Determination of hydrochlorothiazide (Esidrex®) in tablets and in urine

A-43.2

Key words

Instrumental HPTLC - quantitative analysis - densitometry (absorbance) - drugs identification - postchromatographic derivatization - doping control - antihypertonic - diuretic - quality control - hydrochlorothiazide

Scope

Hydrochlorothiazide is a benzothiadiazine-diuretic, often used for treatment of hypertonicity, heart insuffiency and oedemas. Abuse of diuretics of this kind for doping purposes occurs in sports. They are therefore on the IOC (Int. Olymp. Committee) list of banned drugs. For analyzing a large number of samples at the same time a cost effective screening method is demanded.

Extracts of tablets are chromatographed on silica with ethyl acetate - cyclohexane 9:1 and evaluated densitometrically at 270 nm.

Urine extracts are chromatographed on silica with ethyl acetate - methanol - ammonia 85:10:5. Postchromatographic derivatization converts Esidrex® and similar drugs as well as their metabolites into azo dyes. The therapeutic range for hypertonicity patients is0.2-1.6 mg/L urine. In sports every positively identified quantity is regarded as doping. Determination limit of the method is 0.2 mg/L, detection limit <0.1 mg/L.

Advantages of using HPTLC for this analytical task

- Quick identification also in biological samples
- Spectra comparison and specific postchromatographic derivatization
- High sample throughput at low operating costs

Chemicals

Cyclohexane
Diethyl ether
Ethyl acetate
Ethanol
Methanol
N-(1-naphthyl)ethylenediamine dihydrochloride
Amidosulfonic acid ammonium salt

Hydrochloric acid (10% aqueous)
Sulfuric acid (10% aqueous)
Ammonium
Sodium nitrite (10% aqueous)
Sodium sulfate (anhydrous)

Standard: hydrochlorothiazide

Sample preparation

Tablets:

- Dissolve 112 mg pulverized tablets in a 100 mL measuring flask with 5 mL acetone and add 70 mL methanol. Put in an ultra sonic bath for 2 min and adjust to 100 mL.

Urine:

- Adjust 10 mL urine sample with sulfuric acid to pH 2 and extract with 50 mL diethyl ether. Separate ether phase and discard.
- Adjust aqueous phase to alkaline (pH ca. 9) with NH_3 and extract with 50 mL ethyl acetate.
- Separate the organic phase, and dry with Na_2SO_4, filter through cotton wool and evaporate to dryness at 60°C.
- Dissolve residue in 0.2 mL methanol (alkaline extract).

Standard solutions

Tablet: Dissolve 5 mg hydrochlorothiazide in a 100 mL measuring flask with 5 mL acetone and add 70 mL methanol. Put in an ultra sonic bath for 2 min and adjust to 100 mL (50 ng/µL).

Urine: Extract 50 mL urine of a person who has not received Esidrex® analogously (blind extract). Dissolve the alkaline residue in 1 mL hydrochlorothiazide solution (see standard of tablets = 50 ng/µL).

Layer

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

Sample application

Bandwise with CAMAG Automatic TLC Sampler III, band length 6 mm, track distance 10 mm, distance from left edge 12 mm, distance from lower edge 8 mm = 12 applications.

Application pattern:

B	U1	S1	U2	S2	U3	B	S3	U4	S4	U5	S5	
5	5	1	5	2	5	5	4	5	6	5	8	µL/band

B = blind extract
U1-5 = sample
S1-5 = standards in different concentrations

Chromatography

In CAMAG Twin Trough chamber 20x10 cm.

Tablet: with ethyl acetate - cyclohexane 9:1, migration distance 50 mm, R_F ca. 0.5.

Urine: with ethyl acetate - methanol - ammonium 85:10:5; migration distance 60 mm, R_F ca. 0.35.

Derivatization (for doping control only)

Spray plate with HCl with CAMAG TLC Sprayer or (better) put it in a chamber saturated with HCl-gas. Dry plate 5 min at 120°C; let cool it to room temperature; spray with $NaNO_2$ and HCl solution and dry with a hair dryer in a cold air stream. Overspray with 0.5% aqueous solution of amidosulfonic acid ammonium salt and dry with a hair dryer. Overspray with 0.1% solution of N-(1-naphthyl)ethylene-diamine dihydrochloride in ethanol and let the plate dry.

The (red) azo dyes (Esidrex® and metabolites) formed by derivatization are regarded as a doping positive result. The result is verified by a VIS-spectra comparison (400-650 nm) and confirmed by UV-spectra comparison (200 - 350 nm) of the underivatized fractions of a second chromatogram.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning by absorbance at 270 nm, after derivatization at 540 nm, evaluation via peak height.

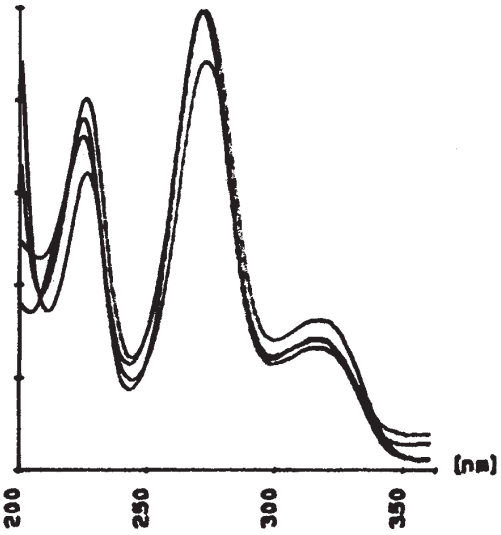

Fig. 1 UV-spectra of hydrochlorothiazide standard and urine sample

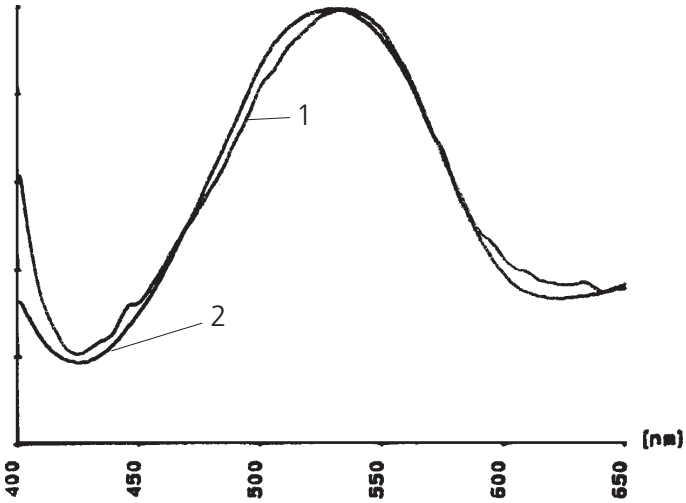

Fig. 2 VIS-spectra of hydrochlorothiazide standard (2) and urine sample (1)

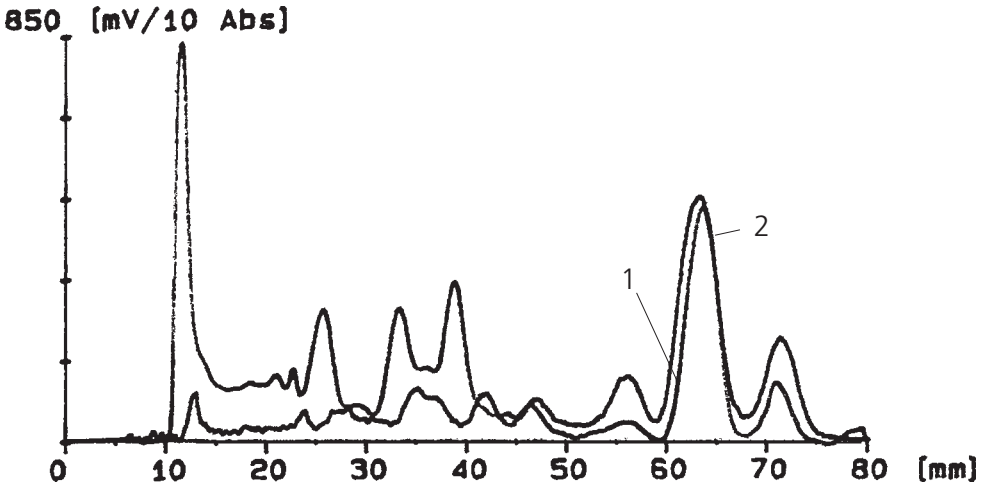

Fig. 3 Densitogram at 270 nm (1) and at 540 nm after derivatization (2). Hydrochlorothiazide, which was identified by UV- spectra, has reacted to an azo dye by derivatization.

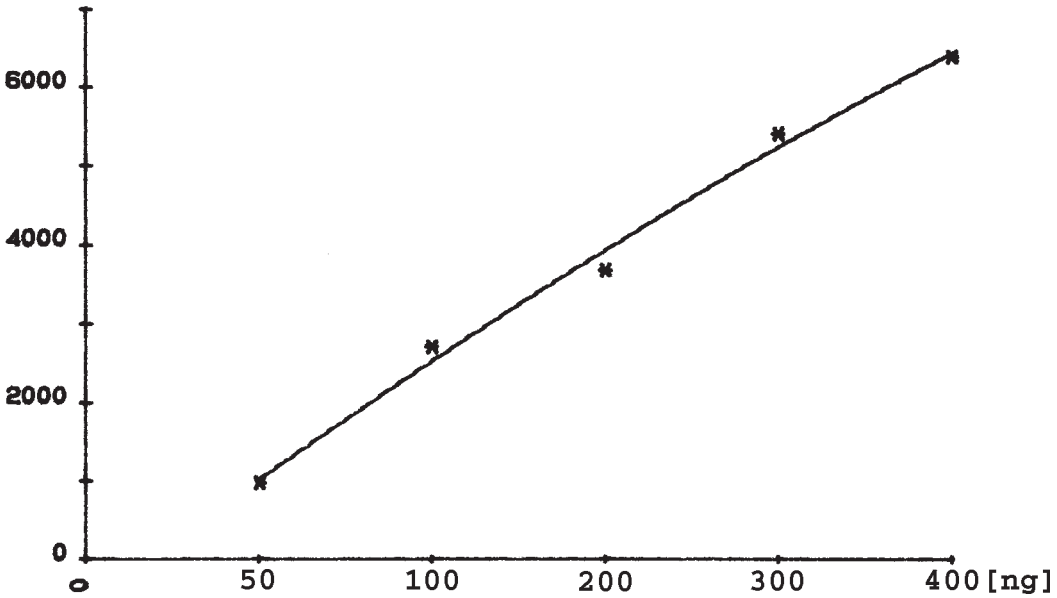

Fig. 4 Calibration curve for hydrochlorothiazide (absorbance at 270 nm): Evaluation via peak height with 2. degree polynomial. Calibration range 100-800 ng, corresponding to 0.2-1.6 mg/L.

Literature

W. Bernhard, S.R. Rippstein, A.N. Jeger (Institute of Forensic Chemistry, Basel), poster presented at Symposium for Clinical and Toxicological Analysis, Salzburg 1987.

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