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Note

Determination of salicylazosulphapyridine and sulphapyridine in plasma using high-performance liquid chromatography

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Salicylazosulphapyridine (Fig. 1a) is a drug used for the treatment of infectious colon diseases, and sulphapyridine (Fig. 1b) is formed from it in the body as a metabolite. Present methods[1,2] for the determination of these two substances in plasma are based on spectrophotometry of plasma extracts, and with sulphapyridine a derivativatization step is necessary before the measurement is made. The methods here proposed utilize high-performance liquid chromatography of plasma extracts, which gives a higher specificity and sensitivity. Salicylazosulphapyridine is analysed by an external standard method. This is considered to be sufficiently accurate as the extraction is quantitative. Sulphapyridine, however, showed a varying extraction yield and an internal standard is therefore preferred.

EXPERIMENTAL

Systems

The liquid chromatograph consisted of a Model M600 pump and a Model 440 detector from Waters Assoc. (Milford, Mass., U.S.A.). Salicylazosulphapyridine was analysed with the detector operating at 365 nm and the column (0.6 m × 2 mm I.D., stainless steel) dry-packed with Bondapak C_{18}/Corasil (Waters Assoc.). The

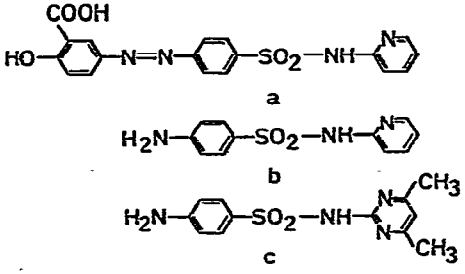

Fig. 1. Structures of (a) salicylazosulphapyridine, (b) sulphapyridine and (c) sulphamethazine.

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mobile phase consisted of 0.01 *M* phosphate buffer (pH 7.7) mixed with 17% of acetonitrile. The system was operated at a flow-rate of 1 ml/min at 23° (room temperature). When sulphapyridine was analysed, the detector was operated at 280 nm and the column (0.15 m × 4.6 mm I.D., stainless steel) was slurry-packed with Li-Chrosorb SI 60, 5 μm (Merck, Darmstadt, G.F.R.). The mobile phase was chloroform–methanol–35% ammonia solution (96.5:3:0.5). The system was operated at a flow-rate of 1 ml/min at 23°.

Extraction of salicylazosulphapyridine

A 0.5-ml volume of plasma was transferred into a 10-ml screw-capped tube, 1 ml of 1 *M* hydrochloric acid added and the mixture extracted with 4 ml of isoamyl acetate (15 min on a shaking board) and then centrifuged for 10 min at 500 *g* (Wifug Doctor). A 3-ml volume of the organic phase was transferred into another tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 500 μl of the eluent mixture (acetonitrile–phosphate buffer). After filtration through a pasteur pipette containing glass-wool, 40 μl were injected into the liquid chromatograph.

Extraction of sulphapyridine

To 0.5 ml of plasma 100 μl of internal standard solution were added (100 μg/ml). This mixture was then mixed with 0.5 ml of 1 *M* acetate buffer (pH 4.7) and *ca.* 0.25 g of sodium chloride. The mixture was extracted (15 min on a shaking board) with 2.5 ml of 4-methyl-2-pentanone. The organic phase was transferred into another tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in the eluent (250 μl) and, after filtration as above, an aliquot (40 μl) was injected into the liquid chromatograph.

RESULTS

Salicylazosulphapyridine

Fig. 2a shows the chromatogram obtained from the analysis of a plasma sample containing 8 μg of salicylazosulphapyridine, and Fig. 2b the chromatogram obtained when a blank plasma sample was analysed in the same way. No interfering compounds were extracted from plasma with isoamyl acetate. Reversed-phase liquid chromatography was chosen because it was considered to be easy and economical to operate with this substance. The pH of the eluent had to be just below the upper limit for the stationary phase (pH 8). A reduction in the pH value resulted in a longer retention time. The use of micro-particle (5–10 μm) reversed-phase columns with this substance resulted in severe tailing of the peak.

The output of the UV detector is linear in absorbance units, and the area under the peak is proportional to the concentration. The calibration graph (Fig. 3) was constructed by analysing samples to which different amounts of salicylazosulphapyridine had been added (0.5–10 μg/ml). The peak areas were plotted *versus* the concentration. The standard deviation was determined by analysing seven plasma samples containing 3 μg/ml of salicylazosulphapyridine. The standard deviation was 4%. The minimal detectable amount (signal-to-noise ratio = 2) was 5 ng.

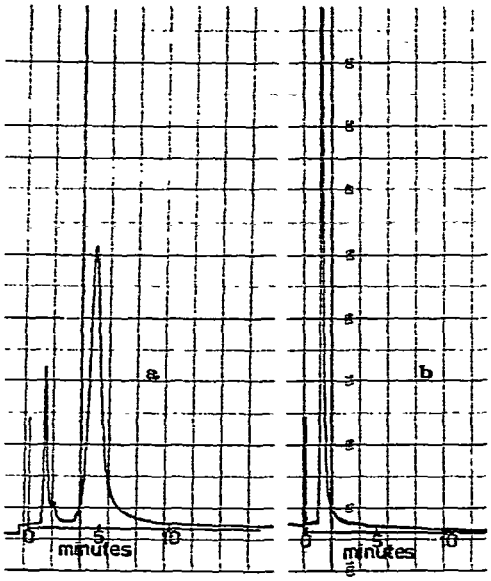

Fig. 2. HPLC determination of salicylazosulphapyridine in plasma. (a) Plasma sample containing salicylazosulphapyridine (8 μg/ml); (b) blank plasma sample [amplification 2.5 times higher than in (a)].

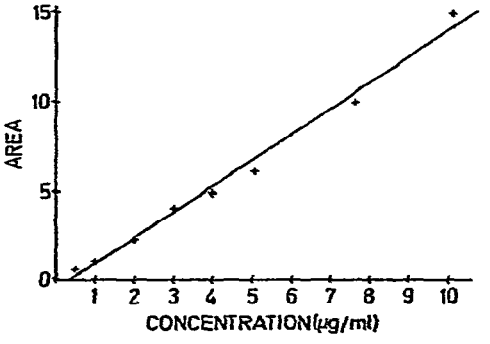

Fig. 3. Calibration graph for salicylazosulphapyridine.

Sulphapyridine

On extraction from plasma, sulphapyridine showed different yields on different occasions. Therefore, sulphamethazine (Fig. 1c), a substance showing similar behaviour, was selected as an internal standard. A mixture of sulphapyridine and this internal standard (1:1) was added to drug-free plasma samples and extracted at pH values ranging from 3 to 6. This was done with several batches of plasma. Despite different yields in these extractions, analysis according to the proposed method resulted in approximately the same ratio between the two compounds.

N^4-Acetylsulphapyridine, which is formed from sulphapyridine in the body, will be co-extracted from plasma with 4-methyl-2-pentanone. However, this metabolite does not interfere in the analysis as it is eluted with the front peak in the chromato-

gram. The peaks corresponding to N^4-acetylsulphapyridine and sulphapyridine will change positions in the chromatogram if the ammonia solution is omitted from the eluent mixture.

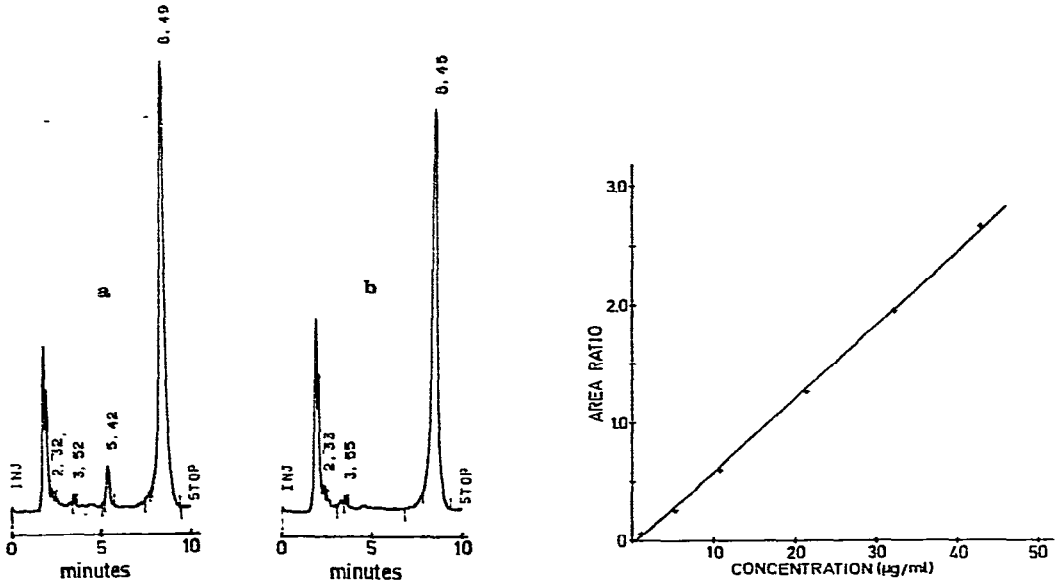

Fig. 4. HPLC determination of sulphapyridine in plasma. (a) Plasma sample containing sulphapyridine (1 µg/ml); (b) blank plasma containing internal standard.

Fig. 5. Calibration graph for sulphapyridine/internal standard.

The chromatogram in Fig. 4a was obtained when a plasma sample containing 1 µg/ml of sulphapyridine was analysed, and Fig. 4b the chromatogram obtained from a blank plasma sample. The calibration graph (Fig. 5) was constructed by analysing samples to which different amounts of sulphapyridine had been added (1.1–43 µg/ml). The peak area ratios (sulphapyridine/internal standard) were plotted *versus* the concentration. The standard deviation was determined by analysing six plasma samples containing 21.4 µg/ml and six samples containing 5.35 µg/ml of sulphapyridine. The standard deviations were 2.9 and 3.6%, respectively. The minimal detectable amount was 700 pg.

REFERENCES

1 K.-A. Hansson and M. Sandberg, *Acta Pharm Suecica*, 10 (1973) 107.
2 K.-A. Hansson and M. Sandberg, *Acta Pharm. Suecica*, 10 (1973) 87.