Journal of Chromatography, 107 (1975) 327–333

CHROM. 8172

DETERMINATION OF SULPHONAMIDES BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY

PREPARATION AND PROPERTIES OF PERFLUOROACYL AND PENTAFLUOROBENZYL DERIVATIVES

OLLE GYLLENHAAL and HANS EHRSSON*

Department of Analytical Pharmaceutical Chemistry, Biomedical Center, University of Uppsala, Box 574, S-751 23 Uppsala (Sweden)

(Received November 22nd, 1974)

SUMMARY

The derivatization of benzenesulphonamide, N-ethylbenzenesulphonamide and N-phenylbenzenesulphonamide with trifluoroacetic and heptafluorobutyric anhydride and pentafluorobenzyl bromide has been studied. A rapid quantitative acylation is obtained in benzene in the presence of trimethylamine. Pentafluorobenzylation is performed by the extractive alkylation technique using tetrabutylammonium as counter ion and methylene chloride as solvent. Less than 20 min are required for a quantitative derivatization.

The derivatized sulphonamides have a hydrophobic character, making them very suitable for gas chromatography. Trifluoroacetylation and heptafluorobutyrylation increase the volatility of the sulphonamides, whereas pentafluorobenzylation decreases it. The derivatives have a high electron-capture detector response (minimum detectable quantity, $1–12 \times 10^{-16}$ moles/sec). A standard curve is given for the determination of N-phenylbenzenesulphonamide as trifluoroacetyl derivative in the range 1.8–90 ng/ml.

INTRODUCTION**

A great number of drugs contain the sulphonamide group. The determination of sulphonamides by GC involves certain problems due to their polar nature, which causes adsorption to the chromatographic support.

* Present address: Department of Pharmacology, Medical University of South Carolina, 80 Barre Street, Charleston, S.C. 29401, U.S.A.

** Abbreviations used: ECD = electron-capture detector; FID = flame ionization detector; GC = gas chromatography; HFB = heptafluorobutyryl; IR = infrared spectroscopy; MDQ = minimum detectable quantity[18]; MS = mass spectrometry; PFB = pentafluorobenzyl; TBA = tetrabutylammonium; TFA = trifluoroacetyl; TMA = trimethylamine.

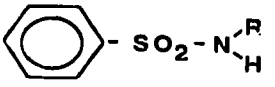
Fravolini and Begliomini[1] hydrolyzed sulfamerazin and related sulphonamides to the corresponding volatile amines, which were quantified by GC. Determinations of intact sulphonamides, *e.g.* sulthiam[2], bumetamide[3] and sulfamerazine[4], have been performed after methylation. Electron-capture gas chromatography has been used for chlorthalidon after extractive methylation[5] and for sulfamerazine after methylation and a subsequent heptafluorobutyrylation of the amino group[6].

The purpose of this investigation is to study conditions for the determination of sulphonamides by GC with electron-capture detection using perfluoroacyl and pentafluorobenzyl reagents.

EXPERIMENTAL

Reagents and chemicals

Three benzenesulphonamides were used as model compounds. They were prepared from benzenesulphonyl chloride and the appropriate amine. After recrystallization from hexane–diethyl ether the purity was checked by GC–FID. The identity was confirmed by IR and MS.



Name	*R*
Benzenesulphonamide	–H
N-Ethylbenzenesulphonamide	$-C_2H_5$
N-Phenylbenzenesulphonamide	$-C_6H_5$

TFA and HFB anhydride and PFB bromide were purchased from Pierce (Rockford, Ill., U.S.A.) and TBA hydrogen sulphate was obtained from Hässle (Mölndal, Sweden). TMA in distilled benzene was prepared from TMA gas supplied by Mathesons Gas Products, East Rutherford, N.J., U.S.A.

Gas chromatographs

An Aerograph Model 600 D gas chromatograph with a FID and a Varian Model 1400 gas chromatograph equipped with an ECD of the ^{63}Ni type were used. The columns, 90 and 150 × 0.2 cm I.D., were packed with 5% OV-17 on 80–100 mesh Gas-Chrom Q and conditioned according to ref. 7. A glass insertion was used in the Model 600 D injector.

A Pye Unicam GCV gas chromatograph equipped with a pulse-modulated ECD of the ^{63}Ni type was also used. The glass column (150 × 0.2 cm I.D.) was packed with 3% OV-17 on 80–100 mesh Gas-Chrom Q. The column was conditioned for 24 h at 300°.

The temperature of the ECDs was maintained at 270°.

Nitrogen carrier gas was freed from contaminants by molecular sieve 13X. A flow-rate of 30 ml/min was used throughout the work.

Preparation of derivatives

Perfluoroacyl derivatives. Acylations were performed with TFA and HFB anhydride. 25 μl of the anhydride, 100 μl of TMA 1 *M* in benzene and 0.5 ml of benzene containing 1 mg of sulphonamide and an internal standard were mixed and kept at 25°.

Pentafluorobenzyl derivatives. 1 ml of an aqueous solution containing 0.1 *M* TBA hydrogen sulphate and 0.2 *M* sodium hydroxide was added to 1 ml of methylene chloride containing 2 mg of sulphonamide and an internal standard. After the addition of 20 μl of PFB bromide the reaction tube was shaken at 25°.

The perfluoroacyl and pentafluorobenzyl reactions were considered complete when no underivatized sulphonamide could be detected (GC–FID) and the amount of formed derivatives was constant.

The structure of the derivatives was established by MS.

Electron-capture studies

Response studies. Reaction mixtures prepared as above were diluted with benzene to a concentration suitable for the ECD. 1-Bromonaphthalene or 9-bromophenanthrene were used as internal standards (except for the TFA derivative of N-ethylbenzenesulphonamide, where 1-bromonaphthalene was used as external standard). A minimum of two separate derivatizations with each sulphonamide and reagent were performed.

Quantitative determinations. 0.1 ml of benzene containing 1.8–90 ng/ml of N-phenylbenzenesulphonamide and 63 ng/ml of 9-bromophenanthrene as internal standards was mixed with 5 μl of TFA anhydride and 10 μl of 1 *M* TMA. After 20 min at 25° excess anhydride was removed by extraction with 0.4 ml of phosphate buffer pH 7 (ionicstrength, 1). After centrifugation at 2000 rpm for 2 min, 2 μl of the organic phase were injected into the gas chromatograph with ECD.

RESULTS AND DISCUSSION

Reaction conditions

Perfluoroacylation. The rate of trifluoroacetylation of N-ethylbenzenesulphonamide at different TMA concentrations is demonstrated in Fig. 1. With 0.15 *M* TMA a complete reaction is obtained within 5 min. The HFB acylation of N-ethylbenzenesulphonamide, as well as the perfluoroacylation of N-phenylbenzenesulphonamide, were also quantitative within 5 min using 0.15 *M* TMA. The catalyzing effect of TMA on the acylation is in agreement with previous findings in our laboratory[8–11].

Benzenesulphonamide is completely derivatized within 5 min, as indicated by complete disappearance of the parent compound in the chromatograms using the same conditions. We did not, however, succeed in chromatographing the formed derivative.

Pentafluorobenzylation. The alkylation of the sulphonamides was performed by a technique developed by Brändström and Junggren[12] and further studied and applied to the PFB alkylation of carboxylic acids and phenols by Ehrsson[13]. Ervik and Gustavii[5] utilized this technique for permethylation of chlorthalidone.

The PFB alkylation of benzenesulphonamide proceeds in two steps, giving a dialkylated derivative as illustrated by Fig. 2. The reaction is quantitative within

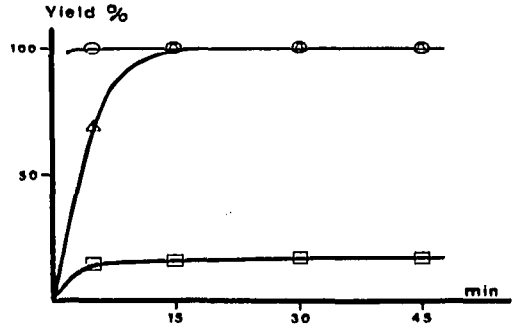


Fig. 1. Influence of TMA on the trifluoroacetylation of N-ethylbenzenesulphonamide. Reactants: 0.01 *M* N-ethylbenzenesulphonamide, 0.3 *M* TFA anhydride and 0.04 *M*, (□) 0.08 *M* (△) and 0.15 *M* (○) TMA. Solvent: benzene. Temperature: 25°. The yields were determined by GC–FID.

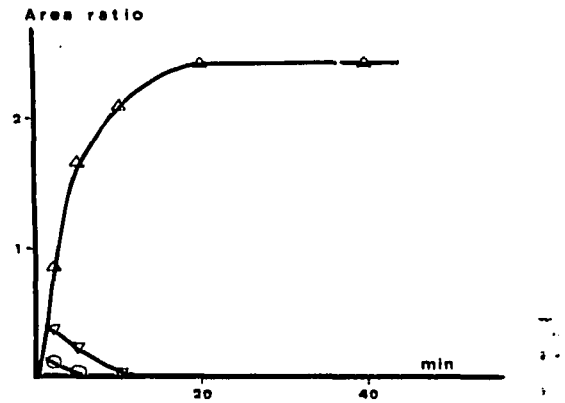


Fig. 2. Pentafluorobenzylation of benzenesulphonamide. Reactants: 0.01 *M* benzenesulphonamide and 0.1 *M* PFB bromide. Aqueous phase: 0.1 *M* TBA in 0.1 *M* sodium hydroxide. Organic phase: methylene chloride. Equal phase volumes. Internal standard: 9-bromophenanthrene. ○, Benzenesulphonamide; ▽, mono-PFB benzenesulphonamide; △, di-PFB benzenesulphonamide. The reaction was stopped with sulphuric acid and the yields were determined by GC–FID. The amount of unreacted benzenesulphonamide is higher than shown in the figure since part of it is also present in the aqueous phase. The partition ratio chloroform to water is 0.57 (ref. 15).

20 min. N-Ethylbenzenesulphonamide was quantitatively derivatized within 15 min and N-phenylbenzenesulphonamide within 2 min. The much shorter reaction time for N-phenylbenzenesulphonamide is probably due to its more lipophilic character (*cf.* ref. 13).

Stability of the derivatives

The derivatives of N-ethyl- and N-phenylbenzenesulphonamide were stable for at least 24 h at 25° in the benzene pH 7 system used for the removal of reagent excess.

The PFB derivatives have previously been demonstrated to be very stable[14,17].

TABLE I

RETENTION TIMES OF SULPHONAMIDE DERIVATIVES RELATIVE TO THE UNDERIVATIZED SULPHONAMIDES

Column: 5% OV-17 (150 cm).

Compound	*Derivative*		
	TFA	*HFB*	*PFB*
N-Ethylbenzenesulphonamide (4.7 min 210°)	0.37	0.27	4.2
N-Phenylbenzenesulphonamide (6.4 min 260°)	0.44	0.33	2.0
Benzenesulphonamide (1.3 min 260°)			7.3*

* Di-derivative.

Gas chromatographic properties

TFA and HFB acylation increased the volatility of the sulphonamides whereas PFB alkylation decreases it (Table I).

All derivatives showed good peak symmetry in contrast to the tailing peaks of the underivatized sulphonamides. Fig. 3 shows a chromatogram obtained after the injection of 36 pg of N-phenylbenzenesulphonamide as TFA derivative.

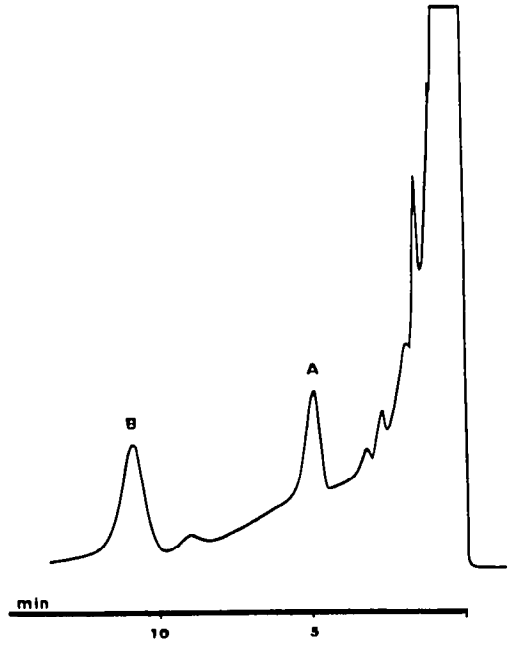


Fig. 3. GC–ECD of N-phenylbenzenesulphonamide as its TFA derivative. Sample: 1 μl of a benzene solution containing the TFA derivative of N-phenylbenzenesulphonamide (36 ng/ml as free amide, A) and 9-bromophenanthrene (63 ng/ml, B); Preparation of sample: see Experimental. GC apparatus: Varian Model 1400 with ECD. Detector temperature: 270°. Column temperature: 190°. Injector temperature: 220°. Sensitivity setting: 2×10^{-10}.

TABLE II

ECD RESPONSE FOR SULPHONAMIDE DERIVATIVES (MDQ $\times 10^{16}$)

Detector temperature: 270°. Internal standards: 1-bromonaphthalene 15×10^{-16} moles/sec, 9-bromophenanthrene 15×10^{-16} moles/sec, 9-bromophenanthrene 8×10^{-16} moles/sec*.

Compound	*Derivative*		
	TFA	*HFB*	*PFB*
N-Ethylbenzenesulphonamide	9	5	2
N-Phenylbenzenesulphonamide	12*	4*	2
Benzenesulphonamide			1**

* Indicates MDQ value determined on the Pye GCV.
** Indicates di-derivative.

Electron-capture response

The MDQ of the derivatives is listed in Table II. The response of the derivatives is in the range $1–12 \times 10^{-16}$ moles/sec. The response ratio between the TFA and HFB derivatives is about three and much lower than reported by Clarke *et al.*[16] for amines and Ehrsson *et al.*[9] for phenols. This indicates that the sulphonamide group itself is contributing to the electron-capturing properties of the derivatives. For quantitative work with biological samples TFA anhydride has definite advantages over HFB anhydride, giving the sulphonamide derivatives a more selective response[19].

Quantitative determination of sulphonamides

A standard curve based on the TFA acetylation of N-phenylbenzenesulphonamide is given in Fig. 4. Each point represents the mean of two separate determinations. The precision at the 18 ng/ml level was 2.3% ($n = 10$).

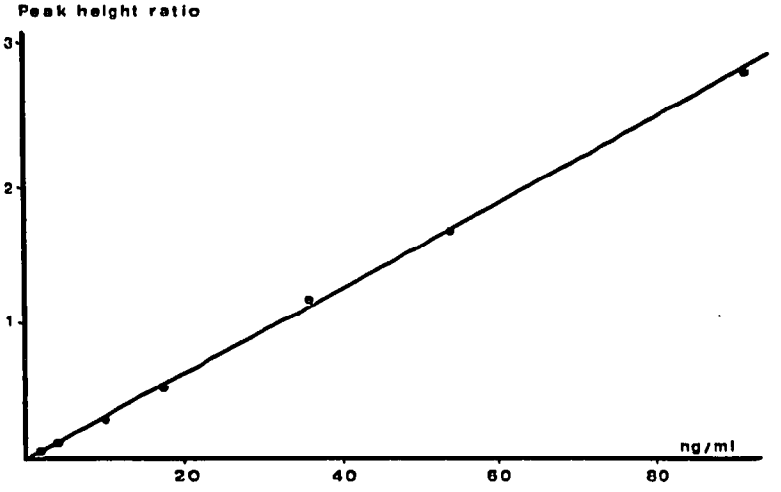


Fig. 4. Standard curve for N-phenylbenzenesulphonamide after trifluoroacetylation. Preparation of samples: see Experimental. GC apparatus, Pye GCV with ECD. Detector current: $1 \cdot 1 \times 10^{-9}$ A. Detector temperature: 270°. Injector temperature: 230°. Column temperature: 200°. Column: 3% OV-17.

ACKNOWLEDGEMENTS

Our thanks are due to Professor Göran Schill for a most valuable discussion of the manuscript and to Miss Barbro Näslund for her excellent assistance in drawing the figures.

REFERENCES

1 A. Fravolini and A. Begliomini, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 767.
2 P. Friel, J. R. Green and H. J. Kupferberg, *Epilepsia*, 13 (1972) 273.
3 P. W. Feit, K. Roholt and H. Sörensen, *J. Pharm. Sci.*, 62 (1973) 375.
4 E. Röder and W. Stuthe, *Z. Anal. Chem.*, 266 (1973) 358.
5 M. Ervik and K. Gustavii, *Anal. Chem.*, 46 (1974) 39.
6 R. J. Daun, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 1277.
7 C. W. Gehrke and K. Leimar, *J. Chromatogr.*, 57 (1971) 219.
8 T. Walle and H. Ehrsson, *Acta Pharm. Suecica*, 7 (1970) 389.
9 H. Ehrsson, T. Walle and H. Brötell, *Acta Pharm. Suecica*, 8 (1971) 319.
10 H. Ehrsson, *Acta Pharm. Suecica*, 9 (1972) 419.
11 H. Ehrsson and B. Mellström, *Acta Pharm. Suecica*, 9 (1972) 107.
12 A. Brändström and U. Junggren, *Acta Chem. Scand.*, 23 (1969) 2204.
13 H. Ehrsson, *Acta Pharm. Suecica*, 8 (1971) 113.
14 F. K. Kawahara, *Anal. Chem.*, 40 (1968) 1009.
15 K. B. Sandell, *Monatsh. Chem.*, 92 (1961) 1066.
16 D. D. Clarke, S. Wilk and S. E. Gitlow, *J. Gas Chromatogr.*, 4 (1966) 310.
17 H. Brötell, H. Ehrsson and O. Gyllenhaal, *J. Chromatogr.*, 78 (1973) 301.
18 R. A. Landowne and S. R. Lipsky, *Nature (London)*, 199 (1963) 141.
19 M. Ervik, T. Walle and H. Ehrsson, *Acta Pharm. Suecica*, 7 (1970) 625.