Detection and quantitative determination of chlortalidone (Hygroton®) in urine

A-47.3

Key words

Instrumental TLC - quantitative analysis - densitometry by absorbance - post chromatographic derivatization - drugs identification - therapeutic drug monitoring - doping control - diuretic - antihypertonic

Scope

Chlortalidone is an often used diuretic for therapy. Moreover, it is abused in sports for doping purposes and is therefore on the IOC (Int. Olymp. Committee) list of banned drugs.

The alkaline urine extract is chromatographed on silica gel. Post chromatographic derivatization (with formaldehyde/sulfuric acid reagent) converts chlortalidone into a yellow dye. Results can be verified by UV absorbance spectra of the underivatized substances. Densitometric quantification is performed by absorbance at 240 nm. Determination limit is 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: Bestimmung von Medikamenten in Urin bei Mischintoxikationen in der klinisch-chemischen Notfallanalytik. Paper presented at a workshop of the Society of Forensic and Toxicological Chemistry (GFTCH, Gesellschaft für Forensische und Toxikologische Chemie), Basel 1988.

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
Ethyl acetate
Ethanol
Methanol
Formaldehyde 37%

Sulfuric acid 98%
Sulfuric acid (10% aqueous)
Ammonia 25%
Sodium sulfate (anhydrous)

Standard: chlortalidone (purum)

Sample preparation

- Adjust 10 mL urine sample with sulfuric acid (10% aqueous) to pH 2 and extract with 50 mL diethyl ether.

> (For simultaneous testing for the diuretic furosemide (Lasix) the ethereal phase can be further processed as described in application note A-46.) Otherwise discard it.

- Adjust the aqueous phase by adding NH_3 to pH 9 and extract with 50 mL ethyl acetate.
- Separate organic phase, dry with sodium sulfate, filter through a cotton ball and evaporate to dryness in water bath at 60°C.
- Dissolve residue in 0.2 mL methanol.

Standard solutions

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

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

Into 5 V-shaped vials pipette 10, 20, 40, 60 and 80 µL stock solution and evaporate under nitrogen. 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.

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 in which a yellow 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

- Spray plate with a solution of 0.2 mL formaldehyde (37%) in 10 mL conc. sulfuric acid.
- Dry plate with a hair dryer and heat for 10 min at 110°C.

Chlortalidone appears as a yellow spot.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning absorbance at 240 nm. Quantification of underivatized chromatograms via peak height, of derivatized via peak area.

Spectra scan of underivatized chlortalidone (Hygroton)-fraction for positive identification of substances (doping analysis) in the 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).

Remark

As chlortalidone does not react with hydrochloric acid to a primary aromatic amine, it cannot be identified via diazotation followed by coupling to a red azo dye, as is usual for many related substances (see A-46). This is why derivatization should be performed as described.

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* Compared with the conventional TLC precoated plate, the HPTLC plate offers a better cost-effectiveness relation, 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 µL of each sample and standard are applied as 10 mm bands, 5 mm apart; migration distance = 80 mm.

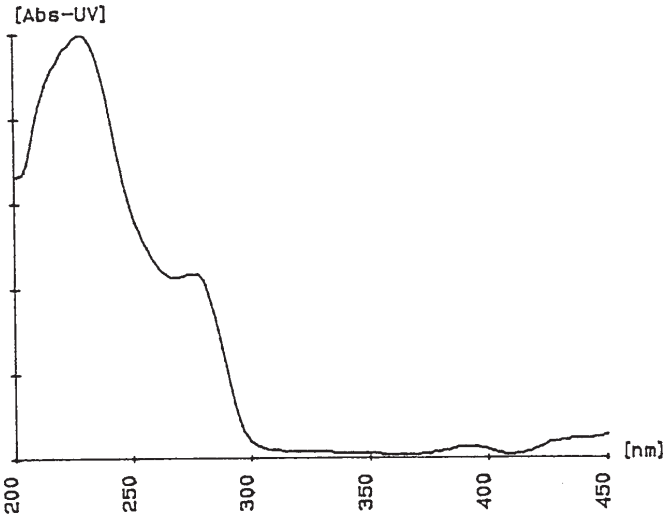

Fig.1 In-situ absorption spectrum of chlortalidone (Hygroton).

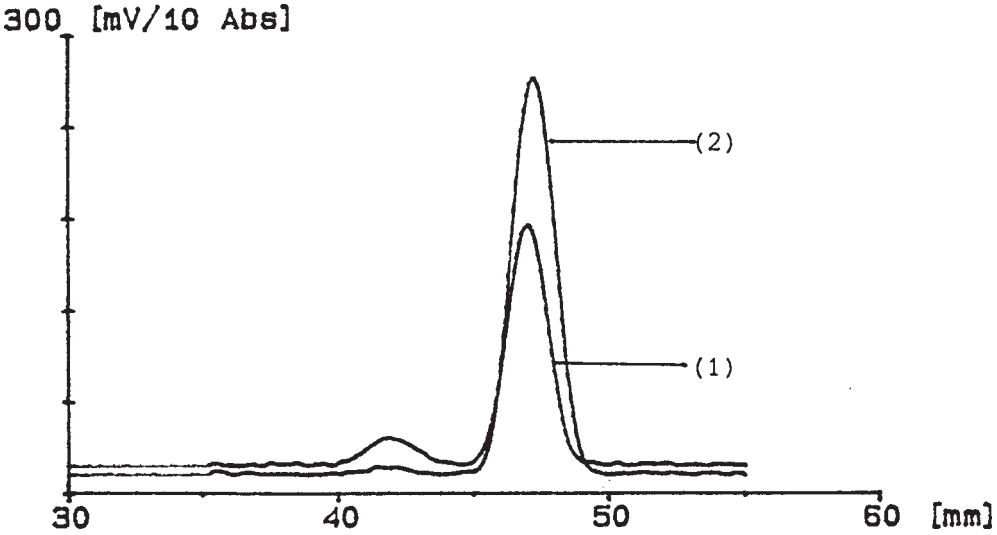

Fig. 2 Superimposed: sample track with urine matrix (1) and standard track (2).

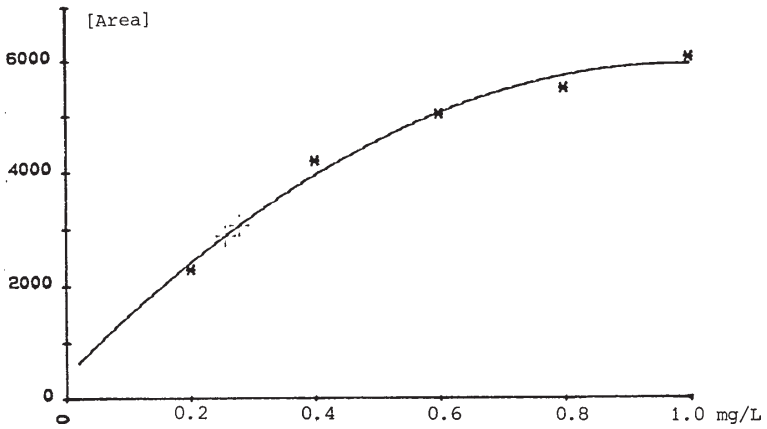

Fig. 3 Calibration curve of Hygroton in the range of 0.2-1.0 mg/L by second degree polynomial.