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Note

Electron-capture gas chromatography of sulphapyridine and its N^4-acetyl metabolite in serum after extractive methylation

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The efficient use of certain drugs such as procainamide, hydralazine or isoniazide requires the classification of patients as slow or rapid acetylators[1,2]. Sulphapyridine has been used as a test substance in acetylation phenotyping[3]. The commonly used assay of sulphonamides[4] is based on the isolation and hydrolysis of the metabolite followed by derivatization and spectrophotometric determination[5,6]. There is a need for a simpler procedure for the simultaneous determination of sulphapyridine and its N^4-acetyl metabolite in biological samples.

The gas chromatographic analysis of low concentrations of sulphonamides is hampered by their polar character, which causes adsorption losses in the gas chromatographic system. Conditions for the derivatization of sulphonamides with perfluoroacyl anhydrides or pentafluorobenzyl bromide for analysis by electron-capture gas chromatography have been studied[7]. Sulphonamide drugs have been determined by gas chromatography with electron-capture detection after methylation[8,9], and after methylation and subsequent heptafluorobutyrylation of the 4-amino group[10,11].

This paper describes the simultaneous determination of sulphapyridine and N^4-acetylsulphapyridine by electron-capture gas chromatography after extractive methylation. The structural requirements for obtaining low minimum detectable concentrations of sulphonamides are discussed.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 5710A gas chromatograph with a constant-current ^{63}Ni electron-capture detector was used. The glass column (120 × 0.2 cm I.D.) was filled with 5% OV-17 on Gas-Chrom Q (80–100 mesh). The carrier gas was argon + 5% methane at a flow-rate of 40 ml/min.

Chemicals and reagents

Sulphapyridine and sulphamerazine (internal standard) were of pharmacopoeial quality. The N^4-acetyl and 5′-hydroxy metabolites were kindly supplied by

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Pharmacia (Uppsala, Sweden). Sulphonamides used as model compounds were prepared as described previously[7]. The methyl derivative of N-decylmethanesulphonamide was synthesized by using methyl iodide in 20% potassium hydroxide in methanol.

Methyl iodide was obtained from E. Merck (Darmstadt, G.F.R.) and tetrabutylammonium hydrogen sulphate from Labkemi (Gothenburg, Sweden). Methylene chloride (Merck) and ethyl acetate (Fisher, Fair Lawn, N.Y., U.S.A.) were of analytical-reagent grade.

Methods

Determination of minimum detectable concentrations. Methyl derivatives of the sulphonamides were prepared at concentrations in the milligrams per millilitre range and diluted with ethyl acetate to concentrations suitable for electron-capture gas chromatography. Minimum detectable concentrations were calculated from the amount that gave a signal three times the noise[12].

Determination of sulphapyridine and N^4-acetylsulphapyridine in serum. The serum sample (0.1 ml) was mixed with 0.1 ml of aqueous internal standard solution (sulphamerazine, 2 μg/ml) and 1.0 ml of tetrabutylammonium (0.1 *M*) in carbonate buffer of pH 10 (0.5 *M*). After addition of 1.0 ml of 0.32 *M* methyl iodide (2%) in methylene chloride, the mixture was shaken for 25 min. After centrifugation, 0.25 ml of the organic phase was evaporated in a stream of nitrogen. Ethyl acetate (0.05 ml) was added and 1 μl was taken for analysis.

RESULTS AND DISCUSSION

Extractive methylation of sulphonamides

Extractive alkylation has found widespread use in the derivatization of organic acids prior to gas chromatographic analysis[13–16]. Extractive methylation of sulphonamides using 0.1 *M* tetrabutylammonium in buffer of pH 10 has been studied[17]. The time course of the methylation of sulphapyridine, N^4-acetylsulphapyridine and sulphamerazine with 0.32 *M* methyl iodide in methylene chloride is illustrated in Fig. 1.

The derivatives were found to be stable in the reaction mixture for at least 6 h. Very long reaction times (greater than 24 h) or a pH of 13 in the aqueous phase was found to give some methylation of the 4-nitrogen group of the acetyl metabolite.

The sulphonamides were methylated at the N^1-position, as confirmed by mass spectral analysis[17].

Minimum detectable concentrations of sulphonamides

The minimum detectable concentrations of methylated sulphapyridine, N^4-acetylsulphapyridine and sulphamerazine were in the range $4 \cdot 10^{-16}$–$12 \cdot 10^{-16}$ mole/sec (Table I). This corresponds to a minimum detectable amount of about 10 pg injected ($N = 1600$, $t_R = 5$ min).

The structural requirement for a high electron-capture response is the presence of two aromatic rings in close conjugation with the sulphonamide group (Table I). If one of the phenyl groups of compound 3 was substituted for a methyl group, the minimum detectable concentration increased considerably (compounds 2 and 4).

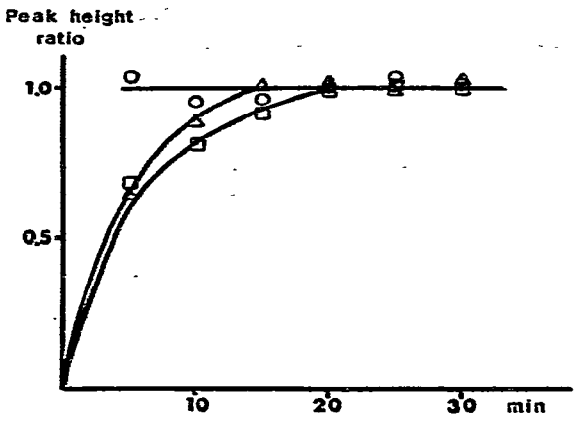
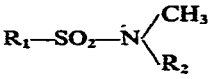
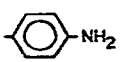
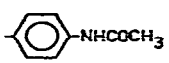
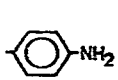


Fig. 1. Extractive methylation of sulphonamide drugs. Organic phase: 0.32 *M* methyl iodide in methylene chloride, 1 ml. Aqueous phase: 0.1 *M* tetrabutylammonium in carbonate buffer, pH 10 (0.5 *M*), 1 ml. Concentration of sulphonamides: 10^{-3} *M*. Internal standard: methylated sulphamethoxydiazine. Temperature: 25°. ○—○, Sulphapyridine; □—□, sulphamerazine; △—△, N^4-acetylsulphapyridine.

TABLE I

MINIMUM DETECTABLE CONCENTRATIONS OF METHYLATED SULPHONAMIDES



No.	*Generic name*	R_1	R_2	*Detector temperature (°C)*	*Minimum detectable concentration (mole/sec × 10^{16})*
1	N-Decyl, N-methyl-methanesulphonamide	CH_3	$C_{10}H_{21}$	270	1000
2	N,N-Dimethylbenzene sulphonamide	C_6H_5	CH_3	270	280
3	N-Methyl, N-phenyl benzenesulphonamide	C_6H_5	C_6H_5	270	8
4	N-Methyl, N-phenyl methanesulphonamide	CH_3	C_6H_5	270	5100
5	Sulphapyridine			350	8
6	N^4-Acetylsulphapyridine			350	12
7	Sulphamerazine			350	4

Application to the determination of serum samples

Sulphapyridine and N⁴-acetylsulphapyridine could be derivatized with the serum sample present. The interference from the biological sample was negligible, as can be seen from the chromatogram in Fig. 2.

Fig. 2. Gas chromatogram from an analysis of sulphapyridine and N⁴-acetylsulphapyridine in serum. Volume of serum sample: 0.1 ml. Column temperature: 290°. Peaks: 1 = sulphapyridine (2 μg/ml in serum); 2 = internal standard (sulphamerazine, 0.2 μg); 3 = N⁴-acetylsulphapyridine (4 μg/ml in serum). Broken line: blank serum.

Selectivity. Sulphapyridine is mainly transformed into the N⁴-acetyl and the 5'-hydroxy metabolites in man[18]. The chromatographic system separated sulphapyridine from the N⁴-acetyl metabolite, as can be seen in Fig. 2. The 5'-hydroxy metabolite was not methylated under the conditions used, and did not appear in the chromatogram.

Precision. The relative standard deviations in the analysis of 0.1-ml serum samples at the 4 μg/ml level of sulphapyridine and the N⁴-acetyl metabolite were 0.8 and 3.4%, respectively ($n = 8$). The corresponding figures at the 1 μg/ml level were 2.3 and 5.4% ($n = 7$).

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