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Note

Confirmation of morphine on thin-layer plates by fluorometry

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Thin-layer chromatography (TLC) is widely used for screening urine for drugs of abuse. The necessity of confirmation of results so obtained is well established[1,2]. Morphine is commonly detected on TLC plates at a minimum concentration of 0.2 μg/ml, about 1/5 the level of barbiturates, amphetamine and methadone[1]. Confirmation requires a method of corresponding sensitivity.

Kupferberg *et al.* developed a method of analysis for morphine dependent on the oxidation of morphine to pseudomorphine by potassium ferricyanide[3]. This reaction has been used to detect morphine in urinary extracts by comparison of fluorescence before and after addition of the oxidant[4]. We have adapted this method to the confirmation of morphine on TLC plates.

EXPERIMENTAL AND RESULTS

Urine was extracted by the method of Finkle[1] or Dole *et al.*[5]. The extract was chromatographed by the method of Davidow *et al.*[6]. Morphine was visualized with iodoplatinate spray[6]. Any spot with an R_F value close to the control morphine was completely scraped off from the plate and transferred to a 12-ml centrifuge tube. One-half millilitre of reagent-grade methanol was mixed with the adsorbent for 1 min with a vortex-type mixer. The tube was centrifuged briefly. The methanol was decanted into 2.5 ml of saturated sodium tetraborate in a 1-cm-pathlength quartz cuvette. Fluorescence was measured with a Farrand Model 104242 grating spectrofluorometer coupled with an Aminco Model 10-222 photomultiplier microphotometer. The excitation wavelength was 254 nm. The emission wavelength was 410 nm. The microphotometer was operated at maximum sensitivity. Two drops of potassium ferricyanide (57 mg/100 ml, diluted 1:3 prior to use) were added and the fluorescence was noted immediately and after 2 min. Typical results are presented in Table I.

The results obtained using methanolic extracts of silica gel were qualitatively similar to those reported for aqueous extracts of urine[4]. Due to photomultiplier noise, the microphotometer constantly fluctuated over a range of ± 0.002 relative intensity units. The upper extremes were used in preparation of the table. The fluorescence of the unoxidized extract at 5 μg/ml presumably represents the native fluorescence of morphine.

TABLE I

FLUORESCENCE OF MORPHINE EXTRACTED FROM TLC PLATES

Urine was extracted by the method of Finkle[1].

Morphine concentration (μg/ml)	*Relative intensity ± S.D. (n = 5)*		
	Prior to $K_3Fe(CN)_6$	*Immediately after $K_3Fe(CN)_6$*	*2 min after $K_3Fe(CN)_6$*
0	0.041 ± 0.001	0.040 ± 0.001	0.041 ± 0.001
0.2	0.048 ± 0.002	0.056 ± 0.006	0.059 ± 0.007
1.0	0.046 ± 0.001	0.070 ± 0.009	0.083 ± 0.012
5.0	0.072 ± 0.005	0.200 ± 0.028	0.324 ± 0.058

The only drugs other than morphine reported to yield greater fluorescence upon addition of potassium ferricyanide are hydromorphone, nalorphine and pentazocine[3]. Only hydromorphone has an R_F value close to morphine[6].

Large amounts of certain chromogenic sprays, including ninhydrin, mercuric sulfate and silver acetate, reduced the sensitivity of this test. When sprays other than iodoplatinate were used, the area where morphine appears was masked. Detection of other drugs was not impaired inasmuch as the R_F value of morphine is smaller than those of drugs visualized with the morphine interfering reagents[6].

REFERENCES

1 B. S. Finkle, *Anal. Chem.*, 44, No. 9 (1972) 18A.
2 R. J. Coumbis, C. C. Fulton, J. P. Calise and C. Rodriguez, *J. Chromatogr.*, 54 (1971) 245.
3 H. Kupferberg, A. Burkhalter and E. L. Way, *J. Pharmacol. Exp. Therap.*, 145 (1964) 247.
4 L. Goldbaum, P. Santinga and A. M. Dominguez, *Clin. Toxicol.*, 5 (1972) 369.
5 V. P. Dole, W. K. Kim and I. Eglitis, *J. Amer. Med. Ass.*, 198 (1966) 349.
6 B. Davidow, N. Li Petri and B. Quame, *Tech. Bull. Regist. Med. Technol.*, 38 (1968) 298.