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Note

Determination of paracetamol, salicylic acid and acetyl salicylic acid in serum by high-performance liquid chromatography

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Salicylic acid derivatives and paracetamol are commonly prescribed as analgesics. Although these drugs are normally administered singly they may be given together in the treatment of certain conditions such as rheumatoid athritis. The blood concentration of salicylate correlates well with the pharmacological actions and adverse effects of this drug[1]. The therapeutic levels of both aspirin (acetylsalicylic acid) and paracetamol are however frequently very close to and often overlap with those concentrations at which toxic manifestations may occur. There are also numerous cases of accidental or self-inflicted poisoning involving the ingestion of one or both of these drugs[2]. In cases of overdose it is important to determine quickly and accurately blood concentrations of salicylate and paracetamol so that adequate treatment may be initiated[3].

Whilst many high-performance liquid chromatographic (HPLC) methods are available for the analysis of either salicylate[4,5] or paracetamol[6] few systems have been described which allow a sumultaneous assay of both compounds[7]. We have developed a simple rapid method for the analysis of salicylic acid, acetylsalicylic acid and paracetamol in serum employing an isocratic HPLC assay with chloroform–acetonitrile (60:40) as the extraction solvent.

EXPERIMENTAL

HPLC conditions

An Altex Hitachi 100-10 pump was used to deliver solvent at 1 ml/min. The eluent was monitored at 234 nm with a Hitachi 100-10 variable-wavelength UV detector. The column was a 25 cm × 4.5 mm I.D. Spherisorb 5-μm ODS (Jones Chromatography) fitted with a Rheodyne injection system incorporating a 20-μl loop. Separation was achieved with a mobile phase of acetonitrile–methanol–water (25:10:65) to pH 3.0 with orthophosphoric acid.

Sample preparation

Human serum was prepared by centrifugation and kept frozen at −20°C until

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required for analysis. Serum (0.5 ml) was acidified with hydrochloric acid (0.1 ml) and extracted by shaking with 2-ml aliquots of an extraction solvent consisting of chloroform–acetonitrile (60:40). The serum sample was extracted three times and the extracts combined. The organic phase was evaporated to dryness under a stream of nitrogen and the residue redissolved in 100 μl of eluent. Samples of 20 μl were injected onto the column.

Straight-line calibration graphs were obtained for salicylic acid, acetyl salicylic acid and paracetamol based on peak area measurements for concentrations of 10, 25, 40, 60 and 120 μg/ml by addition of the drugs to control serum and extraction by the procedure described above. Each point was taken as the average of two determinations.

Materials

Salicylic acid and acetylsalicylic acid were obtained from Thornton and Ross (B.P. grade) and paracetamol from Dista. All solvents used were HPLC grade (Rathburn Chemicals). All other chemicals were analytical reagent grade. Blood samples were obtained from healthy volunteers after oral administration of aspirin and paracetamol.

RESULTS AND DISCUSSION

The use of chloroform–acetonitrile (60:40) as the extraction solvent yielded clean extracts with betweeen 97 and 100% recovery for each of the three compounds under investigation. The acetonitrile in the extraction solvent acts as a protein precipitating agent and was found to give cleaner extracts than any other solvents tested[8].

Straight-line calibration graphs were determined for each of the compounds and found as $y = 1.3x - 2.69$, $y = 0.2x + 0.106$ and $y = 0.537x - 0.109$ for paracetamol, acetylsalicylic acid and salicylic acid, respectively, with correlation coef-

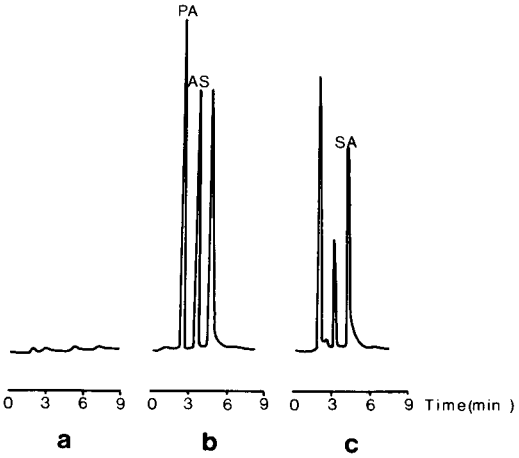

Fig. 1. (a) Chromatogram of a blank serum extract. (b) Serum extract spiked with paracetamol, acetylsalicylic acid and salicylic acid. (c) Serum extract from an individual taking aspirin and paracetamol. Peaks: PA = paracetamol; AS = acetylsalicylic acid; SA = salicylic acid.

ficients of 0.9979, 0.9995 and 0.9985. Limits of detection (signal-to-noise ratio >2) were approximately 2 ng on-column for salicylic acid and acetylsalicylic acid and 1 ng for paracetamol.

The chromatogram of a blank serum extract (from a control subject who had not taken paracetamol or salicylates) is shown in Fig. 1a. The chromatogram of an extract of a serum sample spiked with paracetamol, acetylsalicylic acid and salicylic acid is represented in Fig. 1b and that of an individual taking aspirin and paracetamol is shown in Fig. 1c. Aspirin is hydrolysed in the blood to salicylic acid with a half-life of 10–20 min. No problems with breakdown of aspirin to salicylic acid were encountered using the described extraction procedure.

The described assay has the advantages of giving clean extracts from serum, good resolution and high sensitivity. It provides a rapid method for the routine analysis of paracetamol and salicylate levels in hospitalised patients.

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