Detection and quantitative determination of pemoline [Stimul®] in urine

A-49.3

Key words

Instrumental TLC - quantitative analysis - densitometry by absorbance - post chromatographic derivatization - spectra comparison - therapeutic drug monitoring - doping control - sympathomimetics - stimulants - psychotonics - pemoline [Stimul®]

Scope

Pemoline is abused in sports for doping purposes and is therefore on the IOC (Int. Olymp. Committee) list of banned drugs.

A cost effective screening method is described allowing to analyze a large number of samples at the same time. If the post chromatographic derivatization reaction indicates the presence of pemoline, the result is verified by spectra comparison.

Densitometric quantification is performed by absorbance at 220 nm with the underivatized fraction. Determination limit is 0.1 mg/L, reliable detection limit <0.1 mg/L.

(An independent final verification is possible by gas chromatography/mass spectrometry of a methylated extract.)

Literature

- Report VII of the DFG Commission for Clinical-Toxicological Analysis, Special issue of the TIAFT Bulletin: Thin-layer chromatographic Rf values of toxicologically relevant substances on standardized systems. VCH Publishers, D-6940 Weinheim, 1987
- W. Bernhard, A.N. Jeger (Institute for Forensic Chemistry, Basel, Switzerland), private communication

Advantages of using planar chromatography for this analytical task

- High sample throughput at low operating costs
- Positive identification in doping control; (specific derivatization and spectra comparison)
- Method also suitable for therapeutic drug monitoring and pharmacokinetics

Chemicals

Diethyl ether
1,3-dinitrobenzene
Ethanol
Methanol
Ammonia 10%
Potassium hydroxide
Standard: Pemolin (Sera)

Extrelut® columns (Merck)
Hydrochloric acid 10%
Ammonium chloride
Sodium sulfate
Ethyl acetate

Sample preparation

- Adjust 10 mL urine sample with ammonium chloride - ammonia buffer (pH 9.5) to pH 8.5 - 9.0.
- Add 8 mL water and percolate the alkaline sample through an Extrelut column.
- After 15 min elute 2x with 40 mL diethyl ether.
- Dry combined ether phases with Na_2SO_4, filter through cotton wool and evaporate to almost dryness in water bath at 60°C under normal pressure.
- Remove residual ether.
- Dissolve residue in 0.2 mL methanol (alkaline extract).

Standard solutions

Analogously extract 50 mL urine of a person who has not received pemoline (blind extract). Dissolve alkaline residue in 1 mL methanol.

Stock solution: dissolve 10 mg pemoline with methanol to a volume of 100 mL (10 µL = 1 µg).

Into 5 V-shaped vials pipette 10, 20, 40, 60, 80 µL stock solution and evaporate. Dissolve residue in 100 µL alkaline blind extract. Related to urine the standard levels are:

S1 = 0.20 mg/L, S2 = 0.40 mg/L, S3 = 0.80 mg/L, S4 = 1.20 mg/L, S5 = 1.60 mg/L

Layer

HPTLC plates Merck silica gel 60 F_{254}, 20x10 cm*

Sample application

With CAMAG Linomat as 7 mm bands, track distance 3 mm, distance from left edge 12 mm, distance from lower edge 5 mm, delivery rate 4 s/mL = 18 applications per plate side*.

Recommended application pattern for the quantitative determination in doping analysis and drug monitoring (for doping control screening, considerably less standards are required, e.g. S1 and S3):

Application pattern:

B	U1	S1	U2	S2	U3	B	U4 ... B = blind extract, U = unknown, S = standard
6	6	6	6	6	6	6	6... µL/track

Chromatography

In CAMAG Horizontal Developing chamber 20x10 cm*, in saturated configuration with ethyl acetate - methanol - NH_3 85:10:5; R_f about 0.38.

The further procedure depends on the purpose of the analysis:

For **doping control**, that is in all cases, in which first a qualitative identification is required, post chromatographic derivatization is employed. All samples for which the derivatization is pemoline positive, are chromatographed on a second plate. For result verification spectra comparison of the underivatized fractions is carried out followed by quantitative measurement. This way, two independent detection/identification results are obtained.

For **drug monitoring** densitometric evaluation without prior derivatization is sufficient.

Postchromatographic derivatization

- After spectrum scan, immerse plate in 1,3-dinitrobenzene (5 g/130 mL ethanol),
- dry plate with a hair dryer,
- immerse in a 10 % ethanolic KOH and dry.

Under the hot air stream pemoline appears as a red-violet spot, which disappears under light in a short time.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning absorbance at 220 nm. Quantification of underivatized chromatograms via peak height, of derivatized via peak area.

Spectra scan of underivatized pemoline-fraction for positive identification of substances (doping analysis) in the UV 200-350 nm.

---

* Compared with the conventional TLC precoated plate, the HPTLC plate offers a better cost-effectiveness, even when the modern Horizontal Developing chamber is not available and a twin trough chamber is used instead. In this case, for sample application, the distance from the lower edge should be 8 mm.

In principle, the conventional TLC precoated plate Merck silica gel 60 F_{254}, 20x10 cm can also be used. Then 10 mL of each sample and standard are applied as 10 mm bands, 5 mm apart; migration distance = 80 mm.

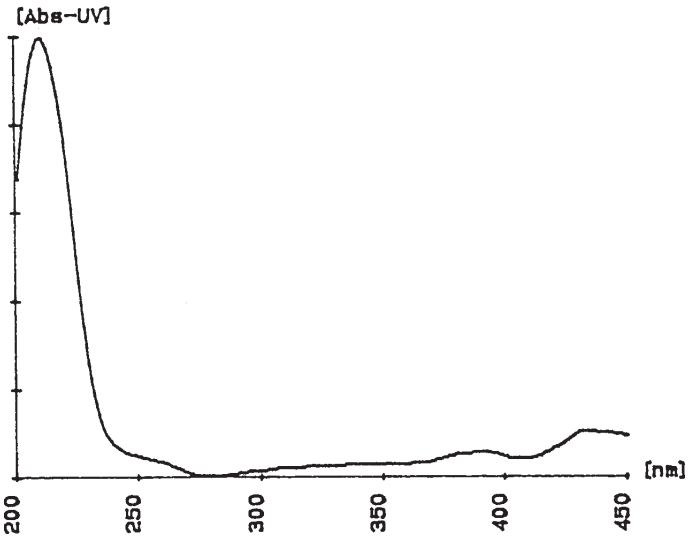

Fig. 1 In-situ UV absorption spectrum of pemoline from an urine sample

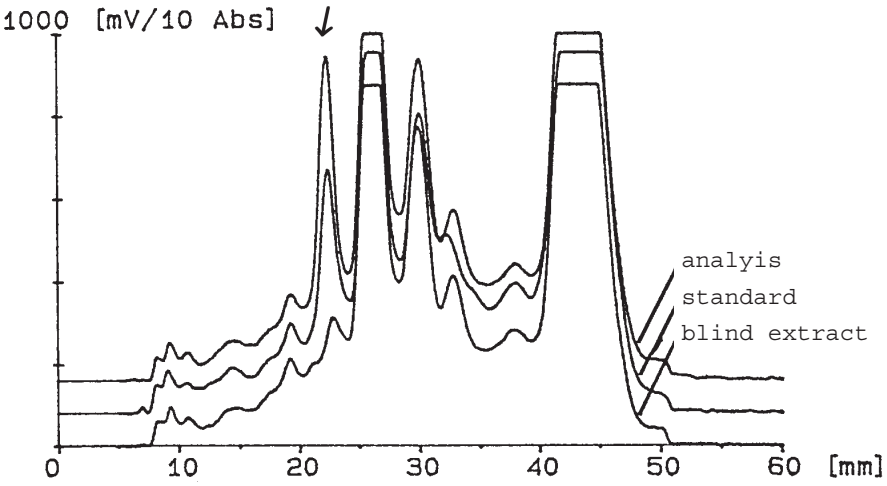

Fig. 2 Superimposed densitograms

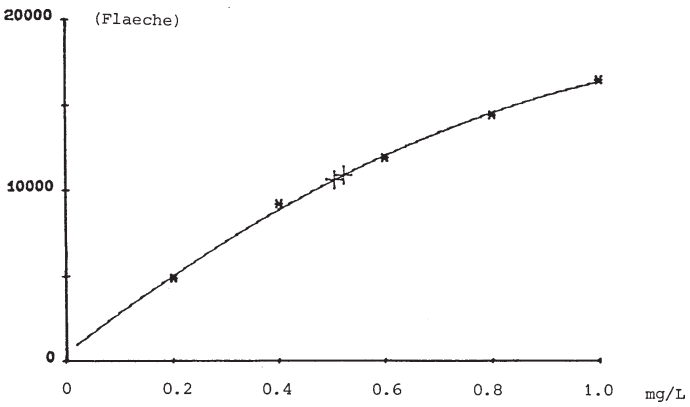

Fig. 3 Calibration curve of pemoline in the range 0.2-1.0 mg/L