Determination of Vitamin B1 in Pharmaceutical Products A-27.4

Key words

Instrumental HPTLC - quantitative analysis - densitometry (fluorescence) - post chromatographic derivatization - quality control - stability tests - vitamin B1 - thiamine

Scope

The method is suitable for vitamin B1 (thiamine hydrochloride) determination in quality assurance, stability tests, etc. The methanolic solution of the drug is chromatographed on silica with methanol - NH_3 - acetic acid - chloroform 18:2:1:1, then derivatized with potassium hexacyanoferrate. After stabilising the fluorescence intensity with paraffin oil the plate is evaluated by densitometry. The determination limit is 500 pg/spot.

Literature

[1] P. Derr, diploma thesis, Polytechnik of Giessen, Faculty of Occupational Health, 1985.

[2] W. Funk et al. in R.E. Kaiser (ed.): Proc. 3rd Int. Symp. Instrumental TLC, Institute for Chromatography, Bad Dürkheim (1985), p. 281-311.

Advantages of using HPTLC for this analytical task

- Simple procedure
- Highly cost-effective, regardless of number of analyses
- Routine method suitable for quality control and stability tests

Chemicals

Water (dist.)
Methanol
Chloroform
Acetic acid
Ethanol
Ammonium hydroxide solution (25%)

Standard: thiamine hydrochloride

Potassium hexacyanoferrate
Sodium hydroxide
Paraffin oil
Triethanolamine
Sulfuric acid (conc.)

Sample preparation

Dissolve unknown samples in methanol so that the expected vitamin B1 concentration is in the working range of 5-40 ng/µL.

Standard solution

Dissolve 10 mg thiamine hydrochloride in 10 mL water and dilute 1:100 with methanol (10 ng/µL).

Layer

HPTLC plates silica gel Merck 60 F_{254}, 20 x 10 cm, prewashed with methanol - chloroform 1:1, then dried for 20 min at 110°C.

Sample application

With CAMAG Automatic TLC Sampler III as 5 mm bands, track distance 10 mm, distance from lower edge 8 mm, distance from left edge 15 mm = 18 applications per plate.

Application scheme:

S1	U1	S2	U2	S3	U3	S4	U4	S5	U5	
0.5		1		2		3		4		µL/band
5		10		20		30		40		ng/band

S1-S5 = standard solution in different concentrations U1-U5 = unknowns

Chromatography

In CAMAG Twin Trough chamber with methanol - NH_3 - acetic acid - chloroform 18:2:1:1 at 58% relative humidity (39.5 mL conc. sulfuric acid + 100 mL water; relevant for separation of thiamine from riboflavin). Migration distance 6 cm, running time 20 min, hR_F = ca. 40.

Derivatization

By dipping for 1 s with CAMAG Chromatogram Immersion Device in potassium hexacyanoferrate solution. Dissolve 100 mg potassium hexacyanoferrate in 70 mL dist. water, add 10 g sodium hydroxide and, after it has cooled down to room temperature, fill up with 200 mL ethanol.

For stabilization and intensification of fluorescence intensity dip the dry plate for 1 s with CAMAG Chromatogram Immersion Device in chloroform - paraffin oil - triethanolamine 6:1:1.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software by fluorescence 366/>400 nm. Quantitative evaluation via peak height with linear regression.

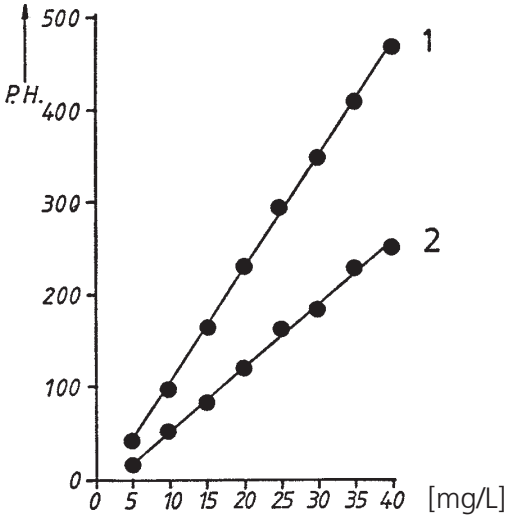

Fig. 1 Calibration curve with (1) and without (2) stabilization and intensification of fluorescence.

By topping up sample matrices (containing no thiamine) with different concentrations of thiamine and measuring recoveries, it was ascertained that the procedure described is free from constant systematic and proportional systematic errors.