Quantitative determination of carbamazepine and two of its metabolites in serum

A-01.4

Keywords

Quantitative TLC - HPTLC - post chromatographic derivatization - densitometry (fluorescence) - biochemistry - clinical analysis - bio-equivalence - bio-availability - doping analysis - carbamazepine

Scope

Carbamazepine (5H-dibenzepine-5-carboxamide) is used as an analgesic and anticonvulsant. The analysis of carbamazepine and its metabolites is required in various fields such as biochemistry, clinical, bio-equivalence, bio-availability and doping analysis. Quantitative determination of carbamazepine and its metabolites is also needed for therapeutic drug monitoring in human medicine.

This method was first developed in the CAMAG Application Laboratory 1979 and last updated at Anchrom Application Laboratory in 1993.

Literature

H.K.L. Hundt and E.C. Clark, J. Chromatogr. **107**, 149-154 (1975).

Advantages of performing this analysis by modern TLC

- No sample clean-up necessary
- The active substances are determined straight from the serum without prior extraction or processing
- Low detection limits (pg/mL) are achieved by spraying-on large volumes of (diluted) serum.
- The method of fluorescence detection used is sensitive and specific

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Reagents

Methanol
Ethyl acetate
Toluene
Hydrochloric acid conc.
Sulfuric acid conc.
Distilled water

Carbamazepine (I),
Carbamazepine-10, 11-epoxide (II)
10,11-di-hydroxycarbamazepine (III)

Sample preparation

Stock solution: Dissolve 10 mg of (I), 2,5 mg of (II) and 2,5 mg of (III) in methanol at 10 mL.

Standard serum solution: Add 2, 4, 8 and 16 µL of the stock solution to 1 mL portions of human serum (in plastic sample tubes). For lowering the viscosity, dilute the serum solutions 1:3 with water before application.

Layer

HPTLC precoated plates silica gel MERCK 60 F 254, 20x10 cm

Sample application

Sample application bandwise with Linomat, sample volume 5 µL, 10 mm bands, 5 mm apart, distance from lower edge 8 mm, 20 mm from the side = 22 samples per plate, 11 on each side; application speed 15 sec/µL.

If 7 mm bands, 3 mm apart are applied, 18 applications on each side = 36 per plate are possible without any loss of quality.

Chromatography

In CAMAG Horizontal Developing Chamber 20x10 cm in sandwich configuration, developing solvent: ethyl acetate - toluene - methanol 5:4:1, migration distance 45 mm, time requirement approx. 10 min.

Post chromatographic derivatization

Dry plate thoroughly with a hair dryer, then place in oven or on CAMAG TLC Plate Heater at 100°C for 5 minutes.

After cooling to room temperature, expose plate to hydrogen chloride gas in a twin-trough developing tank for 5 minutes (one trough empty, the other filled with concentrated sulfuric acid - concentrated hydrochloric acid 10:2).

Subsequent irradiation for 15 minutes with unfiltered light of a 254 nm mercury lamp converts carbazepine and the metabolites into fluorescent compounds.
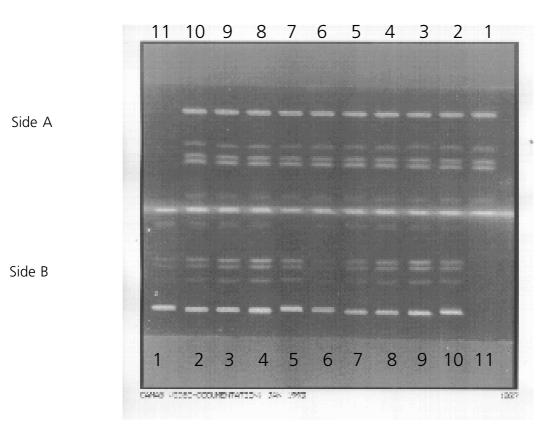
Use fume hood for HCl exposure and during UV irradiation.

Densitometric evaluation

With CAMAG TLC Scanner II with Labdata System and CATS evaluation software.

Scanning by fluorescence with Hg lamp at 366/>400 nm, monochromator bandwidth 10 nm, slit dimensions 0.3 x 5 mm, scanning speed 4 mm/s.

Quantification of carbamazepine and metabolites by peak heights using linear regression.

Chromatogram documented with CAMAG Video Documentation System under UV 366 nm; side A: reproducibility, tracks 1-10 5 µl stock solution per 1 mL; track 11 blank. Side B: see table.

Track No.	Carbamazepine	Carbamazepine-11-epoxide	Dihydroxycarbamazepine
1 & 6	3.3	0.8	0.8
2 & 7	6.7	1.7	1.7
3 & 8	13.3	3.3	3.3
4 & 9	26.7	6.7	6.7
5 & 10	33.0	8.0	8.0

all amounts (ng) absolute

Remark: Since it has been ascertained that linear regression is appropriate, it is sufficient to chromatograph 3 or maximum 4 levels of calibration standard per plate.
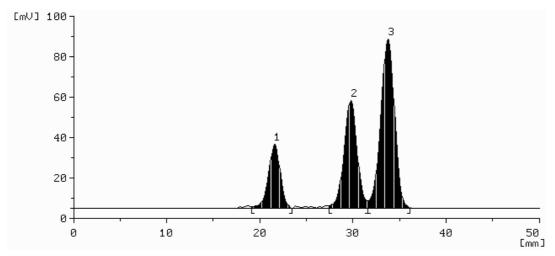
Fig. 1   Densitogram (scanning by fluorescence) of human serum spiked with 6.7 ng 10,11-dihydrocarbamazepine (1), 6.7 ng carbamazepine-10,11-epoxide (2), and 26.7 ng carbamazepine (3) absolute.
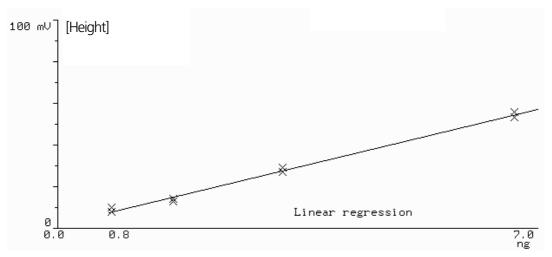

Fig. 2   Calibration curve over 0.8 - 7.0 ng absolute (0.5-4.0 µg/mL) carbamazepine-10,11-epoxide (2), linear regression via peak height.
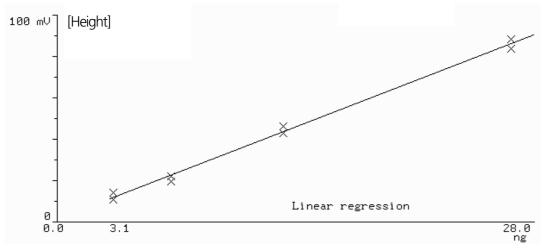

Fig. 3   Calibration curve over 3.3 - 33.0 ng absolute (2.0 - 16.0 µg/mL) carbamazepine (3), linear regression via peak height.