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A note on a simple estimation of amphetamine, methylamphetamine and ephedrine in horse urine

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A chromatographic separation of amphetamine, methylamphetamine and ephedrine from horse urine is possible on alkaline Silica Gel G plates developed with acetone–methanol (1 : 3). After elution, the bases are determined colorimetrically. The intensity of the violet colour resulting from the nitration of amphetamine is measured in a Unicam SP1300 colorimeter using filter No. 1 (sensitivity 50–250 μg). The colour produced by the interaction of methylamphetamine, sodium nitroprusside, acetaldehyde and triethanolamine is measured at 590 mμ (sensitivity 200–2,000 μg). Ephedrine was determined by measuring the intensity of the brown colour of its dithiocarbamate at 440 mμ (sensitivity 100–900 μg).

THE separation of amphetamine and methylamphetamine from the saliva of animals (Bäumler, Brault & Obersteg, 1964) and of amphetamine from contaminants of body fluids (Eberhardt & Debackere, 1965) has been effected by thin-layer chromatography.

We have devised a simple thin-layer method for the estimation of amphetamine, methylamphetamine and ephedrine when present together in horse urine.

BASIC PROCEDURE

Introduce into a separator about 100 ml of horse urine containing variable amounts of amphetamine sulphate, methylamphetamine hydrochloride and ephedrine hydrochloride which either have been added or are suspected of being present as a result of drugging. Render the urine alkaline with ammonium hydroxide (TS). Extract the free bases with chloroform (4 × 20 ml), wash each chloroform extract with the same 5 ml of water. Dry the combined chloroform extract with anhydrous sodium sulphate and concentrate. Repeat the procedure using another 100 ml of horse urine free from stimulant as a control.

Chromatograph the concentrated chloroform extract on thin-layer plates, coated with 0·3 mm of silica gel G along with the authentic bases using acetone–methanol (1 : 3) as developing solvent. Allow the solvent front to travel approximately 15 cm (30–35 min). Remove and air dry the plates and locate the spots by spraying with a reagent of equal volumes of 1% sodium nitroprusside solution and acetaldehyde, followed by a 20% sodium carbonate solution (Wachsmuth & Köckhoven, 1962). Quantitatively transfer the relevant zones to a micro-column (0·4 × 10 cm) and elute with 10 ml of ethanol. After evaporation of the solvent, estimate the residue colorimetrically.

Amphetamine. The nitration test is a modification of that of Rathenasinkam (1951): to the residue, add 0·1 g of potassium nitrate and 2 ml of sulphuric acid. Heat in boiling water for 15 min, cool and quantitatively transfer with distilled water to a separator. Wash with chloroform, make alkaline with ammonium hydroxide and extract with chloroform.

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Evaporate the chloroform, dissolve the residue in 10 ml of acetone and add 2 drops of 0·5% potassium hydroxide in methanol–acetone (97:3). After 10 min, measure the purple colour in a Unicam SP1300 colorimeter using filter No. 1 (370–515 mμ), comparing with the control. The colour obeys Beer's law in the range of 50–250 μg of amphetamine. It reaches its maximum after 10 min and is stable for another 15 min.

Ephedrine. Estimation was by the micro method of Karawya, Wahba & Kozman (1967): dissolve the residue in a few drops of dilute hydrochloric acid and make up to 1 ml with distilled water. Add 5% copper sulphate solution (1 ml), concentrated ammonium hydroxide (3 drops) and carbon disulphide–benzene (1:3) (5 ml). Shake thoroughly for 5 min, separate the organic layer and measure the colour with a Beckman DU spectrophotometer at 440 mμ against the control. The sensitivity is 100–900 μg of ephedrine.

Methylamphetamine. A modification of the test of Wachsmuth & Köckhoven (1962) is used: dissolve the residue in the least amount of dilute hydrochloric acid, make up to 2 ml with distilled water, add acetaldehyde (3 drops), freshly prepared 0·5% sodium nitroprusside solution (1 ml) and 1% triethanolamine solution (1 ml). Measure the blue colour after about 6 min at 590 mμ against the control. The colour obeys Beer's law over a concentration range of 200–2000 μg of base. The colour reaches its maximum after 5 min and remains stable for a further 5 min.

The mean Rf values of the bases and the urine pigments, together with the colours of the corresponding spots produced by the locating reagent, are shown in Table 1, while the results of the quantitative estimation of the bases are shown in Table 2.

TABLE 1. THE MEAN R_F VALUES OF AMPHETAMINE, METHYLAMPHETAMINE, EPHEDRINE AND URINE PIGMENTS

Substance	Rf	Colour developed with	
		Ninhydrin reagent	Sodium nitroprusside reagent
Amphetamine	0·55	Violet	Olive green
Methylamphetamine	0·24	Violet	Blue
Ephedrine	0·03	Violet	Purple
Pigments	0·95	Pale violet	—

TABLE 2. ANALYSIS OF DIFFERENT MIXTURES OF AMPHETAMINE, EPHEDRINE AND METHYLAMPHETAMINE IN SAMPLES OF HORSE URINE

Mixture No.*	Amphetamine sulphate (μg)			Methylamphetamine hydrochloride (μg)			Ephedrine hydrochloride (μg)		
	Added	Recovered	Error %	Added	Recovered	Error %	Added	Recovered	Error %
I	100	100	0·0	200	210	+5	100	100	0·0
II	150	152	+1·3	400	400	0·0	200	196	−4
III	200	205	+2·5	800	760	−5	400	410	+2·5
IV	250	248	−0·8	1,600	1,650	+3·1	800	760	−3

Each value is the average of three experiments.

APPLICATION TO URINE OF DRUGGED HORSES

Urine from five horses of between 250–300 kg weight collected in leather bags tied under their bodies for 24 hr was used as a control. Respective animals were then injected subcutaneously with 100 mg of amphetamine sulphate; with 100 mg of methylamphetamine hydrochloride; with 100 mg of ephedrine hydrochloride; with 50 mg of amphetamine sulphate + 50 mg of ephedrine hydrochloride and with 50 mg of methylamphetamine hydrochloride + 50 mg of ephedrine hydrochloride.

The urine of each animal was collected by catheter after 3, 12, 24, 48 and 72 hr. In addition, a leather bag was used to collect urine passed between the specified collection time, adding it to the next sample collected by catheter. The urine samples were then analysed as described above. The results are shown in Table 3.

TABLE 3. THE EXCRETION OF AMPHETAMINE, METHYLAMPHETAMINE, EPHEDRINE AND THEIR MIXTURES

Time of collection of urine samples (hr)	1st horse	2nd horse	3rd horse	4th horse		5th horse	
	Amphet. %	Me. amphet. %	Ephed. %	Amphet. %	Ephed. %	Me. amphet. %	Ephed. %
3	17·0	7·0	5·5	18·0	6·0	7·4	5·2
12	13·6	13·0	—*	13·7	—	13·0	—
24	18·0	—	—	18·0	—	—	—
48	7·0	—	—	7·0	—	—	—
Total	55·6	20·0	5·5	56·7	6·0	20·4	5·2

* No amine could be detected in corresponding samples.
The percentage of excreted base is calculated with respect to free base content of the administered corresponding salts.

Beckett and others (see Beckett & Rowland, 1965a,b; Beckett & Wilkinson, 1965) found the amount excreted and the rate of excretion of these amines to be pH dependent; larger amounts are excreted at lower pH. The pH of the collected urine samples ranged from 5·9 to 6·7.

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