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Note

High-performance liquid chromatographic method with electrochemical detection for the concomitant assay of sulphadiazine, sulphamerazine and sulphapyridine in plasma

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Sulphadiazine (SD), sulphapyridine (SP) and sulphamerazine (SM) (Fig. 1) are often co-administered in antibiotic preparations widely prescribed in the treatment of bacterial enteritis in sheep and cattle (NuvamideTM and StrinacinTM). For the treatment of systemic infection plasma concentrations of total sulphonamide between 60 and 150 $\mu\text{g}/\text{ml}$ have been suggested [1]. Measurement of plasma concentrations will be required to ensure effective therapy and also to determine withdrawal periods in farm animals.

A great many chromatographic methodologies have been reported for the assay of sulphonamide drugs in biological fluids and tissues [2-11] but none allow the concomitant assay of these three sulphonamides in plasma and none report the use of post-column electrochemical detection. For molecules with functional groups which are oxidisable, electrochemical detection is often more sensitive and specific than UV detection especially at low wavelengths.

In the present paper, we describe a simple specific high-performance liquid chromatographic (HPLC) method for the assay of each of these sulphonamides concurrently. This assay has been applied to the study of the plasma pharmacokinetics of these compounds in lambs.

EXPERIMENTAL

Chemicals and reagents

SD, SP, SM and sulphadimidine (SDM), which was used as an internal standard in the assay procedure, were all synthesised at May & Baker (Dagenham, U.K.). Dipotassium hydrogenphosphate was purchased from Aldrich (Gil-

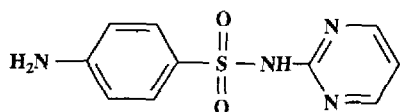
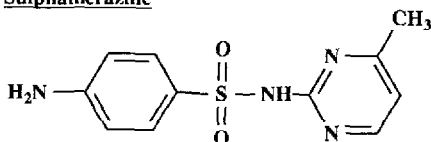
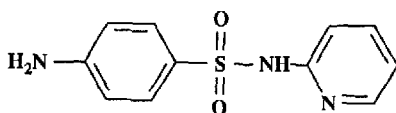
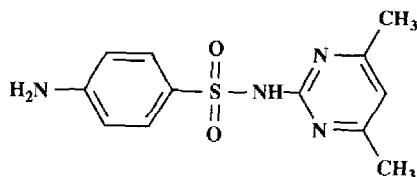
Sulphadiazine**Sulphamerazine****Sulphapyridine****Sulphadimidine**

Fig. 1. Structures of sulphadiazine, sulphamerazine, sulphapyridine and sulphadimidine, the internal standard.

lingham, U.K.). Acetonitrile, ethyl acetate, methanol (HPLC grade), isopropyl alcohol (analytical grade), sodium dihydrogenphosphate and orthophosphoric acid (laboratory grade) were all from May & Baker. Nuvamide and Strinacin are products and trade marks of May & Baker.

Chromatography

The HPLC system consisted of a constant-flow pump (ConstaMetric III G, LDC, Milton Roy, Riviera Beach, FL, U.S.A.), an automated sample injector (WISP 710, Waters Assoc., Milford, MA, U.S.A.), an electrochemical detector (LCA 15, EDT Research, London, U.K.) operated at a potential of +1.0 V and a dual-pen chart recorder (PM 8252, Philips, Eindhoven, The Netherlands). The

stationary phase was 5- μm Spherisorb ODS1 packed in a 250 mm \times 4.6 mm I.D. column (HPLC Technology, Wilmslow, U.K.). Analyses were performed using a mobile phase of aqueous 20 mM sodium dihydrogenphosphate-methanol-acetonitrile (91:4:5, v/v/v) at a flow-rate of 1.5 ml/min and at room temperature (ca. 21 $^{\circ}\text{C}$).

Extraction procedure

To plasma aliquots (1 ml) in glass test-tubes were added SDM, the internal standard (5 μg for drug concentrations up to 20 $\mu\text{g}/\text{ml}$ or 20 μg for drug concen-

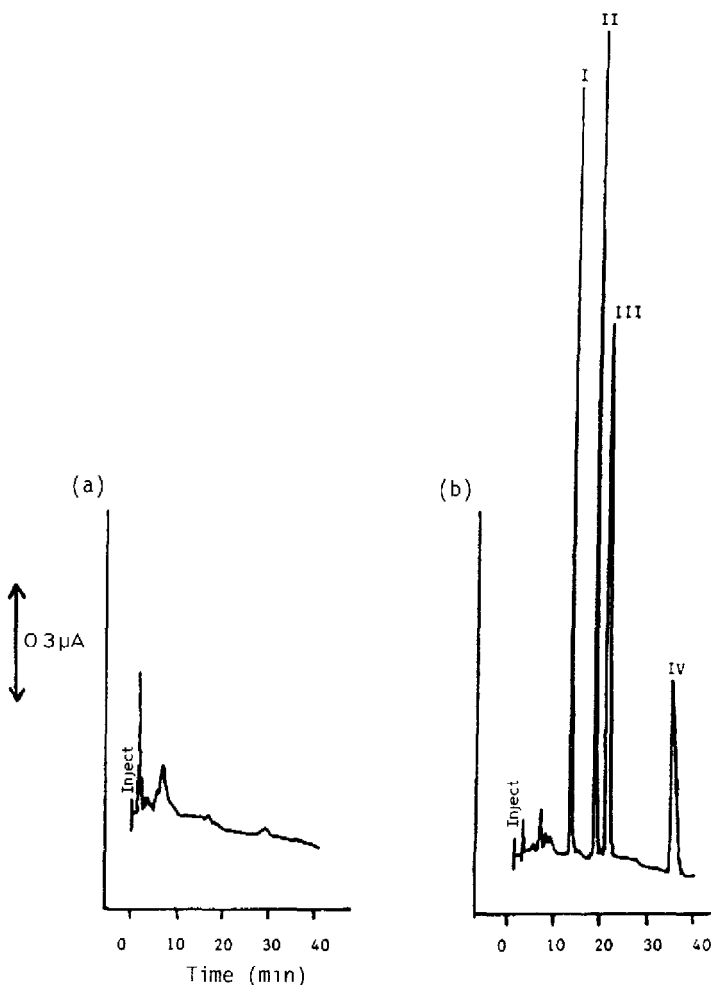


Fig 2. Typical chromatograms of extracts of (a) unmodified blank plasma and (b) blank plasma containing added sulphadiazine, sulphamerazine, sulphapyridine and sulphadimidine at a concentration of 5 $\mu\text{g}/\text{ml}$. Chromatographic conditions: column: 250 mm \times 4.6 mm S5 ODS1; mobile phase: methanol-acetonitrile-20 mM sodium dihydrogenphosphate (4.5:91, v/v/v); flow-rate: 1.5 ml/min; detector: +1.0 V, 3 μA f.s.d.; chart speed: 60 mm/h, pen sensitivity: 0.2 V f.s.d. Peaks: I = sulphadiazine; II = sulphapyridine; III = sulphamerazine; IV = sulphadimidine, internal standard.

TABLE I

ACCURACY AND REPRODUCIBILITY DATA FOR THE DETERMINATION OF SULPHADIAZINE, SULPHAMERAZINE AND SULPHAPYRIDINE IN PLASMA

Compound	Added ($\mu\text{g/ml}$)	Found (mean \pm S.D., $n=5$) ($\mu\text{g/ml}$)	Coefficient of variation (%)
Sulphadiazine	5	5.38 ± 0.6	11.0
	20	21.35 ± 0.47	2.2
	60	55.73 ± 3.7	6.6
Sulphapyridine	5	5.52 ± 0.65	11.8
	20	21.73 ± 0.80	3.7
	60	55.63 ± 5.21	9.3
Sulphamerazine	5	5.66 ± 0.58	10.3
	20	21.78 ± 0.68	3.1
	60	58.57 ± 3.87	6.6

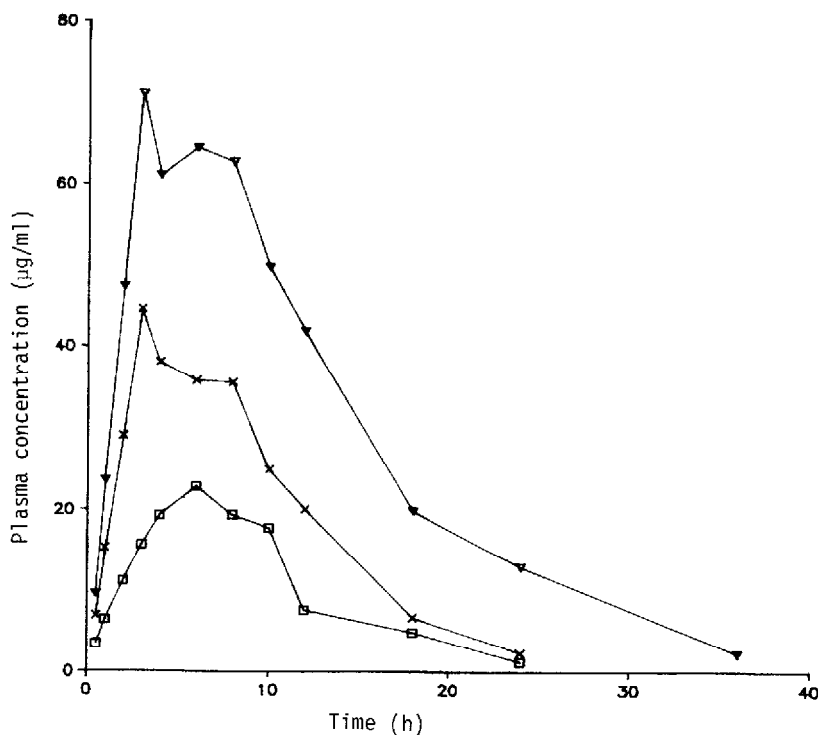


Fig. 3 Plasma concentration versus time profiles of SD (□), SP (×) and SM (▽) following an oral dose of 50 mg/kg in Nuamide suspension to a lamb.

trations $> 20 \mu\text{g/ml}$ in a $0.1 \mu\text{g/ml}$ methanolic solution), 10 mM dipotassium hydrogenphosphate (adjusted to pH 6.8 with orthophosphoric acid, 1 ml) and 10 ml of 2% isopropyl alcohol in ethyl acetate (v/v). After mixing by vortex for 10 s, The tubes were centrifuged for 10 min at approximately 1000 g. The organic phase was removed and evaporated to dryness at 60°C under a stream of nitrogen. The

dry residues were reconstituted in methanol (100 μ l) and aliquots (5–50 μ l depending upon drug concentration) were injected into the chromatograph.

Standard calibration graphs run with each set of unknowns were obtained by analysis of plasma samples to which increasing quantities of SD, SP and SM were added. Nine concentrations (1, 5, 10, 15, 20, 40, 60, 80 and 100 μ g/ml) were analysed on each occasion. Linear correlations between concentration and peak-height ratio of each drug to internal standard yielded in all cases a correlation coefficient (r) of at least 0.999.

RESULTS AND DISCUSSION

Typical chromatograms obtained from lamb plasma before and after addition of SD, SP and SM, together with SDM, the internal standard, are shown in Fig. 2. The chromatographic peaks for SD, SP and SM were well resolved with retention times of 13.0, 18.5 and 21.5 min, respectively. The retention time of SDM, the internal standard, was 36 min. The efficiency of extraction of SD, SP, SM and SDM were calculated to be 70, 61, 49 and 66%, respectively. The intra-assay accuracy and precision for the procedure was determined by replicate analysis ($n=5$) of control plasma to which SD, SP and SM had been added at three levels, 5, 20 and 60 μ g/ml (Table I). These were chosen as being representative levels for lamb or calf plasma after administration of Nuvamide or Strinacin. The limit of quantification was about 1 μ g/ml. This sensitivity was achieved by use of electrochemical detection, after it was found that UV detection was unsuitable due to chromatographic interference from endogenous plasma substances. Furthermore, injection of methanolic solutions of SD, SP and SM showed that UV absorbance at the maximum absorption wavelength (approximately 290 nm) was less sensitive than oxidation at a potential of +1.0 V (in terms of signal-to-noise ratio for a given amount of analyte).

This method has been applied to studies of the plasma pharmacokinetics of SD, SP and SM after their co-administration to lambs. Typical concentration versus time profiles for SD, SP and SM after administration of these sulphonamide drugs (50 mg/kg) as Nuvamide suspension to lambs are shown in Fig. 3.

In conclusion a new HPLC procedure for the concomitant assay of SP, SD and SM in lamb or calf plasma is described. This methodology has been successfully applied to studies of the plasma pharmacokinetics of these drugs in lambs. Furthermore, the described procedure has also been found to be similarly appropriate for the assay of these drugs in calf plasma.

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