CHROM. 3619

DETECTION OF DOPING BY THIN-LAYER AND GAS CHROMATOGRAPHY

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SUMMARY

An analytical procedure for the detection of amphetamine and related drugs in urine is reported. The method consists of a preliminary screening by thin-layer chromatography, followed by scraping the suspicious spots from the plates for confirmation by gas–liquid chromatography on different columns (Carbowax 20 M and Apiezon L). The excretion of amphetamine in man is detectable from 1–2 h after ingestion until 3–4 days later.

In many countries anti-doping control is carried out to prevent the use of various drugs in sporting events. In Italy the Italian Medical Sport Federation (FMSI) undertakes this control, especially in football games and cycle races. Among the drugs most commonly used to improve performance in sport are the amphetamines and related substances. The methods developed for their determination are based on paper[1], thin-layer[2], and gas chromatography[3–5].

The analytical procedure described here, which is used as a routine method for anti-doping control, consists of a preliminary screening by thin-layer chromatography and a subsequent confirmatory test by gas chromatography of a suspicious spot. In this way it is possible to examine a large number of samples in a short time and to support the indication of positive results by gas chromatography with two different liquid phases.

The analyses are carried out on urine, where the unchanged drugs are found at higher concentrations than in the other biological fluids.

EXPERIMENTAL

Extraction procedure

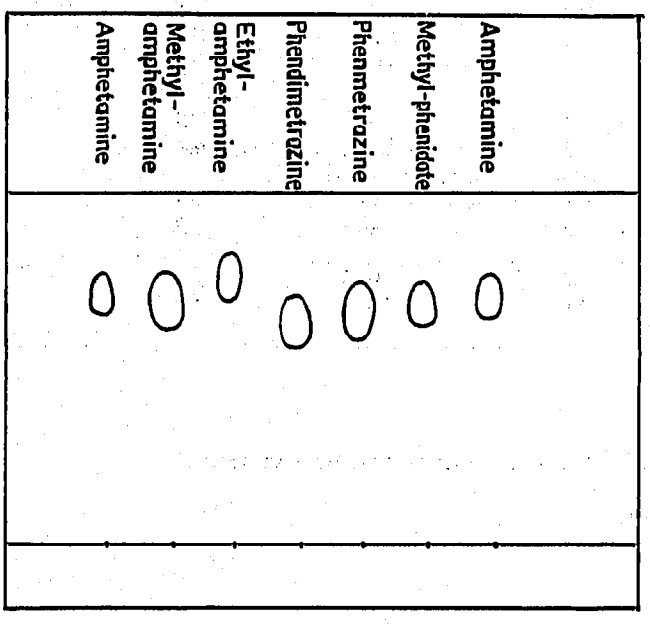
From 5 to 10 ml of urine are placed in a glass-stoppered centrifuge tube and 1 ml of 5 *N* NaOH is added.

The whole is extracted three times with 5 ml of ether with centrifugation always following shaking. The ether extracts are dried over sodium sulphate, transferred to a small test tube having a finely tapered base and then concentrated to 10–15 μl.

Thin-layer chromatography

This technique is used for a rapid analysis of a large number of samples. Only the suspicious spots are scraped from the plate and examined by gas chromatography. For this purpose one must select highly sensitive spray reagents that do not destroy the substances to be tested. Bromocresol green indicator is sensitive to 1 μg of amine and thereby permits recovery of the unchanged drug. This reagent is prepared by mixing a 0.5 *M* phosphate buffer solution of pH 5.5 with a 0.1 % alcoholic solution of the indicator and water in the ratio of 1:2:1.

The plates (20 × 20 cm) are coated with cellulose powder (Whatman CC41) and developed with *n*-butanol–formic acid–water in the ratio of 20:1:2. After drying at 60°, they are sprayed with the above reagent and the spots with R_F corresponding to that obtained for the reference compound are removed for a gas chromatographic confirmatory analysis. Figs. 1 and 2 show plates containing different amounts of amphetamine and some related drugs extracted from urine.



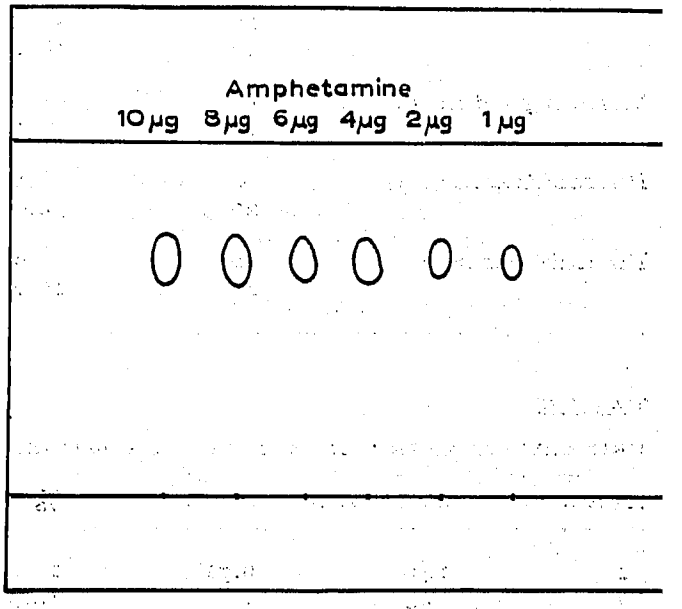


Fig. 1. Thin-layer chromatography of amphetamines and related drugs.

Fig. 2. Thin-layer chromatography of different amounts of amphetamine.

Gas–liquid chromatography

About 1 cm^2 of the positive spots are scraped from the thin layer. The powder is collected in a small test tube, made basic with a few drops of 5 *N* NaOH and extracted as before (three times with 1 ml of ether). The ether extracts, when dried and concentrated, are examined by gas chromatography on two different columns. Glass columns (2 m long, 0.25 cm I.D.) are used on a Carlo Erba (Italy) gas chromatograph model C equipped with a flame ionization detector. The support, Chromosorb 80–100 mesh, washed with 5 % alcoholic KOH solution, is coated with 10 % of the liquid phase. Two different partition liquids are used: a non-polar one (Apiezon L) and a polar one (Carbowax 20 M). Pure nitrogen serves as the carrier gas. The samples plus the standard are injected as solutions in ether (1–10 μl) with a splitter in the ratio of 1:10. Less than 0.1 μg of each amine can be easily detected.

RESULTS

The complete procedure was carried out for some of the amines and the results are reported in Table I. The amine was added to 10 ml of water or urine and, following the procedure described above, the amount recovered was calculated (using N,N-dimethylaniline as internal standard, added to the ethereal extract before the injection).

TABLE I

RECOVERY OF SYMPATHOMIMETIC AMINES AFTER TLC AND DETERMINATION BY GLC

Compound	*μg added*	*μg extracted from water*	*% recovery*	*μg extracted from urine*	*% recovery*
Amphetamine	5	3.7	75	3.0	60
	10	9.0	90	7.4	74
	15	13.2	86	12.8	85
	20	18.2	91	19.5	97
Methamphetamine	10	8.2	82	6.5	65
	20	18.8	94	16.8	84
Phenmetrazine	10	8.1	81	9.0	90
	20	18.0	90	14.0	70
Phendimetrazine	10	8.7	87	6.5	65
	20	16.2	81	16.0	80

TABLE II

URINARY EXCRETION IN MAN AFTER ADMINISTRATION OF 10 mg OF D-AMPHETAMINE

Hours	*ml of urine*	*μg/ml*	*% excretion*
2	140	0.71	1
4	125	1	1.25
8	170	3.79	6.49
12	245	2.18	5.36
16	170	4.61	7.84
20	105	3.81	4.01
24	50	3.56	1.78
28	205	0.31	0.63
32	170	0.44	0.75
36	435	0.04	0.17
40	300	0.04	0.114
44	130	0.05	0.068
48	70	0.03	0.024
52	165	0.02	0.046
56	245	0.02	0.047
60	255	0.03	0.092
64	170	0.02	0.048
68	145	0.03	0.040
72	130	0.002	0.003
76	—	—	—
		Total	29.7

As shown in Table I the method gives a recovery of 60–90 %. These figures can be considered satisfactory because quantitative evaluations cannot be carried out in this anti-doping control since the urinary excretion of the drugs is greatly affected by pH and many other physiological factors.

The method is very specific. When only the "positive" spots obtained from TLC are examined by gas chromatography, the gas chromatograms are simple and the results reliable. After the drug has been well characterized and identified, urinary excretion can be followed directly by gas chromatography, as shown by the data in Table II. These values refer to the amphetamine determination on man following an intake of 10 mg. After 1 h it is already present in the urine and can still be detected 4–5 days later.

With this procedure, which combines the advantages of TLC and GLC, many different amphetamines and related drugs can be detected for anti-doping control. A specific identification excludes the interference of other urinary constituents, making positive results clearly evident.

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