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N-DIMETHYLAMINOMETHYLENE DERIVATIVES FOR THE GAS–LIQUID CHROMATOGRAPHY OF PRIMARY SULFONAMIDES

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SUMMARY

Dimethylformamide dialkylacetals have been found to react readily with primary sulfonamides to form N-dimethylaminomethylene derivatives. These compounds possess excellent gas–liquid chromatographic properties and can be conveniently prepared at the submicrogram level. Their retention times are much greater than those of other sulfonamide derivatives (*e.g.*, N,N-dimethyl) but their ease of preparation and lack of adsorptive properties make them attractive for gas–liquid chromatographic studies. The practical applicability of this derivatization approach to biological studies is illustrated by the gas–liquid chromatographic determination of 3-bromo-5-cyanobenzenesulfonamide in ovine blood using 3,5-dibromobenzenesulfonamide as the internal standard. The method has a detection limit of 25 ppb* with electron capture detection.

INTRODUCTION

Gas–liquid chromatography (GLC) of compounds of biological interest at the nanogram level, with the exception of the chlorinated pesticides, nearly always requires a derivatization step prior to the chromatographic analysis. Thenot and Horning[1] have demonstrated the value of N-dimethylaminomethylene (N-DMAM) derivatives in the GLC of amino acids and other primary amino group containing compounds. We now communicate our findings on the use of N-DMAM derivatives in the GLC of primary sulfonamides**.

EXPERIMENTAL

Derivatization of reference sulfonamides (0.1–200 μg) was carried out by heating in dimethylformamide dimethylacetal (Pierce, Rockford, Ill., U.S.A.) (50–500 μl) for 10 min at 75–80° in stoppered 12-ml glass centrifuge tubes or sealed tapered 200

* Throughout this article the American billion (10^9) is meant.

** Following completion of this work, we noted a patent (H. E. Weinberg, U.S. Pat. 3,121,084 to E. I. DuPont de Nemours and Co., February 11, 1964) on the reaction of dimethylformamide dialkylacetals with amides to form formamidine (*i.e.*, N-DMAM) derivatives.

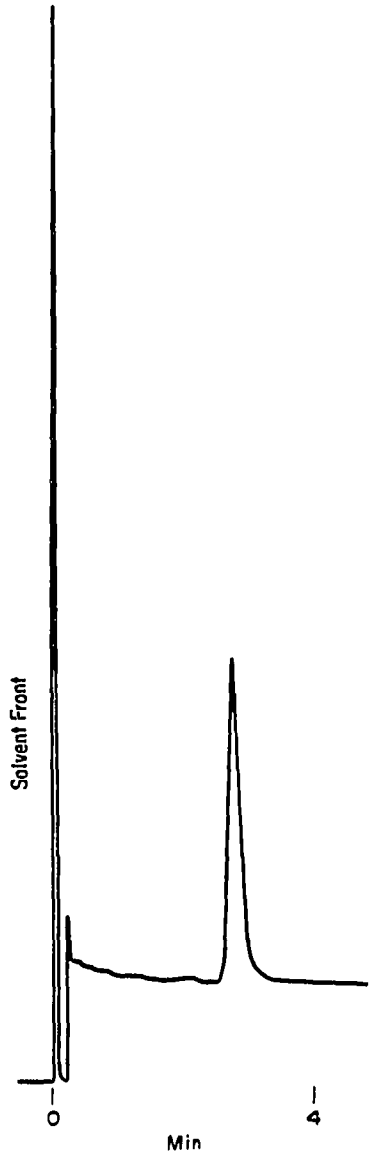


Fig. 1. Gas chromatogram resulting from analysis of an aliquot of a solution of *p*-toluenesulfonamide dissolved and heated in dimethylformamide dimethylacetal. The compound eluted is the N-DMAM derivative. Column conditions (1% OV-17, 210°) are given in the Experimental section.

μl glass sampling/reaction vessels (Hewlett-Packard 4330-0540). Aliquots (1–2 μl) of the reaction mixtures were injected directly for GLC and GLC–mass spectrometry (MS). Methylation of *p*-toluenesulfonamide was carried out by direct injection of a solution of the sulfonamide in 0.1 *M* trimethylanilinium hydroxide in methanol (Southwestern Analytical Chemicals, Austin, Texas, U.S.A.). Trimethylsilylation of *p*-toluenesulfonamide was effected by heating a solution of the sulfonamide in a

TABLE I

METHYLENE UNIT VALUES FOR SULFONAMIDE DERIVATIVES

Compound	*OV-1*	*OV-17*
p-Toluenesulfonamide	15.2	20.4
N-Methyl-*p*-toluenesulfonamide	15.2	20.3
N,N-Dimethyl-*p*-toluenesulfonamide	14.4	19.6
p-Toluenesulfonamide, TMAH*	14.4, 15.2	19.6, 20.3
p-Toluenesulfonamide, BSTFA**	17.2***, 17.7§	19.3§, 20.4***
p-Toluenesulfonamide, N-DMAM§§	21.7	28.6
p-Ethylbenzenesulfonamide, N-DMAM	—	29.7
β-Naphthylsulfonamide, N-DMAM	—	34.1
n-Heptylsulfonamide, N-DMAM	—	26.2

* Derivatized with trimethylanilinium hydroxide in methanol.
** Derivatized with bis(trimethylsilyl)trifluoroacetamide in pyridine.
*** Mono(trimethylsilyl) derivative.
§ Bis(trimethylsilyl) derivative.
§§ Dimethylaminomethylene derivative.

mixture of bis(trimethylsilyl)trifluoroacetamide–pyridine (4:1) for 10 min at 80° prior to injection. Three different columns were used for GLC. Spiral glass columns (4 ft. × 3 mm I.D.) packed with 1% OV-1 and 1% OV-17, respectively, on 80–100 mesh acid-washed and silanized[2] Gas-Chrom P were employed to gather retention and MS data. Methylene unit (MU) values were determined as described by VandenHeuvel *et al.*[3] using long-chain hydrocarbon reference standards (*n*-octadecane, *n*-docosane, *n*-tetracosane and *n*-octacosane). An LKB 9000 combination gas chromatograph–mass spectrometer was used in these studies. The OV-1 column was operated at 120°, whereas the OV-17 column was operated at either 150 or 210°; helium flow-rates were 30 ml/min. Mass spectra were obtained using the following conditions: ionizing potential, 70 eV; accelerating voltage, 3.5 kV; filament current, 60 μA; source temperature, 250°.

Analysis of the blood and plasma extracts was achieved by using an 8 ft. × 3 mm I.D. glass double hairpin column packed with 1% OV-17 on 80–100 mesh Chromosorb W HP. Operating conditions of the gas chromatograph (Hewlett-Packard 7610A) included: column temperature, 250°; flow-rate, 40 ml/min (argon–methane, 95:5); 8 mC ^{63}Ni electron capture detector, 270° with 50 μsec pulse interval;

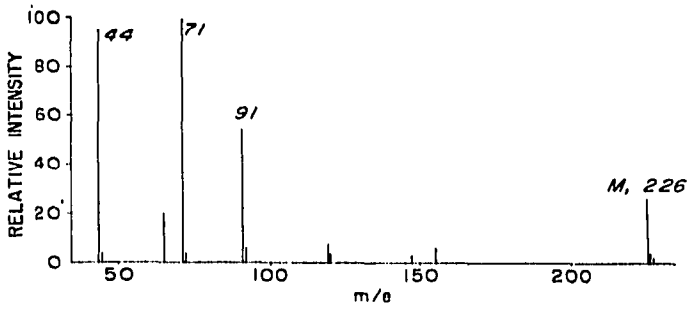
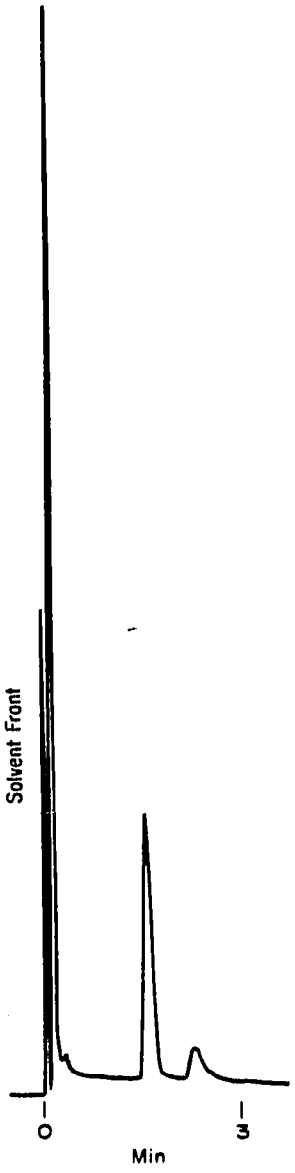


Fig. 2. Mass spectrum of the N-DMAM derivative of *p*-toluenesulfonamide. Spectrometer conditions are given in the Experimental section.



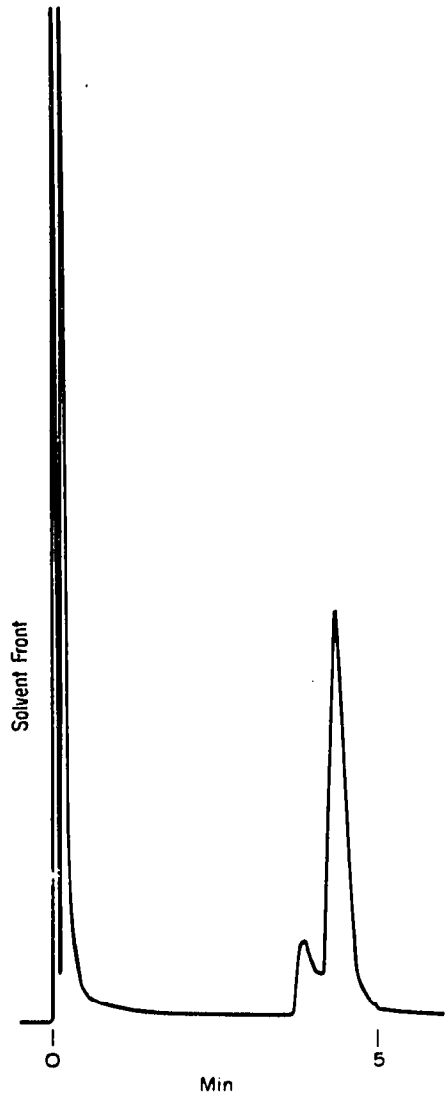


Fig. 3. Gas chromatogram resulting from analysis of an aliquot of a solution of *p*-toluenesulfonamide dissolved in a methanolic solution of trimethylanilinium hydroxide. The earlier eluted component is the N,N-dimethyl derivative; the later eluted compound is the monomethyl derivative. Column conditions (1% OV-17, 150°) are given in the Experimental section.

Fig. 4. Gas chromatogram resulting from analysis of an aliquot of a solution of *p*-toluenesulfonamide dissolved and heated in bis(trimethylsilyl)trifluoroacetamide–pyridine (4:1). The earlier eluted component is the mono(trimethylsilyl) derivative; the later eluted component is the bis(trimethylsilyl) derivative. Column conditions (1% OV-1, 120°) are given in the Experimental section.

electrometer setting 10 × 64; automatic injection (Hewlett-Packard 7671A) of *ca.* 2.0 μl. The isolation procedure for 3-bromo-5-cyanobenzenesulfonamide from blood and plasma was as follows: 0.5 ml of blood or plasma was diluted with 0.5 ml water and then extracted successively with 2 and 1 ml of ethanol. The combined ethanolic supernatant solutions were extracted twice with 2 ml benzene, and then once with 1 ml benzene. The combined benzene extracts were taken to dryness with nitrogen and the residue was dissolved in 0.5 ml ethyl acetate. An aliquot of this solution was taken to dryness in a small tapered glass vessel, and the residue dissolved in 50 μl of a solution of dimethylformamide dimethylacetal containing 0.2 ng per μl of the internal standard. The vessel is sealed (crimp cap), heated at 75–80° for 10 min, and then *ca.* 2 μl of the resulting solution are analyzed. A recovery of *ca.* 60% for drug added to control blood and plasma is observed for this procedure. The observed peak height ratios of drug to internal standard are used with a "working curve" (constructed by adding known amounts of drug to control blood or plasma and subjecting the resulting solutions to the assay procedure) to yield the drug levels.

RESULTS AND DISCUSSION

All the sulfonamides investigated were found to dissolve readily when heated in dimethylformamide dimethylacetal. GLC of an aliquot of the *p*-toluenesulfonamide reaction mixture gave the chromatogram shown in Fig. 1. The single peak exhibited very little tailing and there was no evidence for the presence of parent sulfonamide. Combined GLC–MS demonstrated that the compound possessed a molecular ion of

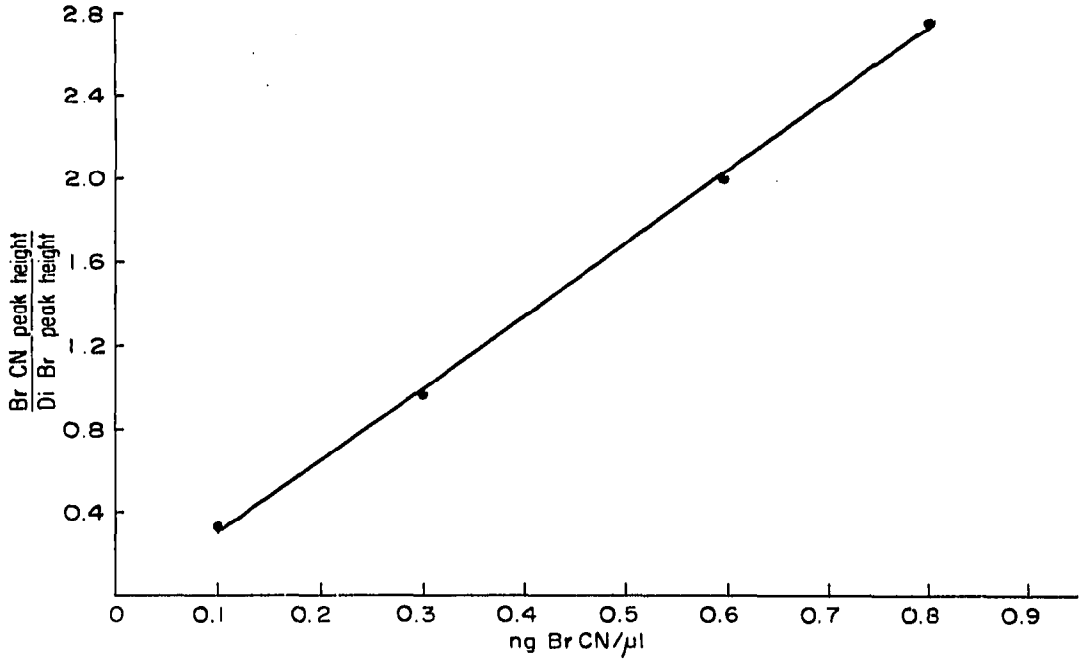
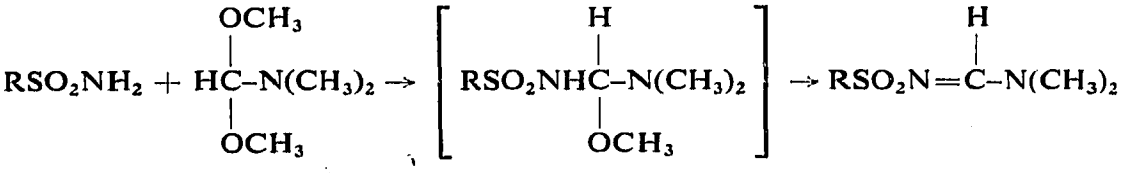


Fig. 5. Relationship between detector response and sample size of 3-bromo-5-cyanobenzenesulfonamide as its N-DMAM derivative.

m/e 226, 55 mass units greater than the molecular weight of *p*-toluenesulfonamide. Similar GLC results were found for the reaction products of *p*-ethylbenzenesulfonamide, *β*-naphthylsulfonamide, and *n*-heptylsulfonamide with dimethylformamide dimethylacetal (MU values for the derivatives are given in Table I), and the molecular ions of these derivatives were also 55 mass units greater than the molecular weights of the parent sulfonamides. Thus a condensation has taken place in each case to form the N-DMAM derivative:



The condensation does not take place with dimethylformamide alone; the dimethylacetal of dimethylformamide is the active agent. The nature of the acetal alkyl group has no bearing on the structure of the condensation product, for the same N-DMAM derivative is formed with dimethylformamide diethylacetal.

The mass spectra of these derivatives are dominated by fragment ions arising

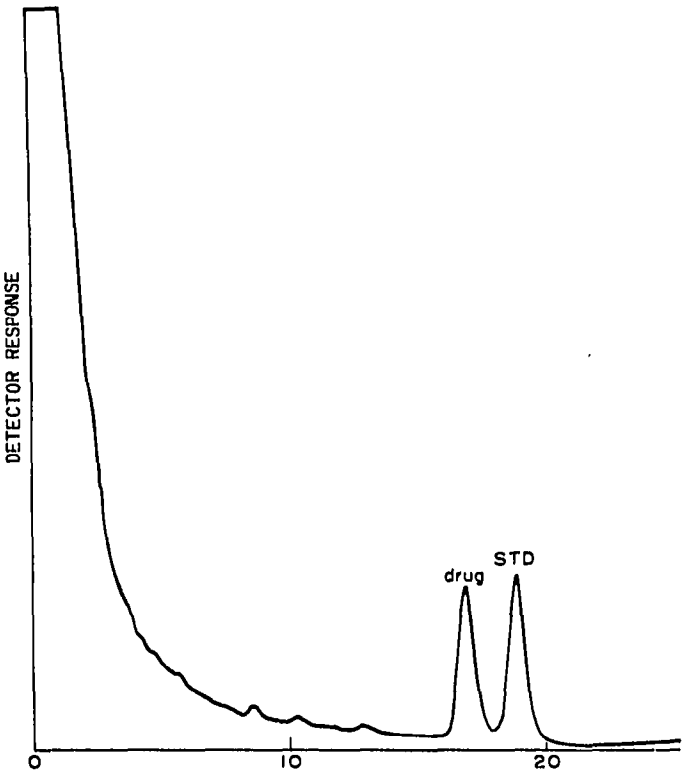
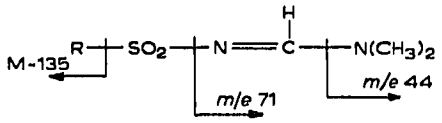


Fig. 6. Gas chromatogram resulting from analysis of an aliquot of the isolate from ovine blood 21 days post-dose (3-bromo-5-cyanobenzenesulfonamide). The drug and the internal standard (STD) are converted to their N-DMAM derivatives.

from the sulfonyl N-DMAM moiety; however, molecular ions of moderate intensity are also observed. This is illustrated by the mass spectrum of the N-DMAM derivative of *p*-toluenesulfonamide (Fig. 2). The ions of *m/e* 44 and 71 arise as shown below. Loss of the entire sulfonyl N-DMAM portion results in formation of the M—135 ion (*m/e* 91 in Fig. 2), the intensity of which is dependent upon the ability of the R group to stabilize the positive charge (*e.g.*, its intensity is moderately great for simple aromatic sulfonamides).



The large MU values presented in Table I indicate that these derivatized sulfonamides possess low volatilities. MU values for methylated and trimethylsilylated *p*-toluenesulfonamide were also determined. Differences in MU values of 7.3 and 9.0 units were observed between N,N-dimethyl-*p*-toluenesulfonamide and the N-DMAM derivative of *p*-toluenesulfonamide on OV-1 and OV-17, respectively. The analogous

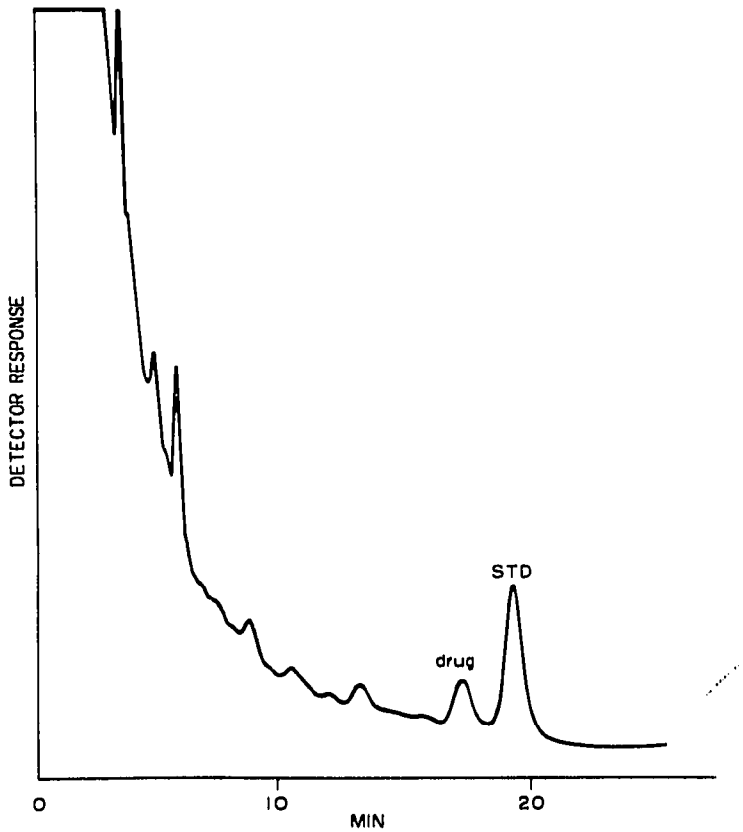


Fig. 7. Gas chromatogram resulting from analysis of an aliquot of the isolate from ovine plasma 21 days post-dose (3-bromo-5-cyanobenzenesulfonamide). The drug and internal standard (STD) are converted to their N-DMAM derivatives.

differences between N,N-bis(trimethylsilyl)-*p*-toluenesulfonamide and the N-DMAM derivative were 3.4 and 6.5 units. Transformation of a polar functional group to a less polar one often results in a shortened retention time, especially with polar stationary phases, even though a significant increase in molecular weight occurs (*e.g.*, note in Table I the MU values for *p*-toluenesulfonamide and its bis(trimethylsilyl) derivative on OV-17). It is clear from the retention data presented in Table I that conversion of a primary sulfonamide to its N-DMAM derivative results in large increases in retention time. Although the resulting long retention times from high-molecular-weight monosulfonamides or those possessing several groups which could condense with the reagent are undesireable, the N-DMAM approach yields only one product. This is in distinction to methylation with trimethylanilinium hydroxide in methanol and trimethylsilylation with bis(trimethylsilyl)trifluoroacetamide. The former approach with *p*-toluenesulfonamide yielded two GLC peaks with both OV-1 and OV-17 (see Table I and Fig. 3). The major component was the N,N-dimethyl derivative, as demonstrated by retention behavior and mass spectrum; the minor component was identified as the monomethyl derivative. Methylation with diazomethane prior to GLC was less satisfactory, with a mixture of mono- and dimethyl-*p*-toluenesulfonamides and also some of the parent compound being eluted. Trimethylsilylation also failed to go to completion (see Table I and Fig. 4) using our conditions.

The practical application of the N-DMAM approach for the GLC of primary sulfonamides has been demonstrated by establishing a GLC assay for 3-bromo-5-cyanobenzenesulfonamide in the blood and plasma of a sheep dosed with 15 mg of this compound per kg. The method has a detection limit of 25 ppb with electron capture detection, a 60% full-scale deflection recorder response (at a retention time of

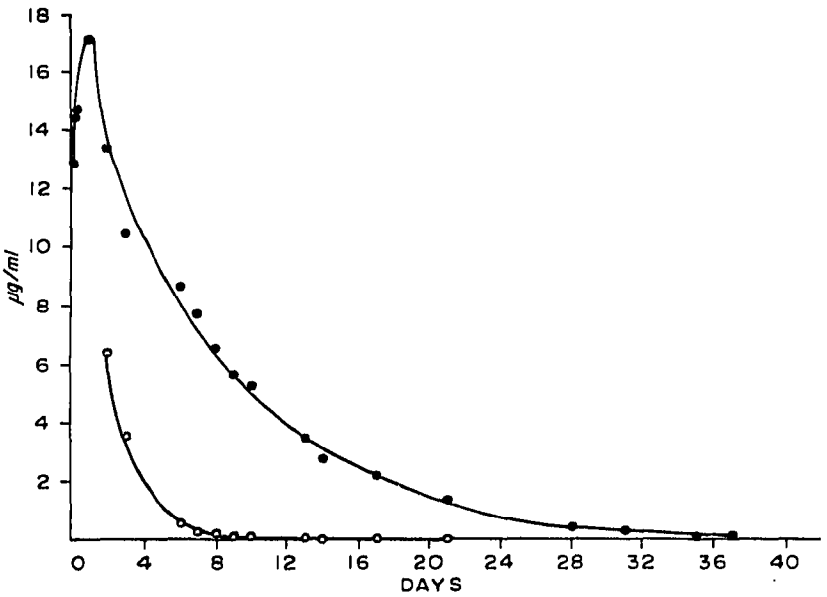


Fig. 8. Plot of ovine blood (●—●) and plasma (○—○) levels of 3-bromo-5-cyanobenzenesulfonamide levels *vs.* days post-dose.

17 min) being observed with 0.2 ng of this sulfonamide as its N-DMAM derivative. A linear relationship was observed to exist between sample size and detector response using 3,5-dibromobenzenesulfonamide as an internal standard (see Fig. 5). The chromatograms resulting from analysis of 21-day post-dose blood and plasma (amounts injected equivalent to 0.0008 and 0.02 ml, respectively) are presented in Figs. 6 and 7. The plots of blood and plasma levels (μg/ml) *versus* days post-dose are presented in Fig. 8.

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