CHROM. 5967

Mini thin-layer chromatography

III. A rapid and sensitive method for the estimation of amphetamine and methamphetamine

Amphetamine, a stimulant of the central nervous system, is included in the list of drugs of abuse. Medico–legal cases have provided an impetus for the increased interest in the examination and determination of this compound in biological specimens. Paper chromatography (PC)[1], thin-layer chromatography (TLC)[2,3], gas chromatography (GC)[2,4–7] and spectrophotometry[8] have all been used to determine the amphetamines. Most of these methods are sensitive only to a microgram scale and all are time-consuming.

Recent advances in mini TLC of a highly fluorescent derivative of 1-dimethylaminonaphthalene-5-sulfonyl chloride (DNS-Cl) on polyamide plates have resulted in an increased sensitivity and better separation of amino acids[9], catecholamines and other psychotropic drugs[10]. The purpose of this communication is to report a rapid and sensitive procedure for the determination of picogram quantities of amphetamine and methamphetamine by dansylation and subsequent chromatography on a mini polyamide plate.

Experimental

Materials. Mini plates were prepared by cutting a thin-layer polyamide plate (15 × 15 cm, Cheng Chin Trading Co., Ltd.*) into 3 × 3 cm sections. [^{3}H]Amphetamine sulfate was a product of New England Nuclear (Boston, Mass.). Dansyl chloride was obtained from Sigma Chemical Company (St. Louis, Mo.).

Procedures. Dansylation was performed in aqueous solutions of the pure substances or other biological specimens. For routine assay, 20 μl of 0.1 *M* $NaHCO_3$ and 30 μl of dansyl chloride (1 mg/ml in acetone) were added to 10–30 μl of drug solutions or unknown specimens. The mixture was heated at 37° in the dark for 2 h. At the end of the incubation two drops of 2 *N* acetic acid were added. A 0.05-μl aliquot of this solution was applied on a mini thin-layer plate (3 × 3 cm) with a fine capillary tube. The plate was developed in a petri dish (diameter 5.5 cm, height 3.5 cm) or a 50-ml beaker containing 1.5–2 ml of chromatographic solvent. When the solvent reached 2 mm from the top, the plate was removed, air-dried and visualized under UV light. For quantitative determination of the amphetamines, the corresponding spots of the unknown compound were scanned for fluorescent intensity by a Farrand Vis-UV chromatogram analyzer.

In some experiments amphetamine and methamphetamine were extracted from test urine specimens containing other drugs by chloroform extraction at pH 13, as well as by the method of DOLE *et al.* which employed an ion-exchange resin paper[11].

In a study to determine the percentage conversion of the drug to its DNS derivative, a known amount of [^{3}H]amphetamine was used. The DNS-[^{3}H]amphetamine spot was identified under UV light after it was separated on a polyamide plate.

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The spots were cut out and radioactivity determined by a Beckman LS-100 scintillation counter.

Results

Table I reports the R_F values of DNS-amphetamine or DNS-methamphetamine in several different solvent systems after they were developed on a polyamide mini (3 × 3 cm) thin-layer plate. With the solvent systems tested, solvents 1 and 2 are suitable for the separation of amphetamine and methamphetamine. In a study of two-dimensional chromatography, with solvent system No. 1 in first dimension and No. 2 in second dimension, we have obtained even better separation of the two drugs. It should be noted that 0.3–0.5 ng of DNS derivatives could readily be visualized under UV light. In order to determine the percentage of amphetamine converted to the DNS derivative under our dansylation conditions, we dansylated a series of known quantities of [^{3}H]amphetamine. The resultant DNS-[^{3}H]amphetamine was separated by mini TLC and its radioactivity measured. Table II indicates that there is almost complete conversion (92 %) of amphetamine to DNS-amphetamine under

TABLE I

R_F VALUES OF AMPHETAMINE AND METHAMPHETAMINE MINI THIN-LAYER PLATES DEVELOPED WITH VARIOUS SOLVENT SYSTEMS

Compound	*No.*	*Solvent system*	R_F *values*
Amphetamine	1	Formic acid–water (1.5:100)	0.26
	2	Water–formic acid–*n*-butanol–ethanol (150:3:4:93)	0.60
	3	Toluene–acetic acid–dimethylformamide (200:10:4)	0.90
	4	*n*-Heptane–*n*-butanol–acetic acid (30:40:2.5)	0.84
	5	Toluene–acetic acid (100:25)	0.80
	6	Benzene–formic acid (200:3)	0.70
	7	Formic acid–methyl acetate–ethyl acetate–*n*-hexane (10:40:50:100)	0.65
	8	Water–ethyl acetate–ethanol (50:25:25)	0.49
Methamphetamine	1	Formic acid–water (1.5:100)	0.12
	2	Water–formic acid–*n*-butanol–ethanol (150:3:4:93)	0.44
	6	Benzene–formic acid (200:3)	0.50

TABLE II

PERCENTAGE CONVERSION OF AMPHETAMINE TO DNS-AMPHETAMINE

	Amount of radioactive amphetamine added	*Amount of radioactivity recovered as DNS-amphetamine*	% *conversion*
	5851	5331	
	5515	5397	
	5596	5202	
	6017	5119	
	5755	5140	
Average:	5745 ± 231	5236 ± 123	92 %

our experimental conditions. With [^{3}H]amphetamine of high specific activity (1 mC per 0.054 mg), we can easily detect amounts of less than 0.1 ng of amphetamine. Fig. 1 indicates that there is a linear relationship with the amount of drug added and the radioactivity recovered from DNS-amphetamine spots.

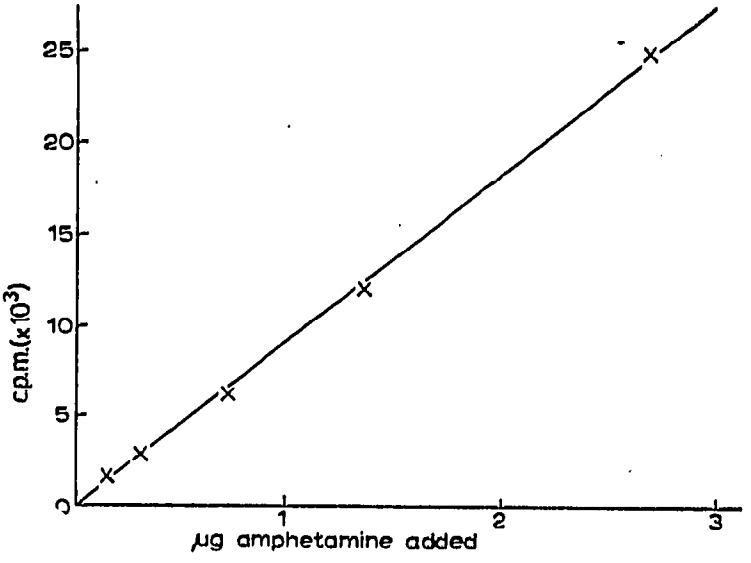

Fig. 1. Quantitative relationship between the amount of [^{3}H]amphetamine added and the amount of DNS-[^{3}H]amphetamine recovered.

The amount of DNS-amphetamine can also be measured directly on the polyamide thin-layer plate by a fluorescence scanner which measures the fluorescent intensity of the spots. Fig. 2 shows a quantitative relationship between the amount of DNS-amphetamine and the intensity of the fluorescence. As little as 1 or 2 ng of amphetamine can be determined by this method.

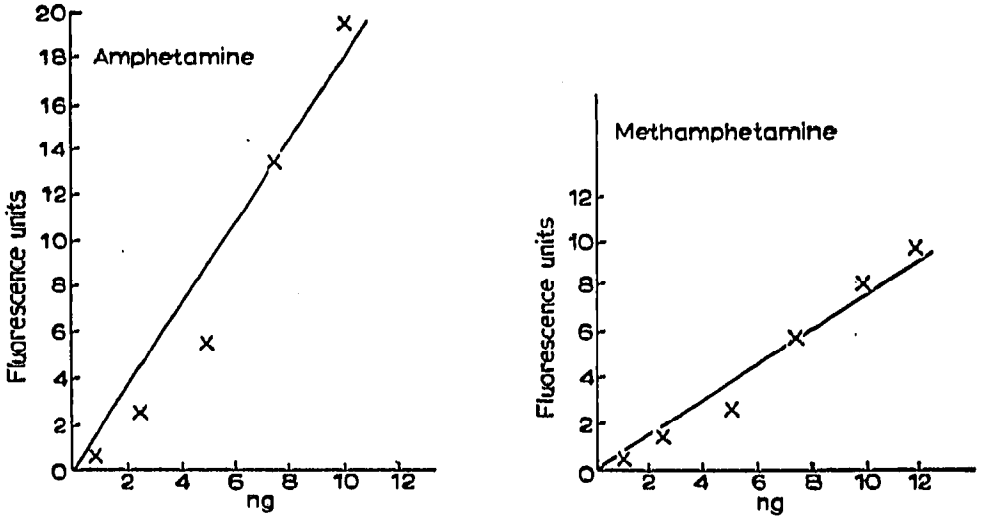

Fig. 2. Fluorescence intensity of DNS-amphetamine and DNS-methamphetamine spots as measured by a Farrand chromatogram analyzer directly on the polyamide mini thin-layer plate.

Discussion

DNS-Cl has been employed successfully in the N-group determination of polypeptide chains[12] and the method is 100-fold more sensitive than SANGER's fluorodinitrobenzene procedure[13].

SEILER AND WIECHMANN[14] have used TLC to separate a large number of amines as their DNS-derivatives. These authors used regular size Silica Gel plates and their studies concerned only qualitative separation of these amines. Recently several mini TLC methods for drug screening have been published[10,15]. The utilization of mini thin-layer plates is not only more simple and rapid, but these advantages are attainable without sacrificing sensitivity and accuracy.

The use of DNS-Cl allows for the detection of picogram quantities of the DNS-amphetamine derivatives after separation on a mini polyamide plate. The highly fluorescent spots were visualized under UV light. Since *d*-amphetamine is converted quantitatively (92%) to DNS-amphetamine, measurement of the fluorescent intensity by thin-layer scanner is a rapid and simple way of quantifying these drugs. The sensitivity of the DNS-amphetamine derivative is extended to the spectrofluorometer where a range of 2 ng may be detected (Fig. 2). By using a radioactive drug with high specific activity, less than 0.2 ng can easily be quantified.

The use of polyamide thin-layer plates offers several advantages: (1) The diminutive pieces (3 × 3 cm) used require less than 5 min and 1.5 ml of solvent for chromatogram to develop; (2) both sides of the polyamide thin-layer plate may be utilized for spotting; (3) a good separation with discrete spots is achieved; and (4) the plate can be re-used after washing.

The qualitative analysis of the amphetamines as performed in our procedure enables one to detect picogram quantities of these drugs in biological fluids. Since DNS-Cl reacts with primary and secondary amines, phenolic hydroxyl groups, thiols, and imidazoles, it is essential to partially purify the amphetamine either by solvent extraction (at pH 13) or by ion-exchange resin paper, in order to reduce the background fluorescent spots. Studies relating to the application of this procedure for the determination of other drugs are being continued in our laboratories.

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