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Note

Detection of sulphonamides in urine by pyrolysis–gas chromatography–mass spectrometry

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We have previously described the pyrolysis–gas chromatography–mass spectrometry of a series of medicinal sulphonamides[1]. It was shown that each sulphonamide yielded a unique pyrogram and the principal mode of decomposition was fission about the labile sulphonamido group. Fragmentation yielded aniline from all medicinal sulphonamides, and a heterocyclic amine which was characteristic of the sulphonamide under test. Here we wish to describe the detection of these drugs in urine samples and to show that on the basis of fragmentations previously described, the identification of sulphonamides in urine may be accomplished using pyrolysis methods.

EXPERIMENTAL

Preparation of samples

An aliquot of urine (≈25 ml), frozen until required, was freeze dried. The residue was taken up in a small amount of water (0.5 ml) and the resulting paste was coated onto the rotating pyrolysis wire[2]. Wires were then placed in a desiccator, under vacuum, for 30 min to yield a firmly adhering coat.

Apparatus and conditions

A Pye Curie Point pyrolyser was connected to a Pye GCV gas chromatograph (dual columns and flame ionisation detector) or a Pye 104 gas chromatograph interfaced to a Micromass 12B mass spectrometer. Pyrolysis was performed at 770°, maintained for 5 sec and chromatography was carried out using 1.5 m × 4 mm I.D. columns packed with 8% Carbowax 20M and 2% KOH on Chromosorb W AW DMCS (100–120 mesh). The temperature was programmed from 100° (5 min hold) at 5°/min up to 245° (8 min hold). The injection port was held at 275° and the detector oven at 350°. The air pressure was maintained at 0.5 kg/cm², the hydrogen at 1.4 kg/cm² and a flow-rate of 50 ml/min (nitrogen, helium) was used. Mass spectra were collected with an ionisation energy of 22 eV, a trap current of 100 μA and an accelerating voltage of 4 kV.

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RESULTS AND DISCUSSION

The pyrolysis of an aliquot of the total solids obtained from lyophilised urine yielded a characteristic pyrogram and typical examples may be seen in Figs. 1 and 2. Samples taken from different individuals or samples taken at different intervals during the day showed no great variation in appearance. The overall pyrograms were surprisingly simple at attenuations necessary for the detection of excreted drugs in view of the complex mixture of biochemicals present[3]. A control sample was established using the 24-h total solids obtained from a normal individual. Pyrolysis of the urine solids from a patient undergoing sulphonamide medication yielded a pyrogram in which the peaks due to the urine components could be established by comparison

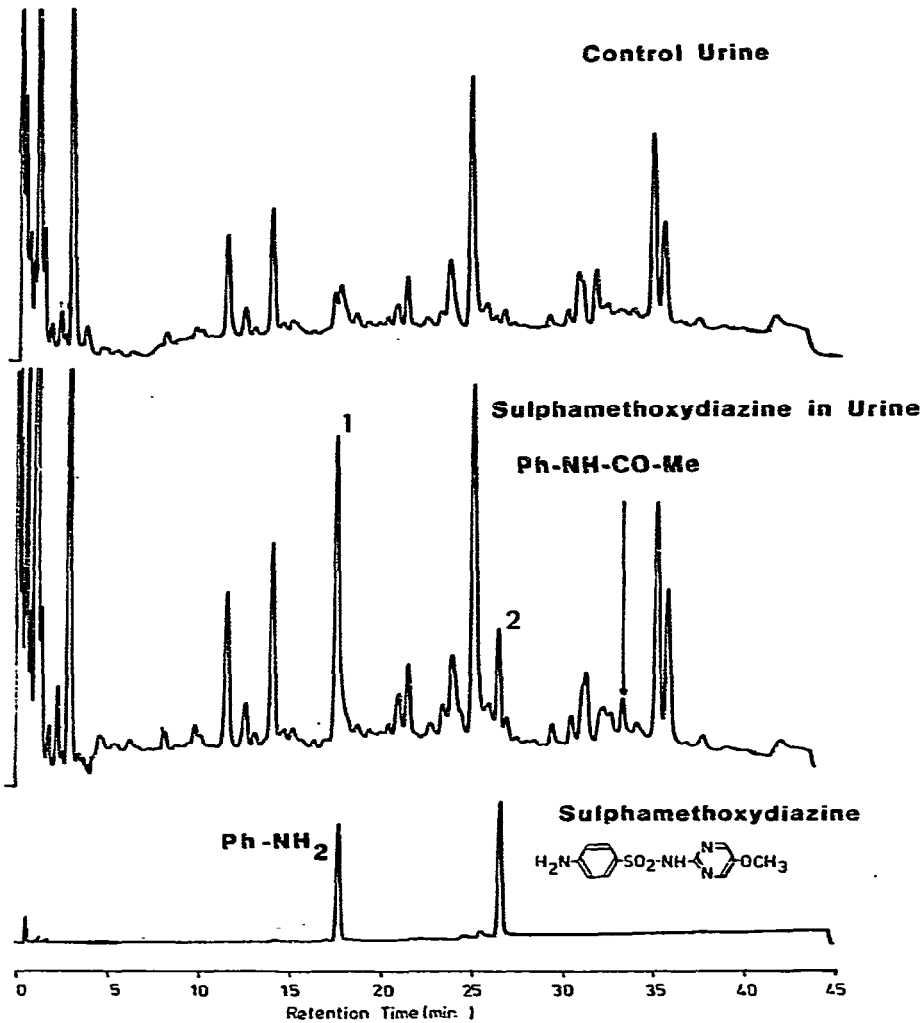

Fig. 1. Detection of sulphamethoxydiazine in urine showing the presence of aniline (1), 2-amino-5-methoxypyrimidine (2) and acetanilide.

with the control sample. In addition, it was seen that the fragmentation of the sulphonamide also present in this sample was essentially identical to that found in the pure drug[1,4]. Thus the sulphonamide could readily be identified in the urine by the presence of the characteristic pyrolysis fragments superimposed upon those of normal urine. Furthermore the presence of the N-acetyl metabolite could also be detected. The pyrolysis mode of this component was similar to that of the parent sulphonamide and yielded the characteristic heterocyclic amine and acetanilide.

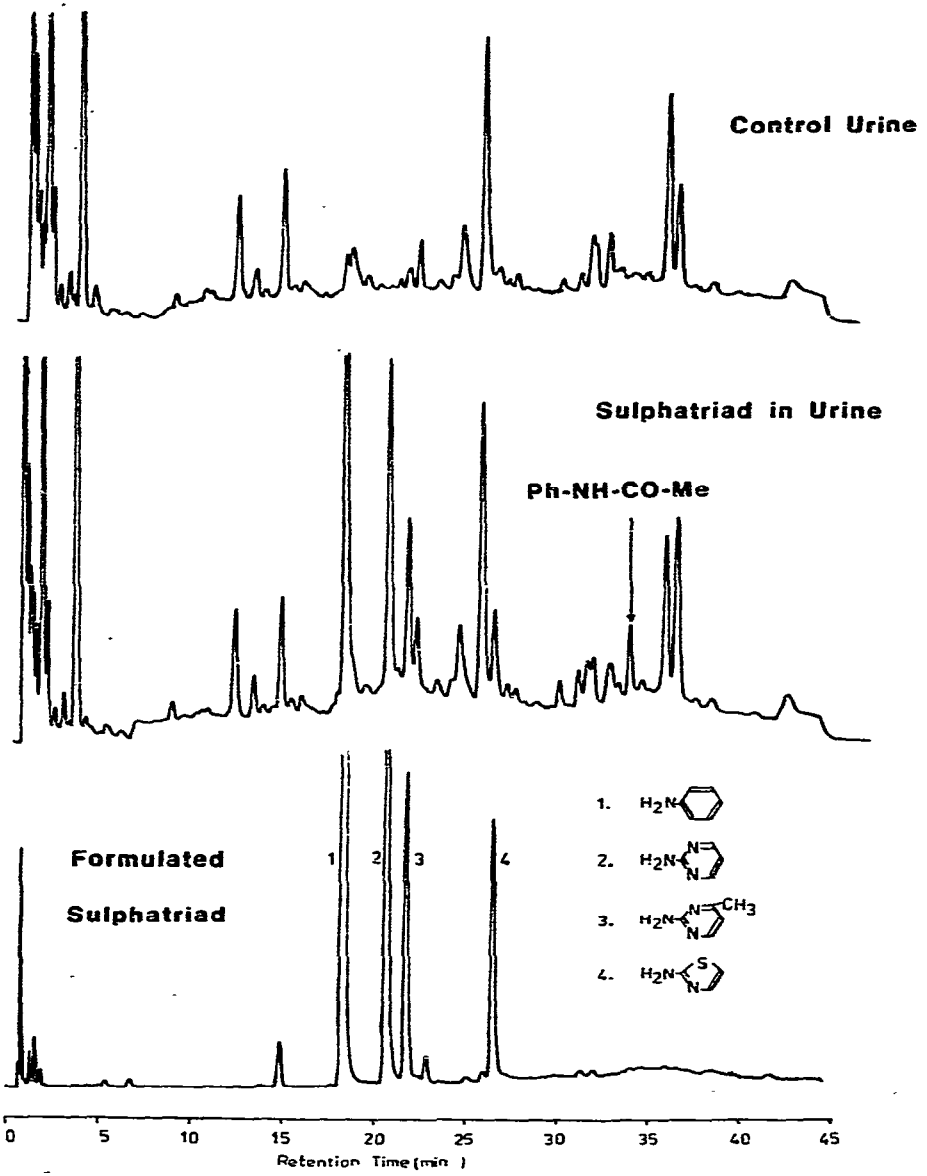

Fig. 2. Detection of sulphatriad in urine showing the presence of aniline (1), 2-aminopyrimidine (2), 2-amino-4-methylpyrimidine (3), 2-aminothiazole (4), and acetanilide.

The factors determining the sensitivity of this method are unusual. The principal consideration is not the detection limit of the sulphonamide (under appropriate conditions 50 ng may be detected and normally 50 μg are pyrolysed) but rather the proportion of drug to total solids which allows the detection of the drug against the urine background. At levels maintained during the clinical use of these drugs, the detection was found to pose no problems. Fig. 1 records the pyrogram obtained from sulphamethoxydiazine. This is relatively low-dosage sulphonamide[5] (maintenance dose 500 mg daily) and a lower than normal attenuation is required in this analysis. The urine background is more significant than in other examples but nevertheless the diagnostic features (*i.e.* aniline, 2-amino-5-methoxy pyrimidine and acetanilide) are clearly visible. The acetanilide is a rather weakly intense peak in this pyrogram as the N-acetyl metabolite is a minor component ($\approx$20%).

This system also enables the presence of mixed sulphonamides to be determined. Fig. 2 shows the pyrogram obtained from the urine of a patient undergoing treatment with sulphatriad. This is a mixed sulphonamide preparation which contains sulphadiazine, sulphamerazine and sulphathiazole to ensure a duration of action. Aniline and acetanilide are again present and act as internal standards. 2-Aminopyrimidine, 2-amino-4-methylpyrimidine and 2-aminothiazole, respectively, are derived from the sulphonamides and enable the identification to be achieved. Quantitative pyrolysis has already been used in the analysis of biologically important molecules and the application of these techniques to the above results may prove of value[6–8].

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