*Journal of Chromatography*, 291 (1984) 471–475
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,546

## Note

# Determination of some doping agents by overpressured thin-layer chromatography

H. GULYÁS* and G. KEMÉNY
*Labor MIM, H-1445 P.O. Box 280, Budapest (Hungary)*
and
I. HOLLÓSI and J. PUCSOK
*Research Laboratory of the National Institute for Medicine of Physical Education and Sports, Alkotás u. 48, 1123 Budapest (Hungary)*
(Received December 28th, 1983)

Methods based on thin-layer chromatography (TLC)[1–3], high pressure liquid chromatography (HPLC)[4,5], gas chromatography[6–8] and gas chromatography–mass spectrometry[9,10] have been described for the separation and identification of doping agents.

Overpressured thin-layer chromatography (OPTLC) combines the advantages of classical TLC, HPTLC and HPLC[11–13], *i.e.*, large numbers of samples, high resolution and speed and the use of selective developing reagents. We have utilized these advantages for the separation and determination of doping agents, *i.e.*, the most frequently used volatile stimulants and sympathomimetic amines strychnine, ephedrine, Coramin, Desopimon, methamphetamine, amphetamine, methylphenidate, phenmetrazine and caffeine.

## EXPERIMENTAL

### *Materials and methods*

The sample was spotted by a Linomat III applicator (Camag, Muttenz, Switzerland). The sorbent layer was HPTLC silica gel 60 $F_{254}$ (Merck, Darmstadt, F.R.G.) with impregnated edges on three sides. For the chromatographic separation a Chrompres-10 chamber (Labor MIM, Budapest, Hungary) was used. As supplied, the chamber has an operating membrane pressure of 1 MPa, via a single head piston pump, delivering water into the membrane system. The eluent is delivered into the sorbent layer by a similar piston pump but with adjustable flow-rate. During the development the layer was covered by a polyethylene sheet 0.2 mm thick.

The quantitative evaluation was accomplished by use of a Shimadzu CS-920 High Speed TLC/HPTLC Scanner (Shimadzu, Japan) at 210 nm.

The standard mixture was prepared by dissolving strychnine (Alkaloida, Hungary), ephedrine (Chinoin, Hungary), methylphenidate and Coramin (Gedeon Richter, Hungary), amphetamine (Chinoin), phenmetrazine and Desopimon (EGYT, Hungary), caffeine and methamphetamine (Sigma, St. Louis, MO, U.S.A.), 10 mg/ml, in methanol. All solvents were reagent grade used without further purification.

0021-9673/84/$03.00 

*Preparation of samples*

To 5 ml urine were successively added 0.5 ml of 5 *M* KOH, 3.2 g of sodium sulphate and 2 ml of diethyl ether. The reagents were thoroughly mixed and extracted for 1 min in a Vortex agitator. After centrifugation at 2000 rpm for 5 min the organic phase was transferred to a 2-ml vial, evaporated in a nitrogen atmosphere and the residue was spotted on the sorbent layer.

## RESULTS AND DISCUSSION

Several solvent systems were tested in various combinations and ratios of *n*-butanol, chloroform, tetrahydrofuran, dichloromethane, methyl ethyl ketone, water and acetic acid. In all these systems the most critical separations were the overlapping pairs of ephedrine–methamphetamine, phenmetrazine–methylphenidate and methylphenidate–amphetamine. The most selective and efficient solvent system proved to be *n*-butanol–chloroform–methyl ethyl ketone–water–acetic acid (25:17:8:4:6).

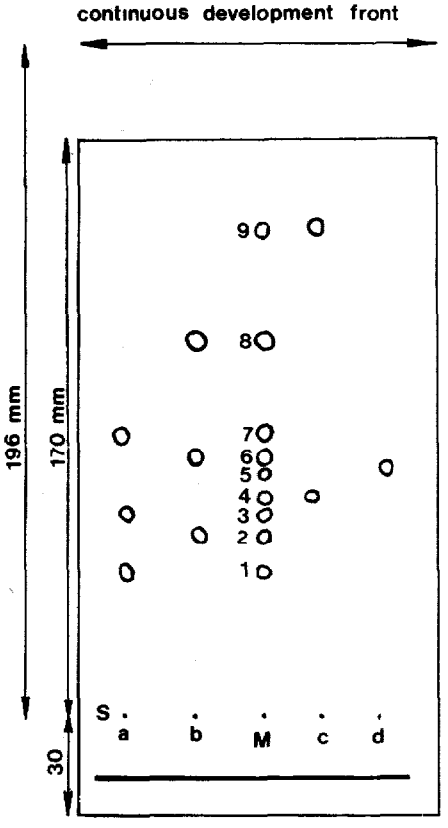


Fig. 1. Separation of a standard mixture (M) of doping agents: 1 = strychnine; 2 = ephedrine; 3 = methamphetamine; 4 = phenmetrazine; 5 = methylphenidate; 6 = amphetamine; 7 = Desopimon; 8 = Coramin; 9 = caffeine; a = 1, 3, 7; b = 2, 6, 8; c = 4, 9; d = 5. S = Start. Sorbent: HPTLC silica gel 60 $F_{254}$ (Merck, Darmstadt, F.R.G.). Eluent: *n*-butanol–chloroform–methyl ethyl ketone–water–acetic acid (25:17:8:4:6); flow-rate 0.85 cm/min. External membrane pressure: 1.0 MPa.

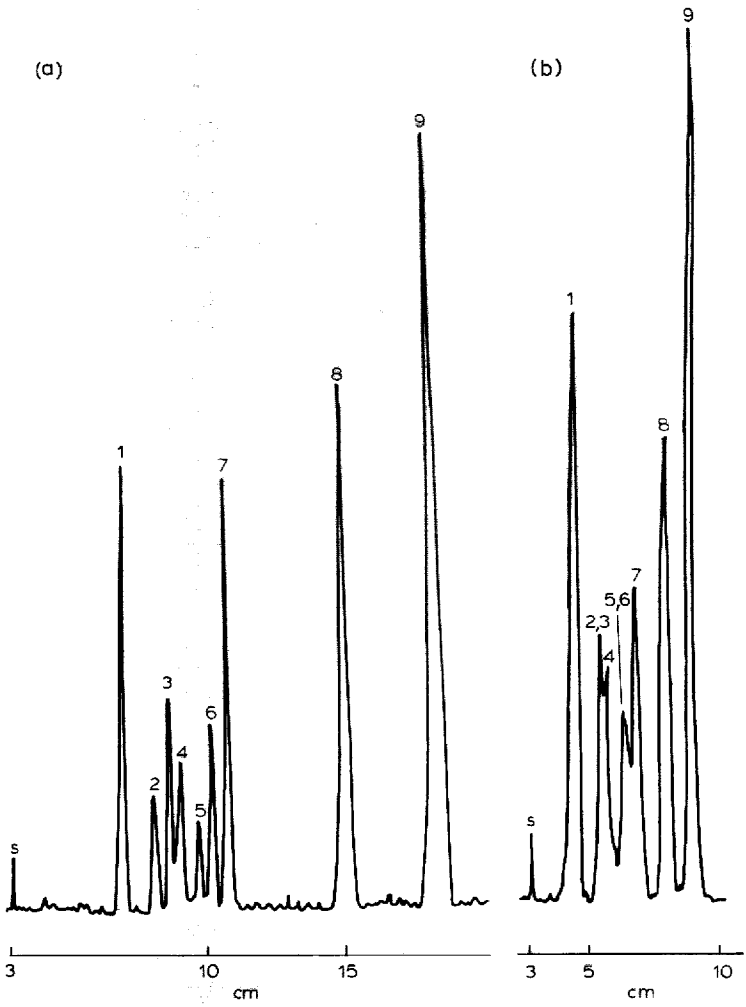


Fig. 2. Densitogram of standard mixture in Chrompres-10 (a) and a conventional chamber (b).

For optimum migration length on the 200 × 200 mm chromatoplate, about 20% continuous development was necessary. The velocity of the solvent front was 0.85 cm/min.

All of the tested doping agents can be separated and determined directly in a short time in Chrompres-10 (Fig. 1).

In comparison with the classical TLC method, the resolution was improved at 1.0 MPa external membrane pressure. The development time was shorter (about 25 min, including the continuous development) than in TLC and the detection limit was lower (0.5–1.0 $\mu$g) than in a normal chamber (95 min for 140-mm development and 10–50 $\mu$g respectively). The quantitative evaluation was carried out on a Shimadzu CS 920 TLC/HPTLC Scanner (Japan) at 210 nm.

Caffeine, Coramin and strychnine gave the most intense signals at 250–280 nm, but the other substances could not be detected at this wavelength. So the quantitative

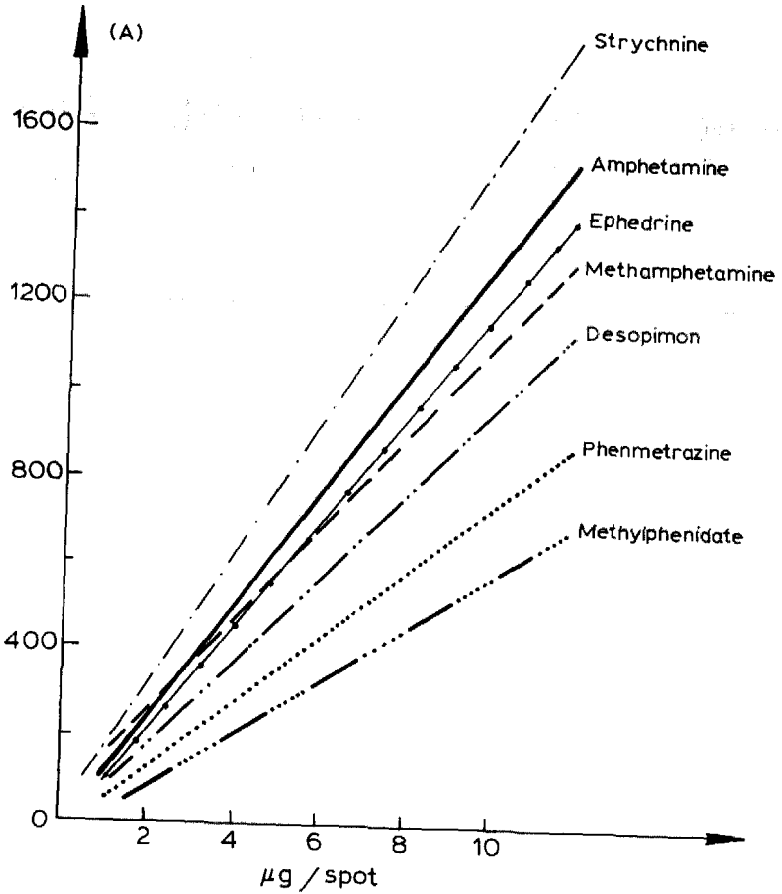


Fig. 3. Calibration curves of some doping agents.

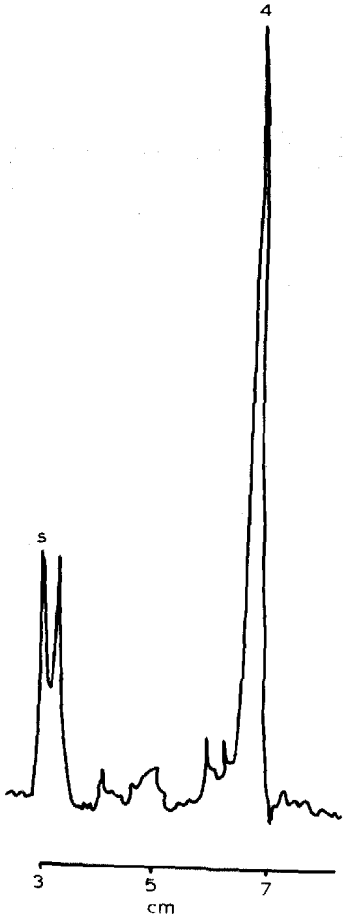


Fig. 4. Densitogram of doping agents in urine sample. Conditions as in Fig. 1. Peak 4 = strychnine.

evaluation was accomplished at 210 nm with an acceptable detection limit for all compounds. Fig. 2 shows the densitogram of the standard mixture at this wavelength.

The preparation of the standard samples used for the calibration curves was accomplished under the same conditions as those for the urine samples. Fig. 3 shows the calibration curves of some doping agents. All of these are approximately linear in the range of investigation. Eleven parallel measurements were used (2 $\mu$g in each) for the reproducibility test. The integrated areas under the peaks of the densitogram on the same plate show a 5% coefficient of variation.

The described method was applied for the determination of strychnine in urine of athletes (Fig. 4).

The average analysis time per sample for a single operator with the described method is about 8–10 min.

## REFERENCES

1 G. Gübitz and R. Wintersteiger, *J. Anal. Toxicol.*, 4 (1980) 141.
2 A. H. Beckett and M. Rowland, *J. Pharm. Pharmacol.*, 1 (1965) 628.
3 G. P. Cartoni and A. Cavalli, *J. Chromatogr.*, 37 (1968) 158.
4 Y. Yamamoto and K. Yamamoto, *Jap. J. Legal Med.*, 34 (1980) 158.
5 I. Jane, *J. Chromatogr.*, 111 (1975) 227.
6 M. Terada, T. Yamamoto, T. Yoshida, Y. Kuroiwa and S. Yoshimura, *J. Chromatogr.*, 237 (1982) 285.
7 R. D. Budd, *J. Chromatogr.*, 245 (1982) 129.
8 A. H. Beckett and G. R. Wilkinson, *J. Pharm. Pharmacol.*, 17 (1965) 104S.
9 R. Dugal, M. Bertrand and R. Masse, *Farm. Tijdschr. Belg.*, 55 (1978) 3.
10 A. K. Cho, B. Lindeke, B. J. Hodson and D. J. Jenden, *Anal. Chem.*, 45 (1973) 570.
11 E. Tyihák, E. Mincsovics and H. Kalász, *J. Chromatogr.*, 174 (1974) 75.
12 E. Tyihák, E. Mincsovics, H. Kalász and J. Nagy, *J. Chromatogr.*, 211 (1981) 45.
13 E. Tyihák, T. J. Székely and E. Mincsovics, in R. E. Kaiser (Editor), *Proc. 2nd Int. Symp. for HPTLC, Interlaken*, Institute for Chromatography, Bad Dürkheim, 1982, p. 159.