Gas Chromatographic Methods for Analysis of Sulfone Drugs Used in Leprosy Chemotherapy

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4,4′-Diaminodiphenyl sulfone (DDS) and 4-acetamidophenyl-4′-aminophenyl sulfone (MADDS) in human or animal plasma can be measured by gas chromatography at the nanogram level following extraction and derivatization. The meta derivatives of both compounds are added to the samples prior to extraction to serve as internal standards. DDS is converted to 4,4′-diiododiphenyl sulfone (I-DDS) and MADDS to 4-aminophenyl-4′-iodophenyl sulfone (I-MADDS). The compounds are separated on 3% Poly-A-103 and measured by an electron capture detector. Acetyl derivatives of DDS can also be measured by reduction of the acetyl groups to alkyl groups, separation of the reduction products on 3% SE-30, and measurement of the nitrogen content of the amines with an electrical conductivity detector.

DDS is the principal drug used for the treatment of leprosy and has been used in combination with other drugs for the treatment of malaria. Man and a few experimental animals can enzymatically acetylate DDS to MADDS, and hydrolyze MADDS to DDS. Consequently, it is necessary to measure both compounds in order to determine therapeutic levels in plasma.

DDS and MADDS, together with other aromatic amines, can be determined by the Bratton-Marshall method *(1)* but the procedure is not specific. DDS and its acetylation products can also be measured by spectrophotofluorometry *(2,3)*. However, a requirement has arisen for a highly sensitive method of analysis because of the experimental use of repository doses of 4,4′-diacetamidodiphenyl sulfone (DADDS) in leprosy chemotherapy. This compound is injected intramuscularly and is hydrolyzed slowly; consequently, levels of DDS and MADDS produced in plasma are much lower than after oral administration of DDS. It has recently been shown that leprosy will develop in the armadillo, the first animal model to contract this disease *(4-6)*. Therefore, a method will be required for measurement of DDS and MADDS in tissue specimens taken from this animal following oral administration of DDS and intramuscular injection of DADDS.

A method is described for the analysis of DDS and MADDS at the nanogram level which is based on converting them to iodo derivatives prior to gas chromatography. The meta derivatives of these compounds, 3,3′-diaminodiphenyl sulfone, *m*-DDS, and 3-acetamidophenyl-3′-aminophenyl sulfone, *m*-MADDS, are used as internal standards in this method and are converted to the corresponding iodo derivatives, 3,3′-diiododiphenyl sulfone, *m*-I-DDS, and 3-aminophenyl-3′-iodophenyl sulfone, *m*-I-MADDS. An alternate but less sensitive analytical method for DDS, MADDS, and DADDS is described which is based on the lithium aluminum hydride reduction of the acetyl groups of the latter two compounds to alkyl groups prior to GLC.

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EXPERIMENTAL

Reagents. DDS and *m*-DDS were obtained from Aldrich Chemical Company, Milwaukee, Wisc. *m*-DDS was recrystallized from hot ethanol. The recrystallized product melted at 175–176 °C. MADDS and DADDS were obtained from Parke, Davis & Company, Ann Arbor, Mich. I-DDS, I-MADDS, *m*-I-DDS, *m*-I-MADDS, and *m*-MADDS were prepared as described below. Sulfamic acid, white label, was obtained from Eastman Organic Chemicals, Rochester, N.Y. All inorganic reagents were Baker Analyzed reagent grade chemicals. The ethanol used was 190-proof purchased from U.S.I. Chemicals. Ethyl acetate of nanograde quality from Mallinckrodt Chemical Company was used for extraction. High purity nitrogen was used for evaporation, and prepurified nitrogen was employed as a carrier gas for chromatography.

Synthesis of Standards. *3,3′-Diiododiphenyl Sulfone* (*m*-I-DDS). To a solution of 200 mg of 3,3′-diaminodiphenyl sulfone (*m*-DDS) in 10% sulfuric acid (10 ml) at 0 °C was added slowly a 20% solution (1 ml) of sodium nitrite. The mixture was stirred for 10 min at 0 °C, following which 15% aqueous potassium iodide (1 ml) was added with stirring. The reaction mixture was stirred at room temperature for 30 min and then rendered colorless by dropwise addition of saturated sodium bisulfite solution. The precipitate was filtered, washed with water, and recrystallized from ethanol. Two more recrystallizations from ethanol gave 250 mg of pure product, mp 122–123 °C. Elemental analysis of the compound was as follows: Anal. Calcd: C, 30.64; H, 1.70; I. 54.04; O, 6.81; S, 6.81. Found: C, 31.06; H, 1.93; I, 53.64; O, 6.96; S, 6.41.

3-Aminophenyl-3′-iodophenyl Sulfone (*m*-I-MADDS). To a solution of 1 gram of 3,3′-diaminodiphenyl sulfone (*m*-DDS) in 15 ml of methanol at room temperature was added, dropwise, acetic anhydride (0.4 ml) with stirring. After stirring for 10 min, the mixture was poured into ice water and made basic with sodium carbonate. The precipitate which formed was filtered and washed with water. The air-dried precipitate was recrystallized twice from ethyl acetate. The crystals were dissolved in ethyl acetate and the 3-acetamidophenyl-3′-aminophenyl sulfone was extracted into 2 × 10 ml of cold 10% sulfuric acid. The combined sulfuric acid extract was washed with ethyl acetate and then made basic with sodium hydroxide. The precipitate which formed was filtered, washed with water, and air-dried. It was recrystallized from a mixture of ethyl acetate and pentane to give 125 mg of pure product, mp 172–174 °C.

A solution of the 3-acetamidophenyl-3′-aminophenyl sulfone (100 mg) in 10% sulfuric acid (7 ml) was cooled to 0 °C in an ice bath and diazotized by the slow addition of a 20% solution of sodium nitrite (0.5 ml). The mixture was stirred for 10 min and a solution of KI-I_2 (0.3 ml) containing 5% iodine in 10% aqueous potassium iodide was added with stirring. The mixture was stirred for 20 min at room temperature, following which a saturated solution of sodium bisulfite was added dropwise with stirring until the iodine color disappeared. The precipitate was filtered, washed well with water, and air-dried. It was dissolved in ethanol, the solution was treated with Darco-G-60 charcoal, and filtered. To the filtrate was added sufficient water to cause slight turbidity. The mixture was held at room temperature to crystallize. Crystals were filtered and recrystallized from ethanol and water to give 8.5 mg of 3-acetamidophenyl-3′-iodophenyl sulfone, mp 151–153 °C.

To a solution of the intermediate acetamide (50 mg) in ethanol (4 ml) was added concentrated hydrochloric acid (2 ml). The mixture was refluxed for 30 min to ensure complete hydrolysis. The solution was cooled and diluted with water (20 ml). It was then rendered basic by slow addition of 10% sodium hydroxide solution. The precipitate which formed was filtered, washed with water, and air-dried. It was recrystallized from ethanol and water to give 15 mg of 3-aminophenyl-3′-iodophenyl sulfone (*m*-I-MADDS), mp 138–140 °C. Elemental analysis of the compound was as follows: Anal. Calcd: C, 40.11; H, 2.79; I, 35.38; N, 3.90; O, 8.91; S, 8.91. Found: C, 40.38; H, 2.63; I, 35.29; N, 3.95; O, 8.70; S, 9.09.

Iodo Derivatives of DDS and MADDS. The iodo derivative of DDS (I-DDS) was prepared by the same procedure used to prepare *m*-I-DDS. The melting point of I-DDS was 211–212 °C. Elemental analysis of the compound was as follows: Anal. Calcd: C, 30.64; H, 1.70; I, 54.04; O, 6.81; S, 6.81. Found: C, 30.67; H, 1.81; I, 54.10; O, 6.66; S, 6.74

The iodo derivative of MADDS (I-MADDS) was prepared from MADDS by the same procedure used to convert *m*-MADDS to *m*-I-MADDS. The melting point of 4-aminophenyl-4′-iodophenyl sulfone (I-MADDS) was 223–224.5 °C. Elemental analysis of the compound was as follows: Anal. Calcd: C, 40.11; H, 2.79; I, 35.38; N, 3.90; O, 8.91; S, 8.91. Found: C, 40.38; H, 2.63; I, 35.29; N, 3.95; O, 8.70; S, 9.08.

Extraction and Derivatization. *Apparatus.* Centrifuge tubes (15-ml, Corning catalog No. 8084) were used for extractions and to carry out the reactions, whereas the 5-ml size was used for the final evaporation and dilution of the extract for injection into the gas chromatograph. Measuring pipets, 10-ml, graduated in 1/10-ml divisions (Corning catalog No. 7060) were used for measuring reagents in volumes of 0.5 ml and upwards while 1-ml pipets graduated in 1/100-ml divisions (Corning catalog No. 7065A) were used for measuring volumes less than 0.5 ml. Pasteur pipets, 6 inches in length, were used for the separation and transfer of liquids after centrifugation. A 10-μl Hamilton syringe was used for injection of samples into the chromatograph.

All glassware was cleaned by soaking in cleaning solution for 24 hours, rinsing with tap water, and soaking in dilute nitrous acid solution for 2 hours. The glassware was rinsed with tap water, distilled water, and finally dried at 100 °C in an oven.

A Vortex-Genie mixer (Scientific Industries, Inc.) was used for mixing and shaking, and a Sorvall Angle Centrifuge, Model NSE, from Ivan Sorvall, Inc., was used for centrifugation.

Procedure. To each sample of plasma (0.5 to 1.0 ml) in a 15-ml centrifuge tube were added internal standards (2 μg *m*-DDS and 0.3 μg *m*-MADDS for the plasma of patients receiving 50 mg of DDS daily; 20 ng of *m*-DDS and 20 ng *m*-MADDS for patients receiving a repository dose of DADDS) and 2 ml of ethyl acetate. The mixture was shaken for 1 min in a Vortex mixer and then centrifuged for 10 min. The ethyl acetate layer was transferred into a second 15-ml centrifuge tube and the serum was re-extracted with additional ethyl acetate (1 ml) as previously described. The combined ethyl acetate extracts were washed successively with 0.1*N* sodium hydroxide (1 ml) and 0.1*N* sulfuric acid (1 ml) by shaking in the Vortex mixer for 1 min and centrifuging for 10 min. The aqueous layers were discarded. The ethyl acetate layer was then shaken with 1 ml of 15% sulfuric acid to extract DDS and MADDS and centrifuged. The ethyl acetate layer was separated and discarded (or sometimes retained for DADDS analysis) and the sulfuric acid extract was washed with ethyl acetate (1 ml). The sulfuric acid solution containing DDS and MADDS was cooled in ice for 15 min and mixed well with a 20% solution of sodium nitrite (0.2 ml) in water. The mixture was kept in ice for 20 min. Excess nitrous acid was destroyed by addition of a 10% solution of sulfamic acid in water (0.5 ml) and shaken carefully until effervescence ceased. To the diazotized mixture was then added a solution of 5% iodine in 10% potassium iodide in water (0.2 ml). The mixture was stirred thoroughly and allowed to stand at room temperature for 20 min. Excess iodine was destroyed by addition of 1–2 drops of a saturated solution of sodium bisulfite in water. The solution was then cooled in an ice bath and mixed well with 10*N* sodium hydroxide (1 ml). The iodo derivatives were extracted into ethyl acetate (2 ml) by shaking in a Vortex mixer for 1 min. The phases were separated by centrifuging for 10 min. The organic layer was transferred to a 15-ml centrifuge tube and the solvent was evaporated at 60 °C under a stream of dry nitrogen. The residue was dissolved in ethanol (0.5 ml) and 15% sulfuric acid (1 ml) was added. The mixture was heated in a boiling water bath for 45 min. It was then cooled in ice, and 10*N* sodium hydroxide (1 ml) was added. The tube was shaken gently and ethyl acetate (2 ml) was added. The mixture was shaken in a Vortex mixer for 1 min and centrifuged for 10 min. The ethyl acetate was then transferred to another centrifuge tube, anhydrous sodium sulfate added, and the tube was stoppered. The tube was shaken gently and held at room temperature for at least 2 hours. The ethyl acetate extract was transferred to a 5-ml centrifuge tube and evaporated to dryness. To the residue was added the desired volume of ethyl acetate. When 1 ml of plasma was analyzed, 1 ml of ethyl acetate was added to the extracts from patients receiving 50 mg of DDS daily, and 100 μl for extracts from patients receiving a repository dose of DADDS. If the original volume of plasma taken was 0.5 ml, the final volume of ethyl acetate to be added was reduced by one-half. An aliquot of the ethyl acetate solution (usually 5 to 7 μl) was injected into the inlet of the chromatograph.

Chromatography. A Micro-Tek Model MT-220 gas chromatograph equipped with a 13.5 mCi ^{63}Ni source was used. The detector was operated at 40 V dc or as required to give 92% maximum standing current. Detector temperature was maintained at 320 °C. A 1 mV full-scale Westronics MT-11 recorder was used.

The column was a 4-foot × 1/4-inch o.d. glass U-tube packed with 3% Poly-A-103 (Applied Science Laboratories, State College, Pa.) on 100–120 mesh Gas Chrom Q (Applied Science Laboratories, Inc.). It was conditioned for 24 hours at 315 °C at a gas flow rate of 50 ml/min of nitrogen. It was operated isothermally at 285 °C with a flow rate of 100 ml/min of nitrogen. When not in use, it was maintained at 240 °C at a nitrogen flow rate of 50 ml/min. Under these conditions, the column had a life span of six weeks or longer.

The inlet temperature was maintained at 305 °C. Sample size varied from 50 pg to 2 ng of compound. Smaller aliquots of samples containing more than 2 ng of compound were reinjected since the response of the detector was not linear above this value. The retention time for I-MADDS under these conditions was approximately 17 minutes.

The area under each curve was calculated by multiplying the height of the peak times its width at half height.

DISCUSSION

Derivatization. As a matter of historical interest DDS was used at one time as a stationary phase for gas chromatography (7). At the high temperature attainable with modern instruments, DDS can be chromatographed satisfactorily. However, MADDS does not chromatograph well, and the method is insufficiently sensitive for the intended use. The only way by which sensitivity at the nanogram level using GLC can be achieved is through the conversion of DDS and MADDS to halogen derivatives and measurement of these with an ECD.

Perfluoracylation is unsatisfactory since the identities of DDS and MADDS are lost through interacylation, and the compounds which are formed chromatograph poorly. The best solution from the standpoints of chromatography, sensitivity, and simplicity was to convert DDS to 4,4′-diiododiphenyl sulfone (I-DDS) and MADDS to 4-acetamidophenyl-4′-iodophenyl sulfone (I-MADDS). This was accomplished by diazotizing the free amino groups of the compounds with nitrous acid, and replacing the diazonium groups with iodine atoms through treatment with iodide. The intermediate derivative formed from MADDS, 4-acetamidophenyl-4′-iodophenyl sulfone, could be chromatographed in the gas phase, but the relative retention time was impractically long. Therefore, it was necessary to remove the acetyl group by acid hydrolysis. Consideration was given to replacing the amino group of I-MADDS with bromine to further improve chromatographic properties and enhance sensitivity to the ECD. However, replacement of diazonium groups with bromine, unlike iodine, requires an elevated temperature. This produced

(7) H. P. Burchfield and E. E. Storrs, "Biochemical Applications of Gas Chromatography," Academic Press, New York & London, 1962.

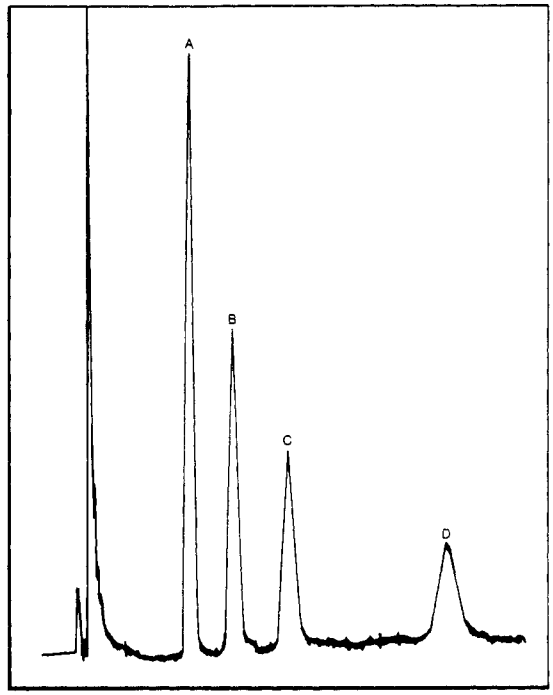

Figure 1. 280 pg each of iodo derivatives of *A. m*-DDS, *B.* DDS, *C. m*-MADDS, *D.* MADDS

many interfering peaks, removal of which would have made the procedure impractically tedious.

Overall yield on derivatization was of the order of 80%. However, since this was a multistep procedure, it was decided to use the internal standard procedure described earlier by Storrs and Burchfield *(8)*. Briefly, this consists of adding to the sample prior to extraction a homolog or analog of the compound being analyzed. The results can then be interpolated from a curve relating nanograms of compound to peak area ratio: compound/standard. The standards chosen for use in this procedure were the meta isomers of DDS and MADDS.

Chromatography. DDS chromatographs well on many high-temperature liquid phases. However, chromatography of MADDS is more difficult because of the presence of the acetamido group. The compounds could be chromatographed on OV-17 or Dexsil 300 gc, but separations were not entirely satisfactory and the compounds and their internal standard did not elute in the order required for satisfactory quantitation.

For a time, it appeared that Versamid 900 might have the characteristics desired. However, it was soon discovered that we apparently had the only batch of this material procurable that was stable at the temperature required for chromatography (270 °C). This seemed unusual since the temperature limit for this phase as listed in vendor's catalogs varies from 300 to 450 °C.

This problem was finally solved by the development of polyamide liquid phases tailored for use in gas chromatography by Mathews *et al (9)*. Columns prepared from one of these materials, Poly-A-103, have a useful life of six weeks, give an acceptable base line, and do not foul the ^{63}Ni ECD. Moreover, the compounds elute in the order required for good quantitations: *m*-I-DDS, I-DDS, *m*-I-MADDS, and I-MADDS (Figure 1). By using this column in combination with a 13.5 mCi ^{63}Ni ECD, 0.28 ng of each compound can be detected with ease.

(8) E. E. Storrs and H. P. Burchfield, *Contrib. Boyce Thompson Inst.*, **21**, 423 (1962).
(9) R. G. Matthews, R. D. Schwartz, J. E. Stouffer, and B. C. Pettit, *J. Chromatogr. Sci.*, **8**, 508 (1970).

Table I. Reproducibility of Peak Area Ratios Using 15 and 30 ng of Each Compound

ng of each compound	Peak area ratios	
	DDS/*m*-DDS	MADDS/*m*-MADDS
15	1.36	0.80
15	1.35	0.84
15	1.34	0.87
15	1.33	0.84
30	1.36	0.86
30	1.33	0.83
30	1.35	0.83
30	1.36	0.86
Average	1.35 ± 1%	0.84 ± 2.6%

Table II. Recovery of DDS and MADDS from Human Plasma Spiked with 20 ng and 50 ng of Each Compound and 20 ng of the Internal Standards

DDS, ng		MADDS, ng	
20	50	20	50
18	47	19	47
22	49	20	48
20	47	19	50
21	49	19	46
17	52	19	48
19	53	20	49
19	...	20	...
20	...	21	...
19.50 ± 1.20	49.50 ± 1.90	19.60 ± 0.88	48.00 ± 1.27

Interferences. During preparation of the sample for chromatography, the plasma is extracted with ethyl acetate, and the extract is washed with dilute acid and alkali to remove potential interferences. DDS and MADDS are then back extracted into aqueous acid for derivatization. This method of sample workup probably eliminates many potential interferences. The high temperature used by GLC probably eliminates others. Even the chlorinated hydrocarbon insecticides, which in any case would be removed during sample preparation, elute far before *m*-I-DDS. Peaks have never been observed which coincide with any of the four compounds of interest. Early in the program, a small peak was observed which had the same relative retention time as DDS. However, this resulted from contamination of the laboratory with DDS as a result of synthetic work utilizing DDS which was in progress in an adjoining laboratory. This problem was eliminated by washing all glassware with nitrous acid prior to use to destroy any DDS and MADDS which might be present.

At very low levels of compound, the peak representing *m*-DDS appears on the tail of the solvent peak which is probably extended by the presence of impurities. However, this does not interfere with manual measurement of peak areas and can be compensated for in electronic measurement by use of a digital type integrator.

Quantitation. Theoretically, it should be possible to compute DDS and MADDS levels from the amounts of standards added and peak area ratios. In practice, however, the compound and its standard do not behave exactly alike during extraction and derivatization so that a calibration curve must be employed. Also, the relationship between concentration of compound and peak area ratio may become nonlinear when the concentration of compound greatly exceeds the concentration of standard. Another complicating factor is that most electron capture

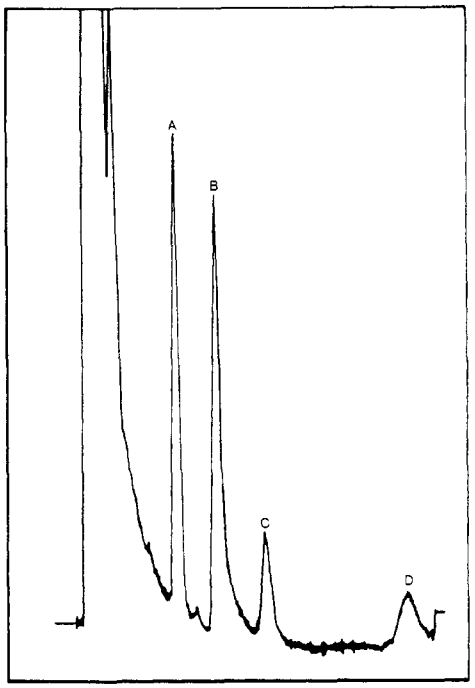

Figure 2. Injection of 1/10th of the extract from 1 ml of human plasma spiked with 2 ng each of *A. m*-DDS, *B.* DDS, *C. m*-MADDS, and *D.* MADDS

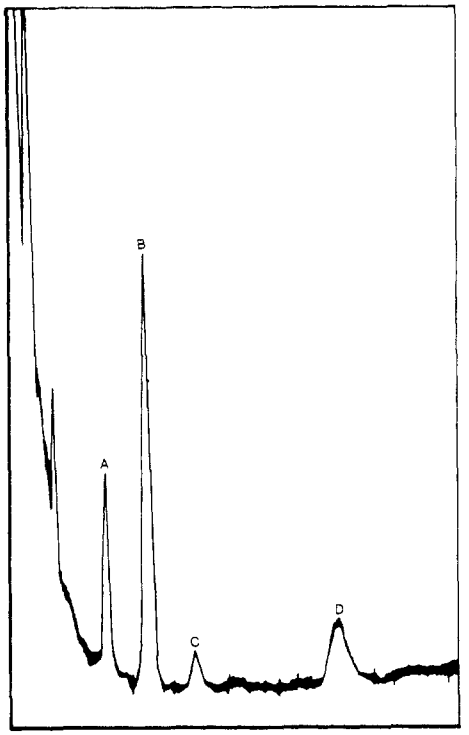

Figure 3. Results obtained on injecting 1.0 μl of the plasma extract from a patient on DADDS therapy. Peaks represent iodo derivatives of *A. m*-DDS, *B.* DDS, *C. m*-MADDS, and *D.* MADDS

detectors in current use become nonlinear as the amount of compound increases. These combined factors make it necessary to use a calibration curve to obtain accurate results.

The key to reproducibility when internal standards are used is the precision with which peak area ratios can be measured. Table I shows that the DDS/*m*-DDS ratio can be measured with a precision of ±1% at levels of 15 and 30 ng/ml of DDS. The ratio of MADDS/*m*-MADDS can be measured to within ±2.6% under the same conditions. These are ideal conditions since the concentration of sample was set equal to the concentration of standard. This situation can always be approached, if required, by running an exploratory analysis and adjusting the standard to optimum concentration in a second run, which, however, doubles the amount of work. Therefore, deviations caused by different ratios of standard to compound and the characteristic nonlinearity of the electron capture detector must be considered in most practical applications.

Recovery of DDS added to normal human plasma averaged 19.50 ng when 20 ng of compound and 20 ng of internal standard were added to plasma (Table II). The coefficient of variation was 6.05%. This is somewhat larger than the coefficient of variation reported in Table I, but the total number of data points are relatively few. Recovery of MADDS averaged 19.60 ng ± 0.88. At the 50-ng level with 20 ng of standard, recovery of DDS averaged 49.5 ng ± 1.90, and recovery of MADDS was 48.0 ng ± 1.27. The slightly low recoveries obtained in this experiment are interesting since the calibration curve was obtained from plasma spiked with a constant amount of the internal standards and para isomers of these compounds.

It was suggested at one point that the amounts of DDS and MADDS found on analysis did not represent the amounts of these compounds present in the plasma because of hydrolysis of MADDS to DDS during the analysis. The most likely step at which this could take place would be during the extraction of DDS and MADDS into aqueous acid followed by diazotization. To eliminate this possibility MADDS and m-MADDS (10 ng each) were extracted from ethyl acetate with 15% sulfuric acid and the aqueous layer was cooled and treated with reagents exactly as specified in the analytical procedure. Only 0.2 to 0.5% of it hydrolyzed. This amount is well within the accuracy of the method. When the acid solution of MADDS was purposely allowed to stand at room temperature for two hours prior to cooling, only 2 to 5% of the MADDS was hydrolyzed.

Applications. As noted earlier, unspiked human and armadillo plasma do not contain compounds which yield peaks with the same relative retention times as the iodo derivatives of DDS and MADDS. However, when these compounds are added to plasma together with the corresponding internal standards, peaks are obtained which represent derivatives of all four compounds (Figure 2). Essentially the same results were obtained on injecting 1.0 μl of derivatized extract from a leprosy patient on DADDS therapy (Figure 3). It will be noted that the peak heights for the derivatives of DDS and *m*-DDS are considerably greater than those for MADDS and *m*-MADDS. This is caused by a combination of shorter retention time and higher sensitivity to the ECD.

This can be compensated for by adjusting attentuation between the elution of compounds *B* and *C*. This does not always require that the sample be rerun after establishing the relative peak heights. In 48 analyses made on plasma from 11 leprosy patients on DADDS therapy, DDS levels varied between 10 and 2480 ng/ml, and MADDS levels between 3.1 and 2610 ng/ml. In general, MADDS/DDS ratios in man range from 0.2 to 1.0 with most of the values clustered around these two figures depending on whether the individual is a fast or slow acetylator.

Figure 4 is a chromatogram obtained on the derivatized plasma extract of a patient on oral DDS therapy in which the attentuation was changed between peaks *B* and *C*. The peak representing MADDS is greatly enhanced, but the sample had to be rerun since insufficient *m*-MADDS was added to the sample prior to extraction.

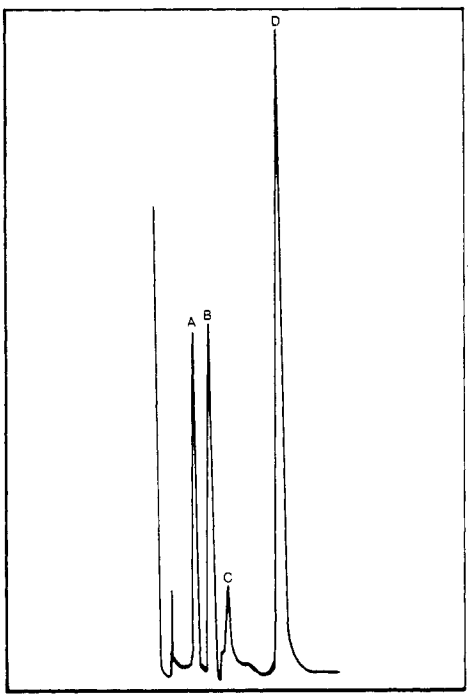

Figure 4. Injection of an aliquot (2.3 μl) of plasma extract from a patient on DDS therapy with attenuation changed between peaks B and C. Peaks represent iodo derivatives of *A*. *m*-DDS, *B*. DDS, *C*. *m*-MADDS, and *D*. MADDS

Table III. Comparison of Fluorescence Intensities of Reduction Products of MADDS and DADDS with Those of DDS and Unreduced MADDS and DADDS

Compound	Excitation, nm	Emission, nm	Relative intensity	R_f[a]
DDS	297	340	1.00	0.2
Ethyl-DDS	302	346	1.45	0.3
Diethyl-DDS	308	346	1.95	0.4
MADDS	297	410	0.74	...
DADDS	290	320	0.18	...

[a] Silica gel TLC plates using benzene material as the mobile phases.

Greater advantage can be taken of changing attenuation in the analysis of plasma from patients on DDS therapy than from those on DADDS therapy because of the higher concentrations of compounds which are present. Consequently, it is necessary to dilute the DDS therapy samples 20-fold. This greatly reduces impurity noise and permits greater amplification of the signal.

Reduction Procedure. Work was discontinued on a reduction procedure for the determination of MADDS and DADDS through derivatization by reduction with lithium aluminum hydride since the method did not have the sensitivity required for this program, in part because of losses of compounds on chromatographic columns, and in part by inherent limitations of the Coulson electrical conductivity detector *(10)*. However, mention of it is made here since it will provide a rapid GLC method available for the analysis of DDS, MADDS, and DADDS at the 100 ng to 1 mg level. Also, reduction substantially increases the fluorescence intensity of MADDS and DADDS.

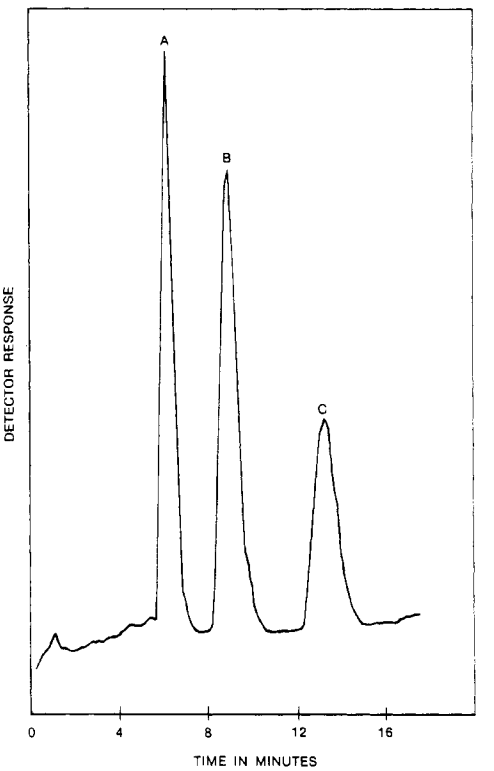

Figure 5. One microgram each of *A*. DDS, *B*. 4-aminophenyl-4′-ethylaminophenyl sulfone, and *C*. bis(4-ethylaminophenyl) sulfone

On reduction with lithium aluminum anhydride, DDS is unchanged, MADDS is converted to 4-aminophenyl-4′-ethylaminophenyl sulfone and DADDS to bis(4-ethylaminophenyl)sulfone. These compounds are well separated on 3% SE-30 at 240 °C and yield reasonably symmetrical peaks at the microgram level (Figure 5), but below the 100 nanogram level, the peaks broaden and become difficult to quantitate.

Reduction increases the fluorescence of MADDS and DADDS by converting the acetyl groups, which tend to quench fluorescence, to ethyl groups which tend to enhance fluorescence (Table III). This could be the basis for methods for the determination of these compounds by TLC or HPLC using a fluorometer for detection.

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