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Letter to the Editor

Determination of sulphapyridine and its N^4-acetyl metabolite in plasma using liquid chromatography

Sir,

Recently, a method for the determination of sulphapyridine using a system based on silica gel was published[1]. When the column packing used for the determination of sulphapyridine had to be replaced with a new batch of silica, it was not possible to obtain a chromatogram showing the same degree of separation as before (several batches of silica were tried). The conclusion must be drawn that the batch of silica used initially could not have been pure. To be able to continue the analysis of sulphapyridine it was necessary to change the eluent to chloroform–acetonitrile–methanol–35% ammonia (65.5:30:4:0.5) at a flow-rate of 1 ml/min. In other respects the chromatographic system and extraction method remained unchanged. This new solvent system made it possible to effect a simultaneous determination of sulphapyridine and its N^4-acetyl metabolite. Fig. 1 shows typical chromatograms.

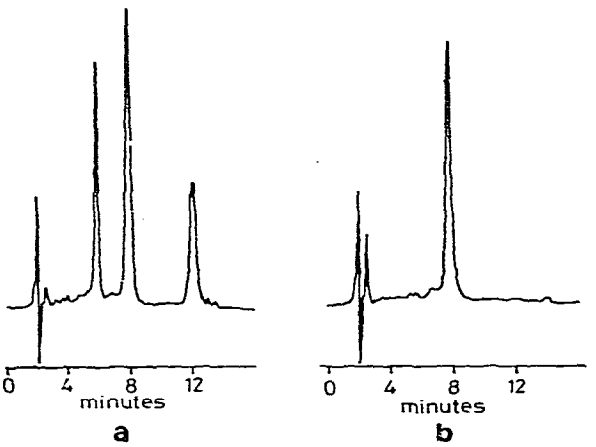

Fig. 1. Chromatograms of (a) plasma sample containing 10.5 μg/ml of sulphapyridine, 16.8 μg/ml of N^4-acetylsulphapyridine and internal standard (sulphamethazine) and (b) a blank plasma sample containing only internal standard. The retention times for sulphapyridine, sulphamethazine and N^4-acetylsulphapyridine were 5.6, 7.2 and 11.7 min, respectively.

When a plasma sample was analysed several times according to the method, the recovery of sulphapyridine sometimes varied considerably. The internal standard (sulphamethazine) behaved in the same manner, *i.e.*, the ratio between the two peaks resulting from sulphapyridine and sulphamethazine remained the same during the

repeated analyses. The metabolite, N^4-acetylsulphapyridine, did not show this variation. This difference may be due to varying adsorption losses caused by the primary amino groups in sulphapyridine and sulphamethazine and slight variations in the work-up procedure.

A calibration graph was constructed by analysing according to the above method samples to which had been added known amounts of sulphapyridine and plotting the peak-height or -area ratios of sulphapyridine to internal standard against concentration. To the samples had also been added different amounts of N^4-acetylsulphapyridine and the calibration graph for the determination of this substance was constructed by plotting the resulting peak heights or areas against concentration. The standard deviation obtained in the analysis of ten samples to which had been added 15 μg/ml of sulphapyridine was 2.7% and that for ten samples containing 15 μg/ml of N^4-acetylsulphapyridine was 2.5%.

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1 K. Lanbeck and B. Lindström, *J. Chromatogr.*, 154 (1978) 321.

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