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Microbore column chromatography of 4,4'-diaminodiphenyl sulfone and its acetylated derivatives

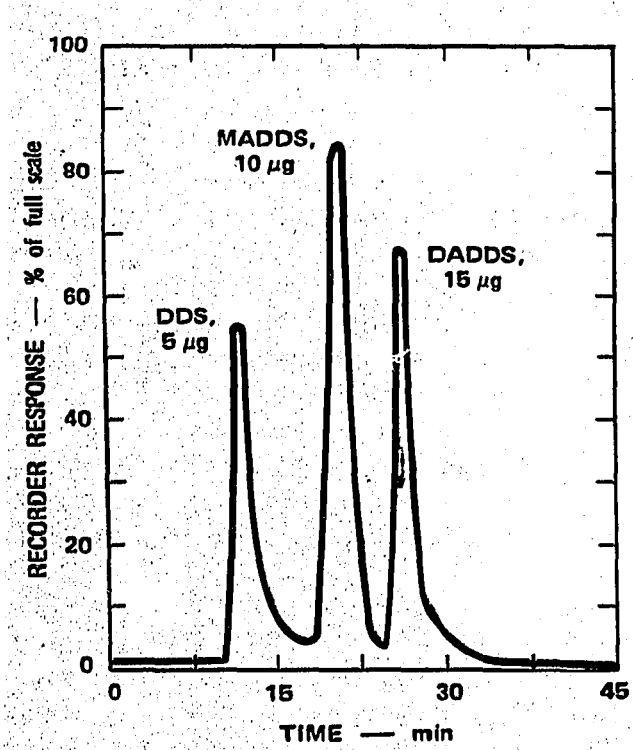
DDS* has emerged, in recent years, as the most efficacious antileprotic drug available[1] as well as an effective antimalarial agent[2]. The need to pursue pharmacologic studies of DDS and related compounds has been met by the development of highly sensitive fluorometric procedures[3–5]. In addition, a rapid qualitative spot test for the detection of DDS in urine, in which a thin-layer chromatographic procedure was employed for confirmation, has been reported[6]. This procedure separated DDS, MADDS and DADDS but was not convenient for quantitative estimation of the compounds. To meet this need and to test results obtained by the direct fluorometric determination of these compounds in urine[5], we adapted the thin-layer chromatographic system to microbore column chromatography. Separation was adequate and quantitative estimation of the compounds in elution fractions was easily accomplished.

Materials and methods

The chromatographic system was composed of a microbore glass column (2.8 × 300 mm), a pressure gauge (200 lbs./in.^{2}), an injection tee, Teflon connecting tubing (Chromatronix, Inc., Berkeley, Ca.), and a controlled volume, piston type pump (Milton Roy Co., St. Petersburg, Fla.). The column was packed with dry silica gel (SilicAR TLC-7, Mallinckrodt, St. Louis, Mo.) by gentle tapping and anhydrous ethyl acetate pumped through at 100 lbs./in^2. The flow rate was 0.25 ml/min. Absorption at 280 nm of the column effluent was monitored by use of an UV monitor (Model UA-2, Instrumentation Specialties Co., Lincoln, Nebr.). Finally, the effluent was collected in 0.5-ml fractions using a fraction collector equipped with a drop counter (Research Specialties Co., Richmond, Calif.). When small air bubbles could no longer be seen in the column effluent and the UV recording gave a stable baseline, 10 μl of ethanol containing 1–20 μg of DDS, MADDS or DADDS, separately or in mixtures, was injected with a Hamilton syringe. Recovery of added compounds was determined by measuring the fluorescence of the fractions at the activation and fluorescence maxima of the compounds (285/350 nm for DDS; 285/415 nm for MADDS; 285/340 nm for DADDS) in a spectrophotofluorometer (American Instrument Co., Silver Spring, Md.). In addition, areas of elution peaks on the UV recording were also estimated by the general method of SCOTT AND GRANT[7] in subsequent, more detailed studies with DDS and MADDS.

Thin-layer chromatography was performed as previously described[6] with the exception that sheets containing silica gel in a glass fiber matrix (ChromAR 500, Mallinckrodt, St. Louis, Mo.) were employed. Determinations of the amounts of DDS, MADDS and DADDS in urine from subjects receiving 100 mg DDS orally were performed by extracting aliquots of urine, made alkaline (pH = 10–11) with 0.1 *N* NaOH, with three volumes of ethylene dichloride. The organic extract was evaporated to dryness at room temperature under a stream of nitrogen and the residue dissolved in 20 μl of diglyme (2-methoxy diethyl ether). Ten-microliter aliquots were subjected to microbore column chromatography as described.

* Abbreviations used: DDS = 4,4'-diaminodiphenyl sulfone; MADDS = 4-amino-4'-acetamidodiphenyl sulfone; DADDS = 4,4'-diacetamidodiphenyl sulfone.



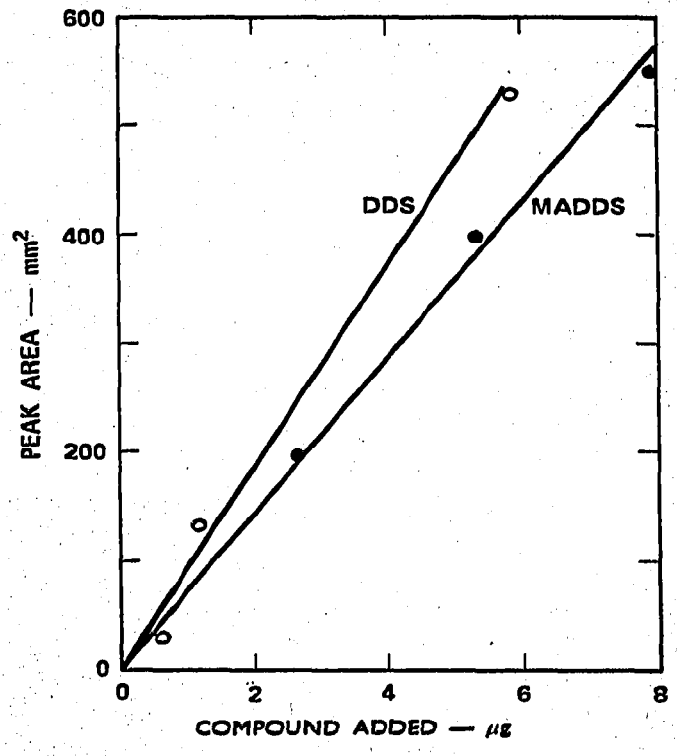


Fig. 1. Separation of DDS, MADDS and DADDS by microbore column chromatography.

Fig. 2. UV-absorbing peak areas versus amounts of DDS and MADDS after microbore column chromatography.

Results and discussion

Fig. 1 shows the plot obtained from the recorder of the UV monitor of the effluent from the microbore column when a mixture of all three compounds was added. Separation was adequate and the elution positions directly paralleled the migrations of the three compounds (R_F values were 0.80, 0.59 and 0.34, respectively, for DDS, MADDS and DADDS) obtained on thin-layer chromatograms. Recoveries of the compounds by fluorometric assay of the fractions were essentially quantitative (99–104%).

The results of direct fluorometric analysis[5] and assay by microbore column chromatography for DDS and MADDS of several urine collections from two subjects receiving DDS (Table I) show that the two procedures gave comparable values. The chromatographic method has the advantage of being more flexible for the determination of small amounts of these compounds. No DADDS could be detected by either the direct fluorometric or the microbore chromatographic methods.

Although UV monitoring was used initially for qualitative identification, we subsequently found that measuring peak areas from the recorder plot yielded a direct estimation of the amounts of DDS and MADDS present in mixtures. Fig. 2 shows a linear relationship between these peak areas and the amounts of DDS and MADDS chromatographed. The approximate limits of sensitivity under the current conditions were 0.5 μg for DDS and 1 μg for MADDS. These limits do not approach the sensitivities attainable by fluorometric assay. However, under some circumstances the more rapid estimations using only UV peak areas may have distinct advantages.

TABLE I

URINARY EXCRETION OF DDS AND MADDS DETERMINED BY FLUOROMETRY AND BY MICROBORE COLUMN CHROMATOGRAPHY

Values are percent of dose (100 mg) of DDS.

Subject	*Collection time (h)*	*DDS*		*MADDS*	
		Fluorometry	*Chromatography*	*Fluorometry*	*Chromatography*
GGO	0–12	1.8	1.2	<0.5	<0.1
	12–24	3.3	2.3	<0.5	0.2
	24–48	2.8	2.6	<0.5	0.2
JPE	24–48	3.3	3.4	<0.5	<0.1

In general, the comparable elution pattern after microbore column chromatography and the migration pattern after thin-layer chromatography of these three compounds suggest that the procedures for the separation of compounds by thin-layer chromatography should be easily adaptable to the microbore column systems. The latter technique has distinct advantages in the quantitation of the separated compounds.

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