Journal of Chromatography, 76 (1973) 505–508

CHROM. 6445

Note

Estimation of morphine by polyamide mini thin-layer chromatography

Morphine has rapidly become one of the most widely abused drugs in the United States. Many methods are available for the detection of this drug in biological specimens. Morphine can be qualitatively detected by paper or thin-layer chromatography (TLC) as well as quantitatively determined by colorimetry, spectrophotometry or fluorometry. Recently, sensitive gas chromatography methods were developed to detect minute quantities of morphine. All these methods have been intensively reviewed by TAYLOR[1]. However, a rapid, simple and sensitive method is still lacking. Using a highly fluorescent marker, dansyl chloride, in combination with polyamide mini TLC, we have developed a rapid and simple method which determines picogram quantities of morphine.

Experimental

Materials. Mini thin-layer plates were prepared by cutting a 15 × 15 cm regular plate into 3 × 3 cm sections. The polyamide plate is a product of Cheng Chin Trading Co., Ltd. (Taipei, Taiwan). [^{14}C]Morphine was obtained from Amersham-Searle Corporation (Ill., U.S.A.) (specific activity = 57 mCi/mmole) and dansyl chloride was obtained from Sigma Chemical Company (St. Louis, Mo., U.S.A.).

Procedure. Morphine from urine or brain (10% homogenate) was extracted with 5 ml of *n*-butanol after 0.3 ml of 16 *N* KOH had been added to the sample. After the samples had been shaken for 10 min, they were centrifuged at 1000 × *g*. The butanol fraction was then pipetted out and dried in a conical tube.

Dansylation was performed in aqueous solution (20–50 μl) by adding 30–50 μl of 0.1 *M* $NaHCO_3$ and 30–50 μl of dansyl chloride (1 mg/ml in acetone). The mixture was heated at 37° in the dark for 2 h after which two drops of 1 *N* acetic acid were added to terminate the reaction. 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 had reached 2 mm from the top, the plate was removed, air dried and visualized under UV light.

Since the DNS derivative is readily soluble in trichloromethane, we have used this solvent extraction to separate the DNS-morphine from the unreacted DNS-Cl. For routine assays, 0.5–1 ml of trichloromethane was added to the test tube after dansylation reaction. The mixture was shaken vigorously for 2 min and then centrifuged at 500–1000 × *g* for 5 min. With the aqueous layer removed by aspiration, the trichloromethane layer was used for spotting. For quantitative studies with [^{14}C]morphine, the DNS-[^{14}C]morphine spots on the mini thin-layer plate were cut out and the radioactivity determined with a Beckman LS-100 liquid scintillation counter.

Results and discussion

Dansyl chloride, a highly fluorescent compound, has been used in the NH_2-group

TABLE I

R_F VALUES OF MORPHINE ON MINI THIN-LAYER PLATES DEVELOPED WITH VARIOUS SOLVENT SYSTEMS

Solvent system	*Morphine R_F value*
Water–ethyl acetate–ethanol (50:25:25)	0.93
Toluene–acetic acid–dimethylformamide (200:10:4)	0.87
Heptane–*n*-butanol–acetic acid (30:40:2.5)	0.91
Acetic acid–dimethylformamide–ethanol–water (25:20:20:50)	0.85
Benzene–acetic acid (9:1)	0.86
Benzene–formic acid (200:3)	0.1

TABLE II

PERCENT CONVERSION OF MORPHINE TO DNS-MORPHINE AND PERCENT EXTRACTION OF DNS-MORPHINE BY TRICHLOROMETHANE

	No. of tests	*Efficiency (%)*
Conversion of [^{14}C]morphine to DNS-morphine	10	77
Extraction of DNS-[^{14}C]morphine by trichloromethane	10	70

TABLE III

AMOUNT OF [^{14}C]MORPHINE RECOVERED FROM BIOLOGICAL SPECIMEN AS DNS-[^{14}C]MORPHINE

[^{14}C]morphine[a] added (ng)	*No. of tests*	*Amount of DNS-[^{14}C]morphine recovered from*		
		Water	*Urine*	*Brain*
5	5	210	107	96
10	5	402	210	182
20	5	751	402	382
50	5	1892	1026	874
100	5	3675	2045	1827

[a] Specific activity of [^{14}C]morphine: 66 μCi/mg.

TABLE IV

SEPARATION OF THE DANSYL DERIVATIVES OF SOME PSYCHOACTIVE DRUGS ON POLYAMIDE MINI THINLAYER PLATES

Solvent system: benzene–formic acid (200:3).

Compound	*R_F values*
Morphine	0.1
Naloxone	0.33
Amphetamine	0.96
Methamphetamine	0.63
LSD	0.22

determination of polypeptide chains (WEBER[2]). Recently, we have employed this procedure in the determination of drugs and catecholamines (HO *et al.*[3]) in picogram quantities.

DNS-Cl can form a derivative with morphine and the latter can readily be separated by polyamide TLC. Table I indicates the R_F values of DNS-morphine in the polyamide mini plates developed with a variety of solvent systems. It should be noted that the R_F value is lower when a non-polar solvent system was used.

Since DNS derivatives are readily soluble in trichloromethane, this was subsequently used to separate the products from the unreacted reagent. Table II shows that the efficiency of trichloromethane extraction is 70% and the percent conversion of morphine to DNS-morphine under our experimental condition is about 80%.

For quantitative measurement, a series of known quantities of [^{14}C]morphine was added to water, urine, as well as a 10% mouse brain homogenate. After shaking, morphine was extracted and dansylated and the DNS-[^{14}C]morphine spot was isolated as described in the method section. Table III clearly indicates a linear relationship between the amount of morphine added and the amount of DNS-[^{14}C]morphine recovered. It should be noted that while the recovery is much lower (about 50%) when morphine was isolated from the biological specimens, as little as 5 ng of [^{14}C]-morphine can be detected and quantitatively recovered. Better sensitivity can be achieved if [^{14}C]morphine with higher specific activity is available.

Application of the dansylation procedure to other psychoactive drugs was also tested. Table IV shows that morphine, naloxone, amphetamine, methamphetamine, as well as LSD can be dansylated. The DNS-derivatives of these drugs can easily be separated in one-dimensional TLC with polyamide plates. In a separate study, we have extended this procedure to the identification of mescaline, Bromo-LSD, catecholamines, serotonin, as well as some of their precursors and metabolites (HO AND LOH[4]).

Since morphine is quantitatively converted to DNS-morphine, the latter compound shows a fluorescence spectrum with an activation peak at 360 nm and a fluorescent peak at 520 nm. With a Farrand chromatogram analyzer, the intensity of the fluorescent spot was determined and a linear relationship between the amount of drug tested and the intensity of the fluorescent spots was found. Published results have shown as little as 2 ng of amphetamine and methamphetamine can be qualified by this method (LOH *et al.*[5]).

SEILER AND WIECHMANN[6] 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 (HO *et al.*[3,7]). 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 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 the chromatogram to develop. (2) Both sides of the polyamide thin-layer plate may be utilized for spotting. (3) Good separation with a discrete spot is achieved. (4) The plate can be re-used after washing.

The qualitative analysis of the narcotic as performed in our procedure permits detection of a picogram quantity of this drug in the biological fluid. Since DNS-Cl

reacts with primary and secondary amines, phenolic hydroxyl, thiols, and imidazoles, it is essential to purify partially the drug 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 precudure for the determination of other drugs are being continued in our laboratories.

We thank Miss BARBARA HALPERIN for her help in typing this manuscript. The financial support of USPHS Grant DA-00564 is also gratefully acknowledged.

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Received October 31st, 1972