Detection and quantitative determination of atenolol [Tenormin®] in urine

A-48.3

Key words

Instrumental TLC - quantitative analysis - densitometry by absorbance - post chromatographic derivatization - drugs identification - therapeutic drug monitoring - doping control - beta- receptor-blocker - atenolol [Tenormin®]

Scope

Atenolol is a beta-receptor-blocker often administered in combination with diuretics [e.g. hydrochlorothiazide [Esidrex®].

Beta-blockers are abused in sports where tranquillity rather than physical strength is required (e.g. shooting, curling, stunt-flying). They are therefore on the IOC (Int. Olymp. Committee) and SLS (Schweiz. Landesverband für Sport) lists of banned drugs.

Urine extracts are chromatographed on silica gel. Post chromatographic derivatization shows the presence of this and other beta-blockers administered in normal dosages. Results can be verified by UV spectra comparison. Densitometric quantification is performed by absorbance at 223 nm. Determination limit of the method described is 0.05 mg/L, reliable detection limit about 0.025 mg/L.

Literature

W. Bernhard, A.D. Fuhrer, A.N. Jeger and S.R. Rippstein, Fresenius Z. Anal. Chem. **330**, 458 (1988)

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

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Chemicals

Diethyl ether
Ethyl acetate
Ethanol
Methanol
Fast blue salt B
1,2-naphthoquinone-4-sulfonic acid ammonium salt

Standard: atenolol (purum, ICI-Pharma)

Formaldehyde solution (37%)
Sulfuric acid (10% aqueous)
Ammonia (25%)
Sodium sulfate (anhydrous)

Sample preparation

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

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

- Adjust aqueous phase to alkaline (pH ca. 9) by adding NH_3 and extract with 50 mL ethyl acetate.

- Separate organic phase, dry with sodium sulfate, filter through a cotton ball and evaporate to dryness at 60°C under vacuum.

- Dissolve residue in 0.2 mL methanol.

Standard solutions

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

Stock solution: dissolve 10 mg atenolol 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...	mL/track

Chromatography

In CAMAG Horizontal Developing Chamber 20x10 cm*, in saturated configuration with methanol - NH_3 100:1.5.

For simultaneous testing for hydrochlorothiazide the solvent system ethyl acetate - methanol - NH_3 85:10:5 is used.

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 spot in the critical area occurs and behaves as described, 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 derivatization is sufficient.

Postchromatographic derivatization

1. Spray plate with fast blue salt B (0.5% in water). Dry with a hair dryer. Atenolol appears as a yellow spot.

2. Spray with 1,2-naphthoquinone-4-sulfonic acid ammonium salt. The color of the yellow spot intensifies.

3. Spray with formaldehyde-sulfuric acid reagent (0.2 mL formaldehyde solution (37%) in 10 mL sulfuric acid 98%). Dry plate with a hair dryer and heat at 110°C for 10 min; the yellow spots turn violet.

The violet spots formed by derivatization are regarded as a doping positive result.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning absorbance for atenolol 223 nm and for Esidrex 270 nm (see CAMAG application note A-43). Quantification of underivatized chromatograms via peak height, of derivatized via peak area.

Spectra scan of underivatized atenolol-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).

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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.
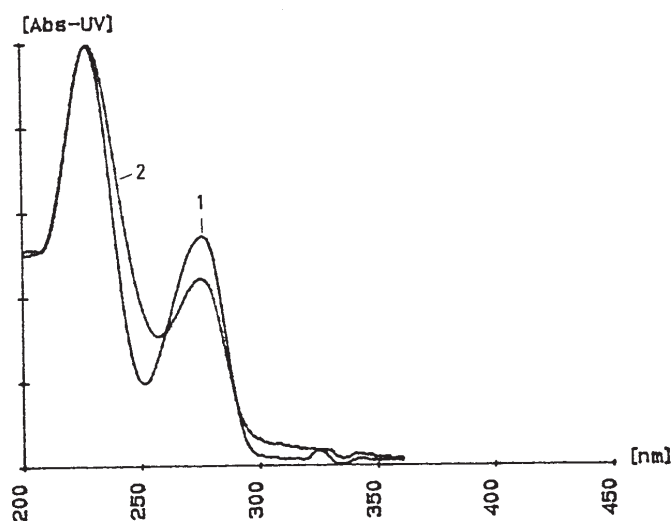
[Abs-UV]

[nm]

Fig. 1 Superimposed absorption spectra of the atenolol fraction of the unknown (1) and the identification standard (2). Due to the precise correspondence of both maxima the fraction is regarded as identified.
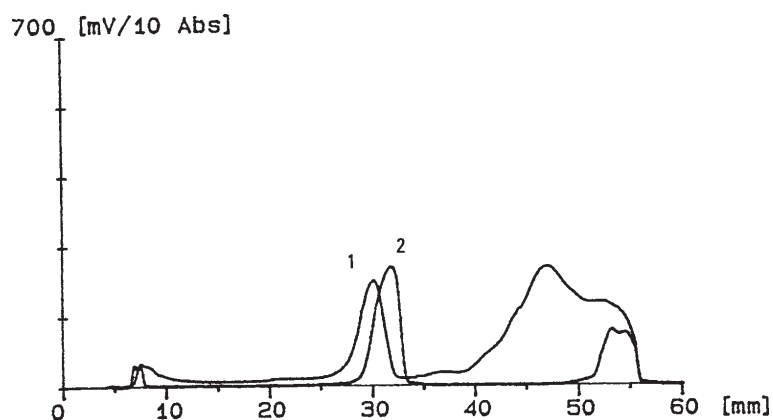
700 [mV/10 Abs]

[mm]

Fig. 2 Superimposed densitograms of an unknown (1) and a standard track (2). Due to the very different matrices of the proband's urine extract and the one of the blind extract the Rfs are different. In such case, verification is possible by chromatography of a sample spiked with authentic atenolol .
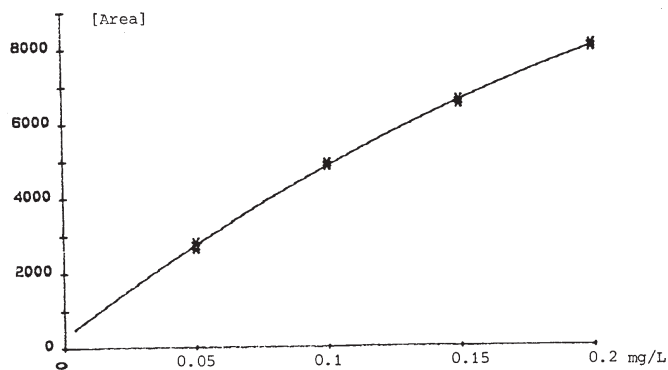
[Area]

Fig. 3 Calibration curve for atenolol in the range 0.05-0.2 mg/L via 2. degree polynomial.