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GAS–LIQUID CHROMATOGRAPHIC DETERMINATION OF SULPHONAMIDES

NORIHIDE NOSE, SUSUMU KOBAYASHI, AKIKO HIROSE and AKINOBU WATANABE
Saitama Institute of Public Health, 639-1 Kamiookubo-higashi, Urawa, Saitama (Japan)
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SUMMARY

Simultaneous qualitative analysis of fourteen sulpha drugs and their individual quantitative determinations have been performed by gas–liquid chromatography on solutions of dimethylformamide dialkylacetal derivatives of the drugs in acetone.

Linear calibration curves were obtained for each sulpha drug under appropriate conditions. The structures of the above derivatives are assumed to be N^1-methyl, N^4-acetal sulphonamides, which can be detected with an electron capture detector with highly sensitive response. Columns used were 10% OV-101, 5% XE-60 and 5% OV-225.

INTRODUCTION

Various sulpha drugs, such as Prontosil, sulphamine, etc., have been synthesized and although their use decreased as more effective antibiotic agents were developed, they have recently been increasingly used.

More than thirty sulpha drugs have been used for medicinal purposes, and methods for their assay include paper chromatography[1–3], thin-layer chromatography[4–7] and spectrofluorimetry[8–11]. Gas–liquid chromatography (GLC) has also been used; volatile derivatives of sulphonamides were prepared by acylation of the amino group with heptafluorobutyric anhydride[12,13], and by iodination after diazotization[14], and GLC of the amino substituents was carried out following acid hydrolysis[15,16].

Meerwein[17,18] prepared dimethylformamide dialkylacetals, which were found to react with amino groups. Using this principle the authors were able to prepare volatile derivatives of sulpha drugs with dimethylformamide dimethylacetal (DMFA) and subjected them to qualitative and quantitative analysis by GLC.

EXPERIMENTAL

Procedure

An acetone solution (1 ml) containing 1–10 μg, of the sulpha drug was placed in a test tube (8 × 60 mm) with a screw cap and 100 μl of DMFA were added; the test tube, with the cap tightly screwed down, was heated at 120° in a liquid paraffin

bath for 60 min. After cooling it, 1–2 μl of the reaction mixture was directly introduced into the GLC column. (1 ml of 1.5 ppm of decachlorobiphenyl or 2 ppm of diethyldithiocarbamic acid 2-benzothiazolyl ester (DDBE) in acetone was added, after the above reaction, as an internal standard.)

Gas chromatography

A Shimadzu GC-4BMPFE gas chromatograph equipped with a flame ionization detector (FID) and an electron capture detector (ECD) was used. The U-shaped glass columns (1 m × 0.3 cm I.D.) used were packed with 10% OV-101 on Chromosorb G HP (80–100 mesh) and operated at 230°, 5% XE-60 on Gas-Chrom Q (80–100 mesh) at 220° and 5% OV-225 on Gas-Chrom Q (80–100) at 240°. The temperature at the injection port was 250° and at the detector 260°. The flow-rate of the carrier gas (nitrogen) was 110 ml/min.

RESULTS AND DISCUSSION

The fourteen sulpha drugs examined in the present work are listed in Table I.

The solvents tried in the reaction of the drugs with DMFA were acetone, acetonitrile, pyridine, methanol and methyl acetate, and the reactivity of the reactants in each of these solvents and without solvent was examined. It was found that the reaction proceeded well in acetone and pyridine, but that the reactivity was approximately 20% lower in the other solvents. Acetone was therefore used in the experiments.

A slight excess of DMFA is usually added to the sample. To obtain an adequate amount of DMFA, 1 ml of a 10 μg sulpha drug solution was mixed with 50, 100, 150 and 200 μl of DMFA. The reaction was performed under the above conditions and it was found that the same amount of derivative was obtained when using amounts of DMFA above 75 μl. Therefore, 100 μl of DMFA was used for the reaction.

Thenot *et al.*[19] reported the temperature of the reaction of DMFA with amino acids to be 100°, so we carried out the reaction at this temperature (water-bath) and at 120° (paraffin-bath).

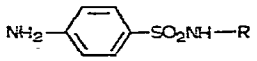
As the reaction at 120° gave better products, it was decided to use this temperature. Use of a higher temperature might be advantageous, but the necessary equipment was not available and it was not therefore tried. A reaction time of 60 min was found to be sufficient. Differences in reaction temperature and time gave slightly different results for the sulpha drugs tested, but the conditions given above were found to be satisfactory.

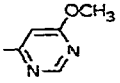
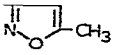
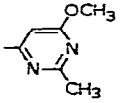
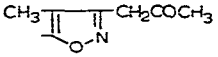
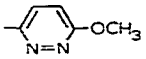
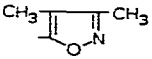
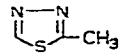
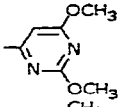
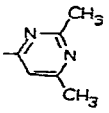
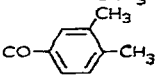
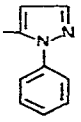
The degree of stability of the reaction products was also examined as it had an effect on the results for precision, and it was found that they were stable at room temperature for at least for 6 h and then gradually decomposed. This phenomenon was common to all fourteen sulfa drugs.

Sensitivity of detection of DMFA derivatives of various sulpha drugs differs, and they could be classified into two groups. The first group, less than 10 ppm, included sulphisomezole, sulphamethomidine, sulphadimethoxine, sulphamerazine, acetosulphamine, sulphisomidine, sulphathiazole and sulphamonomethoxine, and the second group, less than 20 ppm, sulphisoxazole, sulphamethoxypyridazine, sulphaphenazole, sulphamethizole and acetylsulphisoxazole. Calibration curves for some

TABLE I

CHEMICAL STRUCTURE OF SULPHONAMIDES



Drug	*Structure*	*Drug*	*Structure*
	R		*R*
Acetosulphamine	$COCH_3$	Sulphamonomethoxine	
Sulphisomezole		Sulphamethomidine	
Acetylsulphisoxazole		Sulphamethoxypyridazine	
Sulphisoxazole		Sulphamethizole	
Sulphathiazole		Sulphadimethoxine	
Sulphisomidine		Xyloylsulphamine	
Sulphamerazine		Sulphaphenazole	

of these compounds are shown in Fig. 1; xyloylsulphamine showed exceptionally low sensitivity and its curve was drawn up to 200 ppm. Calibration curves were linear under the above conditions. As the temperatures used in GLC procedures differed, the same internal standard could not be used. In most instances use was made of decachlorobiphenyl, but this compound was not always suitable and DDBE was chosen for the determination of sulphisoxazole, sulphisomezole, sulphathiazole, sulphisomidine and sulphamethomidine.

All fourteen sulpha drugs could be analyzed simultaneously, but because of the same limitations of experimental conditions, *i.e.*, use of different column temperatures and stationary phases, each drug was assayed separately and quantitatively under the most suitable condition given above so as to obtain more accurate results.

In order to identify the sulpha drugs and their reaction products, analysis by GLC–mass spectrometry (MS) was carried out. The fragmented spectral data for sulphathiazole are shown in Fig. 2. It was assumed from these spectra that the di-

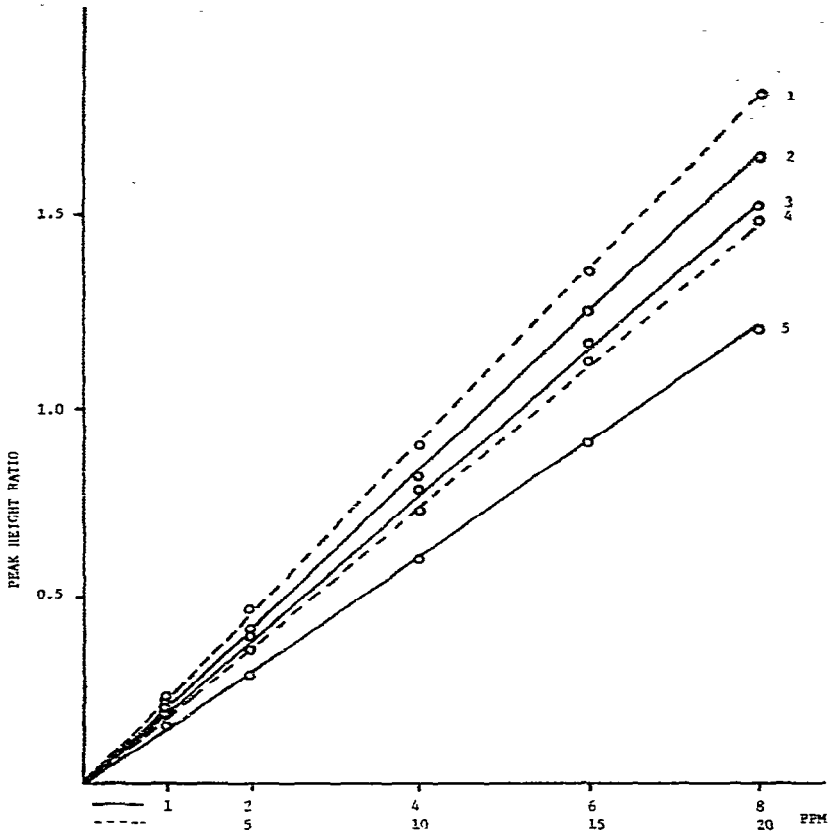


Fig. 1. Calibration curves for derivatised sulphonamides with five data points. 1 = sulphaphenazole; 2 = sulphadimethoxine; 3 = sulphamerazine; 4 = sulphisoxazole; 5 = acetosulphamine.

methylacetal was introduced into the N^4- and the methyl group into the N^1-position, that is, the acetal was introduced into the amino group and the methyl into the imino group. Similar phenomena were shown by other sulpha drugs.

Sulpha drugs that include two amino groups, such as sulphamine, homosulphamine, etc., showed no gas chromatographic peak, by ECD or FID, which might indicate poor reactivity of the compound or thermal decomposition of the reaction products.

Among the various GLC columns tried: OV-1, OV-17, OV-101, OV-225, SE-30, XE-60 and SP-1000, the liquid phases OV-101, OV-225 and XE-60 gave good results. If the liquid layer was too thin, reproducibility of the GLC results was poor when the temperature was 280°, so that 5–10% of liquid phase was employed in this experiment.

Relative retention times of sulpha drugs with internal standard are shown in Table II. These data were recorded individually; when all of the drugs were chromatographed simultaneously, the retention times were slightly different. Gas chromatograms obtained by the simultaneous GLC of all fourteen drugs are shown in Fig. 3.

In the present experiments, microscale analysis using ECD was the main pur-

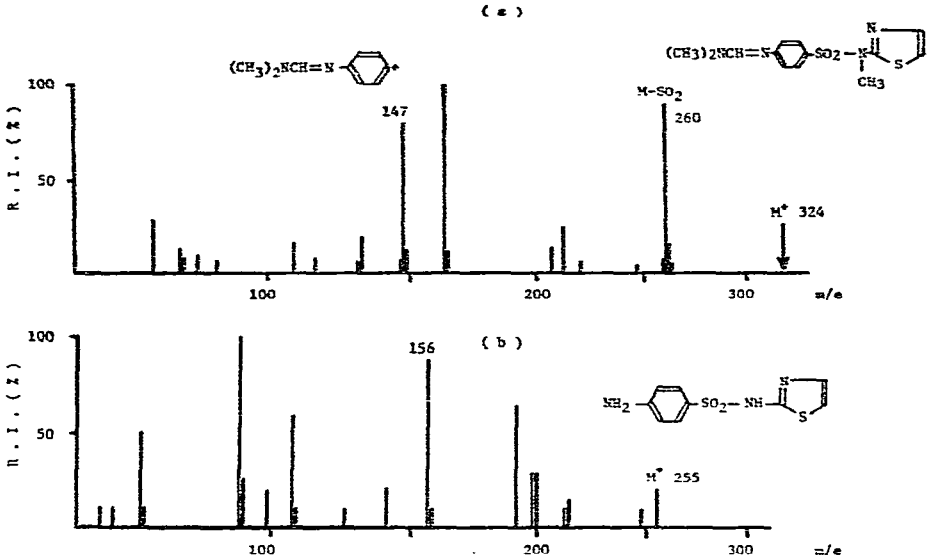


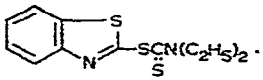
Fig. 2. Mass spectral data obtained (a) on gas chromatographic effluent at a retention time corresponding to derivatized sulphathiazole and (b) on direct inlet system of sulphathiazole. GLC–MS conditions: chamber voltage 70 eV; chamber temperature 290°; column 10% OV-101, 1 m × 0.3 cm at 230°; helium carrier gas flow-rate 30 ml/min.

TABLE II

RETENTION INDICES OF DERIVATIZED SULPHONAMIDES IN THREE SYSTEMS

Drug	*Stationary phase*		
	OV-101 (230°)	*OV-225 (240°)*	*XE-60 (220°)*
DDBE*	1.0	1.0	1.0
Acetosulphamine	0.9	1.1	1.2
Sulphisomezole	2.3	3.3	3.9
Acetylsulphisoxazole	2.4	3.7	4.2
Sulphisoxazole	2.4	3.8	4.2
Sulphathiazole	2.9	3.6	3.9
Sulphisomidine	3.1	3.0	3.7
Suphamerazine	4.1	6.5	7.4
Sulphamonomethoxine	4.3	4.8	5.7
Sulphamethomidine	4.4	4.3	5.4
Sulphamethoxypyridazine	4.8	8.1	9.0
Sulphamethizole	4.8	9.3	10.8
Sulphadimethoxine	7.2	10.7	13.2
Xyloylsulphamine	8.4	14.3	18.1
Sulphaphenazole	9.2	16.5	19.1

* Internal standard: diethyldithiocarbamic acid 2-benzothiazolyl ester



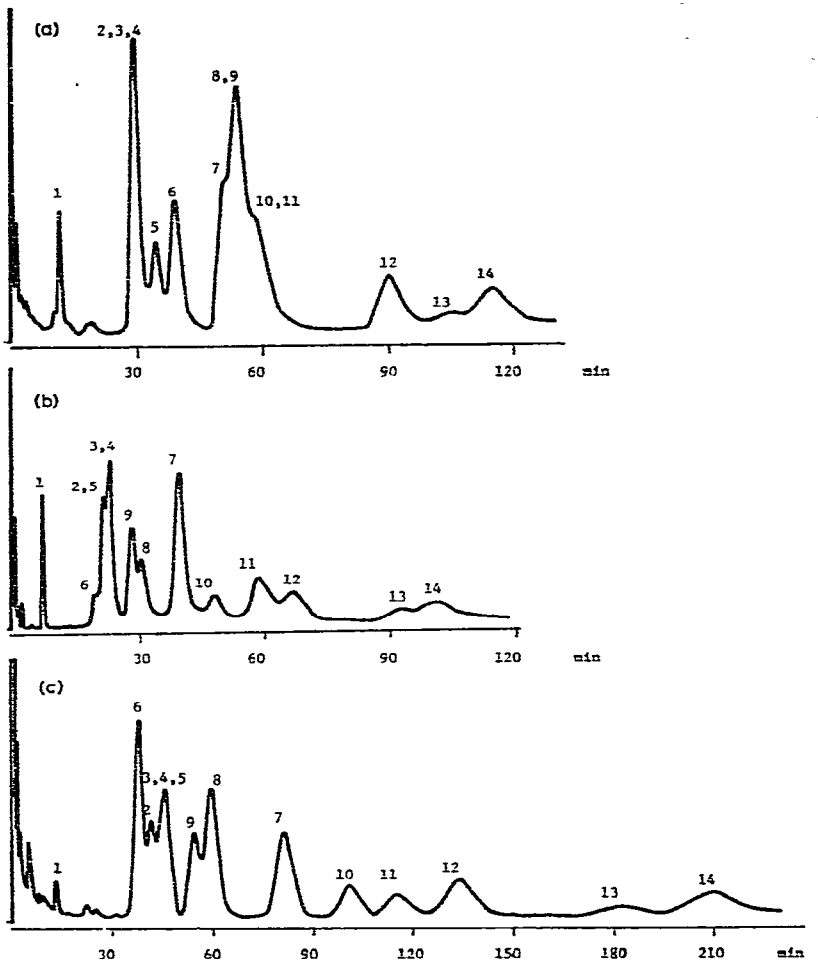


Fig. 3. Gas chromatograms showing the separation of derivatized sulphonamides obtained (a) on a 1 m × 0.3 cm I.D., 10% OV-101 column at 230°; (b) on a 1 m × 0.3 cm I.D., 5% XE-60 column at 220°; and (c) on a 1 m × 0.3 cm I.D., 5% OV-225 column at 240°. Peaks: 1 = acetosulphamine; 2 = sulphisomezole; 3 = acetylsulphisoxazole; 4 = sulphisoxazole; 5 = sulphathiazole; 6 = sulphisomidine; 7 = sulphamerazine; 8 = sulphamonomethoxine; 9 = sulphamethomidine; 10 = sulphamethoxypyridazine; 11 = sulphamethizole; 12 = sulphadimethoxine; 13 = xyloylsulphamine; 14 = sulphaphenazole.

pose. Other detectors, FID and a flame photometric detector (FPD), were also used in the determination of sulphisomezole; the gas chromatograms obtained are shown in Fig. 4. Sensitivity was increased in the order FID, FPD and ECD; that of FPD was different for each sulpha drug.

CONCLUSION

Based on the consideration that the electron-attracting ability of sulpha drugs might be increased by introducing a methyl group in the N^1-position, thus making

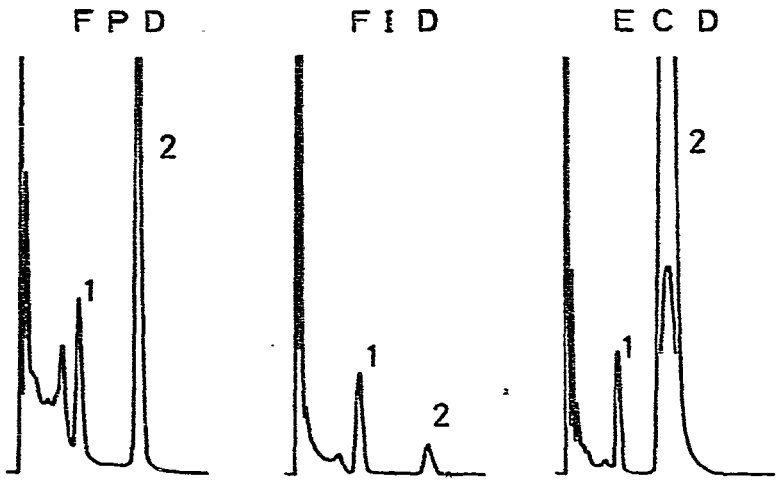


Fig. 4. Relative gas chromatograms showing response values for the same concentration of sulphisomezole; 1 = internal standard; 2 = reaction product of sulphisomezole.

possible micro-analysis using electron capture detection, GLC determination of fourteen sulpha drugs was performed successfully by use of their DMFA derivatives. Linear calibration curves were obtained.

Sulphamine, homosulphamine, etc., which bear amino groups in the molecule, may yield unstable reaction products that are decomposed by heat, lowering the sensitivity with ECD. This problem is worthy of further study.

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