Detection and quantitative determination of furosemide (Lasix®) in urine

A-46.3

Key words

Instrumental HPTLC - quantitative analysis - densitometry by absorbance - post chromatographic derivatization - drugs identification - therapeutic drug monitoring - doping control -diuretic - antihypertonic - furosemide (Lasix®)

Scope

Furosemide is the most often prescribed diuretic. Combined with beta-receptor-blockers it is used for medical treatment of hypertonicity. Moreover, furosemide is abused in human as well as in horse sports for doping purposes and is therefore on the IOC (Int. Olymp. Committee) list of banned drugs.

The acidic urine extract is chromatographed on silica gel. Postchromatographic derivatization converts furosemide into azo dyes. Results can be verified by UV absorbance spectra of the underivatized substances. Densitometric quantification is performed by absorbance at 275 nm. Determination limit is about 0.2 mg/L, reliable detection limit about 0.1 mg/L.

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, S.R. Rippstein, A.N. Jeger, Institute for Forensic Chemistry, Basel: Screening and detection of diuretics by instrumental TLC. Paper presented at the 5. Cologne Workshop on Doping Analysis, Deutsche Sporthochschule, 1987.

Advantages of using planar chromatography for this analytical task

- High sample throughput at low operating costs
- Positive identification in doping analysis
- Method also suitable for therapeutic drug monitoring and pharmacokinetics

Chemicals

Diethyl ether
Ethanol
Ethyl acetate
Methanol
N-(1-napthyl)-ethylenediammonium dichloride
Amidosulfonic acid ammonium salt
Hydrochloric acid 37%
Sulfuric acid (10% aqueous)
NH_3 25%
Sodium nitrite
Sodium sulfate (anhydrous)

Standard: furosemide (purum)

Sample preparation

- Adjust 10 mL urine sample with sulfuric acid (10% aqueous) to pH 2 and extract with 50 mL diethyl ether.
- Separate organic phase, dry with sodium sulfate, filter through a cotton ball and evaporate to dryness in water bath at 60°C under normal pressure.

> (For simultaneous testing for the diuretic chlortalidone (hygrotone) the aqueous phase can be extracted alkaline and further processed as described in application note A-47.)

- Dissolve residue in 0.2 mL methanol.

Standard solutions

Extract 50 mL urine as described above of a person who has not received Lasix, dissolve residue in 1 mL methanol = "acidic blind extract".

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

Into 5 V-shaped vials pipette 20, 40, 60, 80 and 100 µL stock solution and evaporate under nitrogen. Dissolve residue in 100 µL acidic 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 8 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 of furosemide is about 0.28.

The procedure depends now 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 in which a red colored fraction in the critical area occurs, 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

- Expose plate to HCl gas in a chamber for 10 min.
- Dry for 5 min at 120°C and let cool to room temperature.
- Spray with $NaNO_2$ solution (10% aqueous).
- Then spray with HCl solution (10% aqueous), dry plate with a hair dryer in a stream of cold air.
- Overspray with 0.5% aqueous solution of amidosulfonic acid ammonium salt, dry with hair dryer.
- Overspray with 0.1% solution of N-(1-naphthyl)-ethylenediammonium dichloride solution; dry with a hair dryer.

The red azo dyes formed by derivatization are regarded as a doping positive result for Lasix in the urine sample.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning absorbance at 275 nm.

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

If a documentation of the derivatized sample is desired - normally the qualitative finding resp. photodocumentation is sufficient - it can be done by spectra scan in the visible range (400-650 nm).

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* 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 silica gel Merck 60 F_{254}, 20x10 cm can also be used. Then 10 µL of each sample and standard are applied as 10 mm bands, 5 mm apart; migration distance = 80 mm.

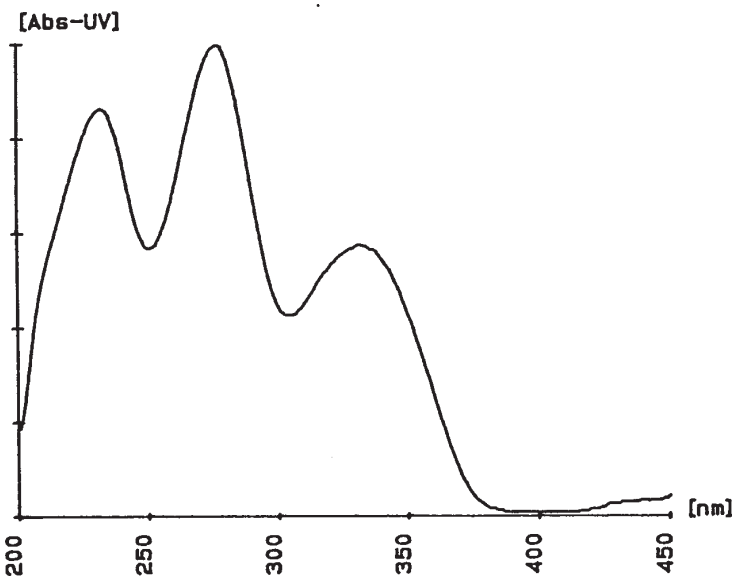

Fig. 1: In-situ absorption spectrum of furosemide (Lasix).

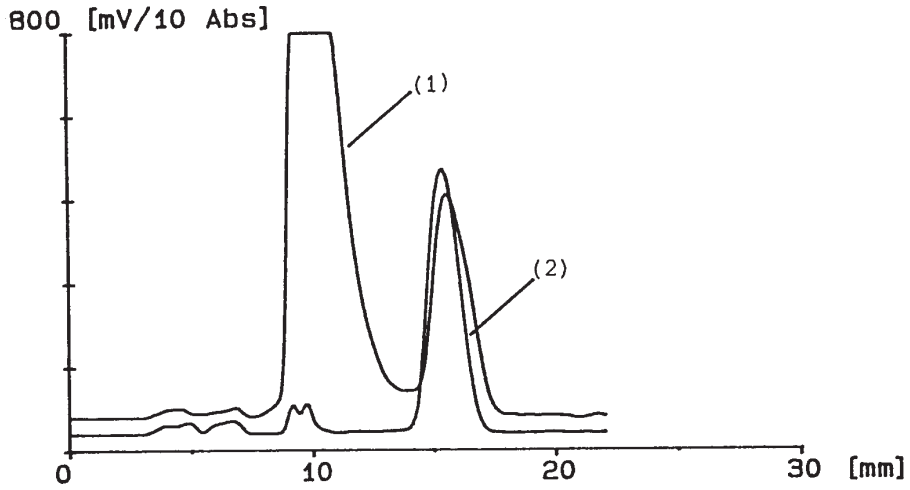

Fig. 2: Superimposed: sample track with urine matrix (1) and standard track (2).

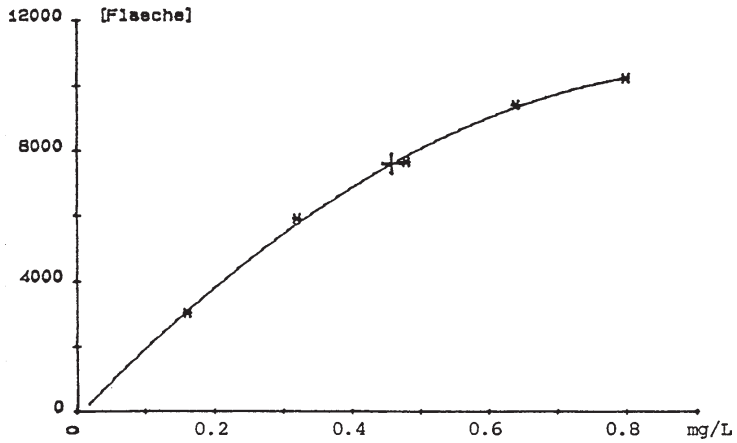

Fig. 3: Calibration curve of furosemide in the range of 0.16-0.8 mg/L (160-800 ng absolute) by second degree polynomial; (+) = found concentration of the curve depicted in fig. 1 0.48 mg/L.