HPTLC determination of caffeine in urine A-44.2

Key words

Instrumental HPTLC - quantitative analysis - densitometry by absorbance - doping control - stimulant - caffeine

Scope

Sports associations allow athletes to consume food containing caffeine during competitions. Because stimulants like amphetamines and ephedrines can be readily detected in urine, athletes often resort to caffeine as a stimulant.

In 1987, the Medical Commission of the International Olympic Committee (IOC) determined the limit for caffeine in urine at 12 mg/L.

Caffeine is extracted from urine with chloroform after increasing the ionic strength by the addition of sodium chloride. The extract is chromatographed on silica gel. Densitometric quantification is performed by absorbance at 254 nm. Recovery (n=9) was found to be 100.8%, the precision of the method is 1.0%. The caffeine fraction in the unknown is positively identified by in-situ spectroscopy.

Literature

K. Schwarten, D.K. Baron: Leistungssport (2), 11-14 (1986)

Advantages of using planar chromatography for this analytical task

- Simple procedure; short analysis time;
- Reliable result verification by positive identification;
- Cost effectiveness.

Chemicals

Methanol p.a.
Chloroform p.a.
6N sodium hydroxide solution
Sodium chloride p.a.
Standard: caffeine

Sample preparation

- Pipette 2.0 mL urine into a 10 mL centrifuge vessel, add 0.2 mL 6N sodium hydroxide solution and mix.
- Add 2 g sodium chloride*.
- Then mix with 2.0 mL chloroform, close the vessel airtight and shake for about 5 min.
- Centrifuge 5 min; use the organic phase for chromatography.

Standard solution

Methanolic solution of 4.0 mg caffeine in 100.0 mL.

Layer

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

Sample application

With CAMAG Linomat as 8 mm bands, track distance 6 mm, distance from left edge 12 mm, distance from lower edge 8 mm, delivery rate 8 s/µL = 13 applications per plate.

Application pattern:

S1	U1	U2	S2	U3	U4	S3	U1	U2	S4	...	
4	20	20	5	20	20	6	20	20	7	...	µL/band
8			10			12			14	...	≙ mg/L urine

S1-S4 = standards; U1 - U4 = unknowns

Alternatively the Automatic TLC Sampler can be used.

* The extraction procedure described is considerably more efficient than extracting with ethyl ether after adding anhydrous sodium sulfate, which often yields only 50% recovery and a precision around 5-8%.

Chromatography

In CAMAG Horizontal Developing chamber or in Twin-Trough chamber 20x10 cm with chloroform - methanol 9:1 after 10 min pre-equilibration; migration distance 50 mm.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning absorbance at 254 nm

The recovery was found = 100.8% (n=9), the precision of the method is about 1%.

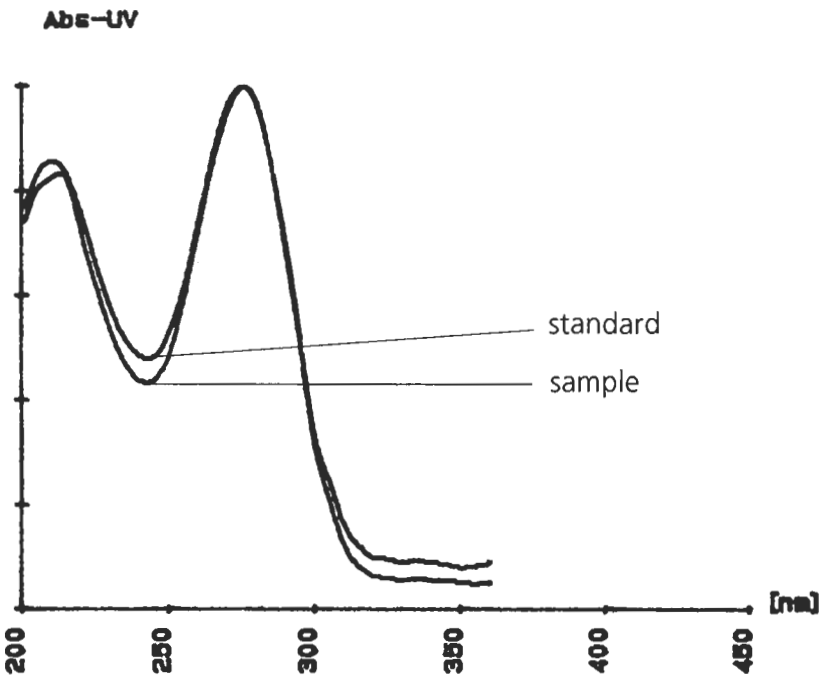

Fig. 1 UV absorption spectra of caffeine standard and the respective fraction of the unknown

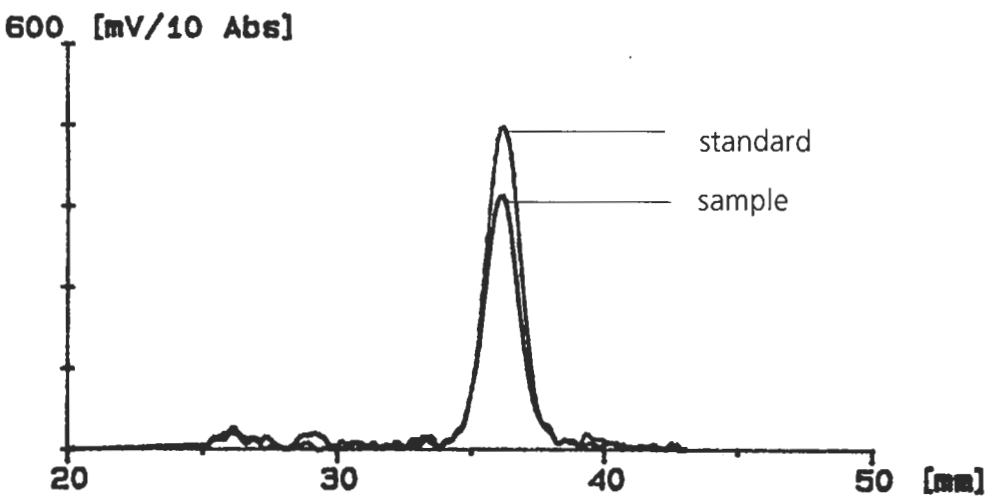

Fig. 2 Plotted superimposed: analog curves (absorbance at 254 nm) of chromatogram tracks with caffeine standard and with unknown; calibration standard with 60 ng corresponding to a concentration of 3.0 mg/L, i.e. about 25% of the tolerated value.

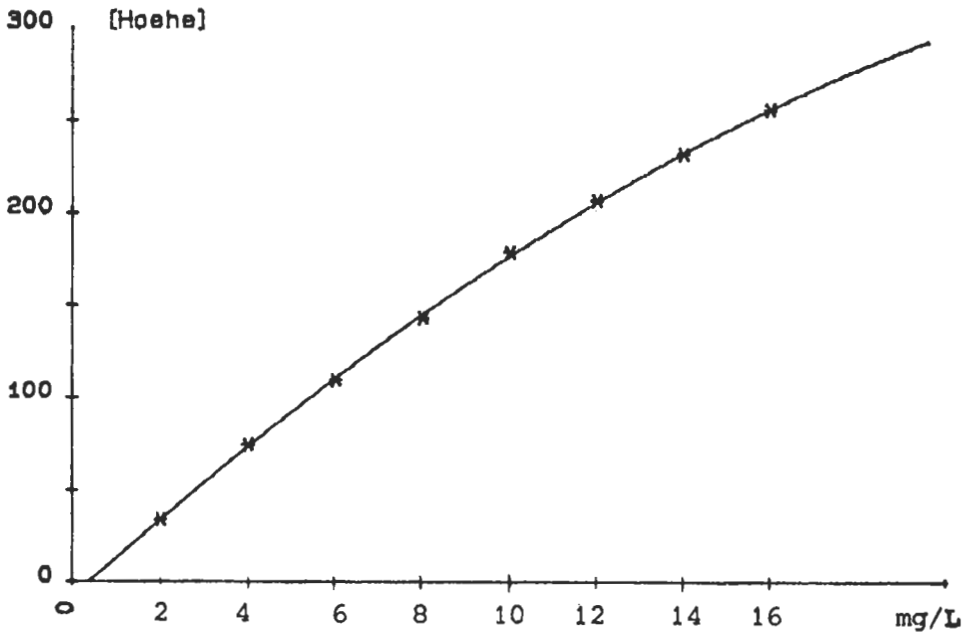

Fig. 3 Calibration curve for caffeine; calibrated range of 40-320 ng corresponding to 2-16 mg/L in the urine sample; polynomial 2nd degree, regression quality 1%.