. The results obtained from an investigation on the rat are briefly discussed.

INTRODUCTION

Salicyloyl pyridixol (SP) is a new non-steroid antiinflammatory agent which in previous pharmacotoxicological investigations has been shown to possess the activity of salicylates with very low gastrointestinal side-effects, in agreement with a series of previous investigations on drugs containing the carboxylate in esterified form[1–5].

SP is the product of esterification of salicylic acid (SA) and 3-pyridixol (or pyridoxine) (Fig. 1). Any pharmacokinetic investigation of an ester such as SP must be based on an analytical method capable of evaluating both the parent drug and its metabolite SA. This paper describes an analytical method which evaluates SP and SA in plasma, tissues and urine. Pharmacokinetic results in the rat are also discussed.

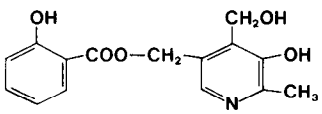

Fig. 1. Chemical structure of salicyloyl pyridixol (SP), $C_{15}H_{15}O_5N$. Molecular weight = 289.293.

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EXPERIMENTAL

Materials

The solvents and chemicals, all of analytical or HPLC grade, were supplied by Merck (Bracco, Milan, Italy), Fluka (Schrepfer, Milan, Italy) and Sigma (Prodotti Gianni, Milan, Italy).

SP was synthesized by Erregierre (S. Paolo D'Argon, Bergamo, Italy). SA was available in the Real laboratory and was shown to meet the USP XXI (1985) specifications. An Hitachi Model 655A-11 liquid chromatograph (Bracco) and an Hitachi Model 655A variable-wavelength UV detector were used for analysis. The column was a μBondapak C_{18}, 5 μm, 300 mm × 4.6 mm I.D., from Waters Assoc. (Millipore, Segrate, Milan, Italy). The statistical computations were performed on a Macintosh Plus personal computer (Apple, Reggio Emilia, Italy).

Methods

A 1-ml volume of plasma was diluted in 1 ml of phosphate–citrate buffer at pH 7.4. The tissues were homogenized with the same buffer at a tissue/buffer ratio of 1:4 (w/v), and 50 μl 30% perchloric acid and 0.5 ml methanol were then added to deproteinize the plasma or tissue sample. After stirring for 10 min and centrifuging at 2400 *g* for 5 min, an aliquot of the supernatant was separated. 3-Methylbenzoic acid was used as an internal standard (I.S.), added after extraction to monitor recovery and before extraction in the routine analysis. The mobile phase consisted of acetonitrile–1% phosphoric acid (17:83) at pH 3.25. The flow-rate was 2 ml/min. Absorbance was monitored at 238 nm. Retention times were 5.25 min for SA, 6.78 min for SP and 12.31 min for I.S. Fig. 2 shows typical chromatograms.

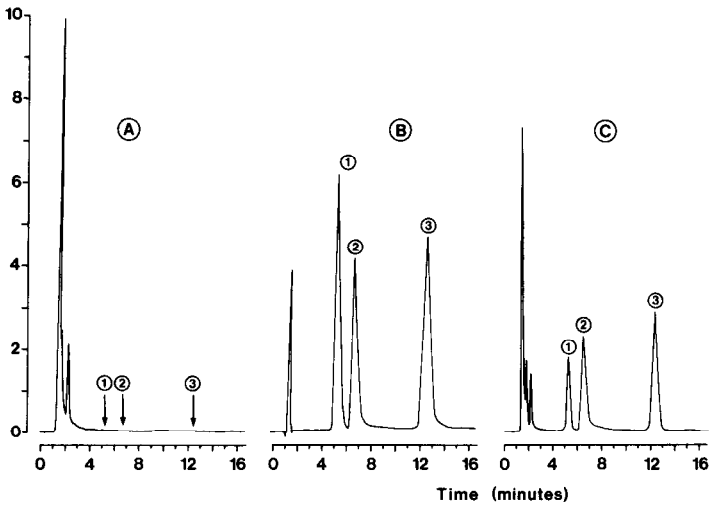

Fig. 2. HPLC of salicylic acid (1), salicyloyl pyridixol (2) and 3-methylbenzoic acid (I.S.) (3). (A) Blank plasma; (B) authentic standards; (C) plasma of a rat treated with the drug.

RESULTS AND DISCUSSION

Linearity was verified in terms of detector response factor for both the parent drug and SA in the 50–1000 ng range, five replications being performed. The intra-assay coefficient of variation was 1.12 for SP and 1.35 for SA.

The inter-assay coefficient of variation was 4.05 for SP and 4.37 for SA when a fixed analyte/I.S. ratio (1:1) was used. It was 4.78 for SP and 4.92 for SA when the method was validated at variable analyte/I.S. ratio ranging from 1:4 to 4:1 (Table I).

TABLE I

ANALYTICAL LINEARITY OF DETECTOR RESPONSE EVALUATED WITH BOTH A FIXED ANALYTE/I.S. RATIO OF 1:1 AND A VARIABLE RATIO RANGING FROM 1:4 TO 4:1

$$DRF = \frac{\text{analyte peak area}}{\text{I.S. peak area}} \quad \frac{\text{I.S. concentration}}{\text{analyte concentration}}$$

Amount injected (ng)			*DRF*	
Analyte	*I.S.*		*SP*	*SA*
50	50		1.667	1.504
125	125		1.669	1.573
250	250		1.728 ± 0.0195 (S.D.)* (C.V. = 1.12%)	1.433 ± 0.0194 (S.D.)* (C.V. = 1.35%)
500	500		1.839	1.606
1000	1000		1.725	1.516
		Mean	1.726	1.526
		S.D.	0.0698	0.0668
		C.V. (%)	4.05	4.37
250	1000		1.850	1.443
500	1000		1.635	1.629
1000	1000		1.710	1.538
1000	500		1.682	1.580
1000	250		1.764	1.476
		Mean	1.728	1.533
		S.D.	0.0826	0.0755
		C.V. (%)	4.78	4.92

* Five replicates.

The recovery of SP and SA from rat plasma was investigated in the range from 10 to 100 μg/ml, the analysis being performed in quadruplicate at each concentration. The average recovery was 90.5% for SP and 91.5% for SA, with linear correlation coefficients, r^2, of 0.9928 and 0.9966 respectively (Table II). Extraction recovery in urine and tissues was very close to the values obtained in plasma.

This method has been used for a pharmacokinetic study of SP in the rat after oral administration of the drug at a dose of 100 mg/kg (48 mg/kg in terms of SA). Sprague-Dowley male rats, weighing 225–250 g, supplied by Charles River (Calco,

TABLE II

RECOVERY OF SP AND SA FROM RAT PLASMA IN QUADRUPLICATE ANALYSES

Internal standard was added in such a way as to have a drug/I.S. ratio of 1:1 both for SP and SA. During routine analysis, the internal standard used was such as to remain in a drug/I.S. ratio of 1:4–4:1.

	Amount added (= x) (μg/ml)	*Amount recovered (= y)*				*Recovery (%)*
		Mean (μg/ml)	*S.D.*	*C.V. (%)*		
SP	10	9.5	0.23	2.39		95.0
	20	18.6	0.63	3.40		93.0
	50	44.0	2.38	5.41		88.0
	100	86.0	5.26	6.11		86.0
					Mean	90.5
					S.D.	4.20
					C.V. (%)	4.64
SA	10	8.7	0.43	4.90		87.0
	20	18.0	0.51	2.83		90.0
	50	48.5	2.59	5.34		97.0
	100	92.0	2.07	2.25		92.0
					Mean	91.5
					S.D.	4.20
					C.V. (%)	4.59

Italy), were used. Fig. 3 shows the mean plasma SA concentration *vs.* time curve obtained according to the open one-compartment model for the oral route, using the non-linear fitting method. Table III shows the main pharmacokinetic parameters obtained from the mean plasma concentrations of SA in the rat.

The lowest detectable amount was 20 ng (1000 ng/ml in terms of plasma concentration) for SP and 10 ng (500 ng/ml) for SA.

Considerable care was given to specificity in terms of evaluating both the parent drug and SA, in that SP was hydrolysed at a very slow rate, giving a concentration of the drug as such in the gastric and intestine walls and of SA in the systemic circulation.

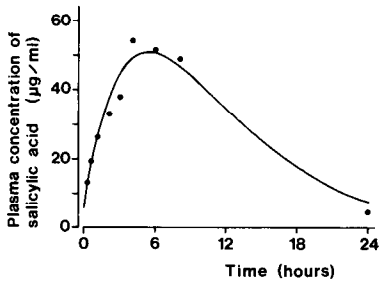

Fig. 3. Plasma concentration *vs.* time behaviour of salicylic acid after oral administration of salicyloyl pyridixol in the rat (100 mg/kg).

TABLE III

PHARMACOKINETIC PARAMETERS REFERRING TO THE PLASMA CONCENTRATION OF SA AFTER ORAL ADMINISTRATION OF SP (100 mg/kg, 48 mg/kg AS SA)

t_{max} = peak time; C_{max} = peak concentration; $t_{1/2}$ = half-life; K_a = absorption rate constant; K_{el} = elimination rate constant; AUC = area under the plasma concentration *vs.* time curve.

t_{max}	(h)	:	4
C_{max}	(μg/ml)	:	58.7
$t_{1/2}$	(h)	:	4.24
K_a	(h^{-1})	:	0.178
K_{el}	(h^{-1})	:	0.170
$AUC_{0-24\ h}$	(μg/ml · h)	:	770.78

In conclusion, the method described possesses the specifications required for pharmacokinetic and bioavailability investigations in human beings and in laboratory animals. It is rapid, requiring only one extraction for the parent drug and SA, and simple and inexpensive reagents. A skilful operator can process more than 25 analyses a day without an autosampling injector. The method is also proving to work very well in human pharmacokinetic studies, which are now in progress.

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