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Letter to the Editor

Simultaneous liquid chromatographic determination of aspirin and the metabolites in human urine

Sir,

Several high-performance liquid chromatographic (HPLC) methods have been described for the simultaneous analysis of salicylic acid (SA), salicyluric acid (SUA) and gentisic acid (GA) in biological fluids [1—10]. The problem often encountered in such analysis in urine is the interference of endogenous substances with the peak due to GA. Several workers found the extraction process and sometimes changes in the detection wavelength inevitable in order to determine GA in urine by HPLC simultaneously with other SA metabolites [5—10]. It is reported here that a modification of the conditions used by Cham et al. [1] was sufficient to provide a separation of GA from the endogenous substances in urine, thus providing a rapid HPLC method for the simultaneous analysis of acetylsalicylic acid (ASA), SA, SUA and GA in urine. The method was used in the analysis of urine samples from patients with self-administered aspirin overdose admitted into the Clinical Pharmacology Unit of the Royal United Hospital (Bath, U.K.).

The pumping system was Costa Metric III and detection at 245 nm was by the variable-wavelength Spectromonitor III (both from Laboratory Data Control, LDC, Milton Roy). The prepacked column was Spherisorb ODS-5 25 cm × 4.6 I.D. mm). Injection into the column was through a Rheodyne injector valve, LDC Model 7125, fitted with a 100-μl loop. The mobile phase of water—methanol—acetic acid (71:25:4), pH 2.5, was degassed and pumped through the column at 1.2 ml/min. The analysis was carried out at ambient temperature.

In the preparation of standard curves in urine, varying amounts of stock solutions (500 mg/l) of ASA, SA, SUA and GA were placed in centrifuge tubes, followed by the addition of 1 ml of blank urine (from apparently healthy normal subjects) and 1 ml of 500 mg/l *o*-anisic acid (in acetonitrile) as the internal standard (I.S.). Water was added to the tubes to make up to 10 ml, so as to give 5—100 mg/l for each compound except ASA, 2.5—10 mg/l. After whirlimixing for 30 s, the solution was injected onto the liquid chromatograph.

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Peak-height ratios were used to construct the calibration curves, except for ASA where peak heights were used. Urine was collected at predetermined intervals from patients with self-administered ASA overdose throughout the period of their stay in the Clinical Pharmacology Unit of the Royal United Hospital. Aliquots of the urine were kept frozen until analysed. To 1 ml of the urine was added 1 ml of I.S. solution followed by 8 ml of water. After whirlimixing for 30 s, 100 μl were injected onto the HPLC column. Incubation of urine samples at pH 5 with β-glusulase for 18 h before subjecting it to the above process afforded the amounts of salicylic acid glucuronides (SAG) present.

The retention times (t_R) of GA, SUA, I.S., ASA and SA were 5.8, 7.5, 9.8, 11.1 and 20.8 min, respectively. The detector response was linear in the concentration ranges tested, with $r > 0.995$ for the calibration curves obtained for all the compounds. The inter-assay precision (coefficient of variation) was lower than 4% at 50 mg/l and the sensitivity was 1 mg/l for SA and the metabolites.

The endogenous peak, always found interfering with the GA peak, was well resolved from the metabolite peak (Fig. 1). The resolution of the endogenous peak and the GA peak was, however, found to be affected by the room temperature. It was observed that when the temperature was 14°C or lower, the system could not resolve the GA peak from that due to the endogenous sub-

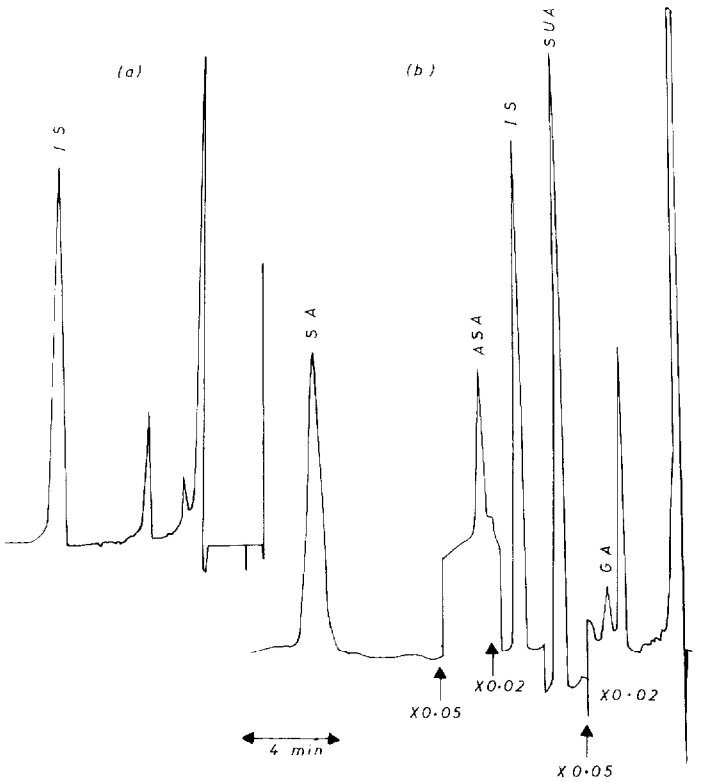

Fig. 1. Chromatogram of (a) blank urine sample and (b) test urine from a patient after aspirin overdose.

TABLE I

URINARY RECOVERY OF ASPIRIN AND METABOLITES IN PATIENTS WITH SELF-ADMINISTERED OVERDOSE

Patient	Sex	Aspirin ingested* (g)	Period of urine collection** (h)	Total salicylate recovered in urine (g)	Recovery (%)				
					SUA	SA	SAG	GA	ASA
V.Y.	F	5.0	24	1.87	47.22	33.67	15.70	3.42	—
H.W.	F	6.0	36	1.88	76.16	0.88	22.18	0.78	—
S.B.	M	9.0	22	3.01	50.78	10.90	29.50	8.82	—
B.F.	F	9.0	60	3.16	52.62	8.22	23.01	9.31	—
R.G.	F	13.5	42	2.52	58.02	14.36	18.38	9.25	—
L.C.	F	13.5	48	3.77	26.39	64.85	2.58	4.96	1.22
B.C.	F	15.0	54	9.41	28.62	34.13	27.99	7.60	1.66
M.H.	M	19.2	42	4.63	45.94	7.01	26.07	19.56	1.41
N.H.	M	21.0	10	5.10	32.39	56.11	7.70	3.03	0.73
K.B.	M	25.5	36	4.37	52.13	22.26	16.38	8.58	0.65
D.D.	F	30.0	72	5.67	49.84	11.76	24.12	13.74	0.53
N.M.	M	60.0	72	8.13	47.90	7.81	27.08	15.34	1.87
Mean					47.33	22.66	20.06	8.70	1.15
S.D.					13.58	20.51	8.32	5.44	0.53

*As claimed by the patient.
**This was the period of stay in the Unit.

stance(s). In the previous work of Reidl [8], separation of ASA and its metabolites was effected at 45°C. In this work, a room temperature of 18 ± 1°C, the usual temperature of operation, always gave good resolution of the peaks for all of the compounds.

This direct injection method proved adequate in the analysis of urine from patients with self-administered ASA overdose and who participated in the investigation of glycine in the treatment of ASA overdose [11]. The urinary excretion of ASA and the metabolites in twelve of such patients was as shown in Table I.

The method presented is simple, fast, accurate and quite adequate for the simultaneous analysis of ASA, SA, SUA and GA in urine.

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