Rapid content uniformity test of 6 batches of Coenzyme Q10 in soft gel capsules by HPTLC

A-96.1

Key words:

HPTLC, densitometry, Coenzyme Q10, Ubichinone-10, content uniformity test, uniformity of dosage units

Introduction:

Coenzyme Q10, found naturally in the body, is involved in the production of body energy. Thus heart, lung and liver have the highest concentration of Q10. The substance is active in many ways but primarily assumed to enhance the immune system and to work as an antioxidant protecting against free radicals that damage cells. Coenzyme Q10 is an expensive material. As dietary supplement it is mostly sold in soft gelatin capsules. For quality control of such products the CUT (content uniformity test) and the assay are of high interest.

Scope:

This method is suitable for the quantification of coenzyme Q10 in gel capsules but may be adapted for liquid and solid dosage forms.

Required or recommended CAMAG devices:

Automatic TLC Sampler 4 or Linomat 5, Automatic Developing Chamber ADC2 or Horizontal Developing Chamber or Twin Trough Chamber 20 x 10 cm, Visualizer, TLC Scanner 4 and winCATS software

Derivatization reagent:

No reagent is used.

Sample:

One soft gel capsule is placed into a flask and 50.0 mL of toluene are added. While submerged in the liquid the capsule is cut with a blade. The flask is placed on a shaker for 15 min. Based on the target content of the capsule an aliquot of the extract is diluted to a concentration of about 15 µg/mL. Do not expose the samples to bright light.

Standards:

A standard solution containing 1 mg/mL of Coenzyme Q10 in toluene is prepared. Dilute the stock solution with toluene to 10, 15 and 20 µg/mL of Coenzyme Q10. Do not expose the standards to bright light. The solutions are stable for about one week at 4 °C.

Chromatography:

Stationary phase:	HPTLC Si 60 F_{254} 20 x 10 cm (Merck).
Sample application:	2 µL each of test solution and standards are applied as 2 mm-bands, min 2 mm apart, 8 mm from lower edge of plate; 20 mm from the left and right edges.
Developing solvent:	toluene
Development:	20 x 10 cm Horizontal Developing Chamber (HDC) or Twin Trough Chamber (TTC) or ADC2, no saturation, For HDC: 8 mL developing solvent for each side (36 / 72 samples). For TTC: 10 mL of developing solvent in front trough.
Developing distance:	40 mm from lower edge of plate.
Plate drying:	10 min in a stream of cold air.
Derivatization:	none
Evaluation:	Examination under UV 254 nm

Densitometry:

With CAMAG TLC Scanner 4 and winCATS software in absorption mode at 282 nm using a deuterium lamp; slit dimension: 3.00x0.20 mm; evaluation via peak height, linear regression (20-50 ng) [alternatively: 20-150 ng polynomial regression]. Reproducibility of a sample or of a standard < 5 %.

Results:

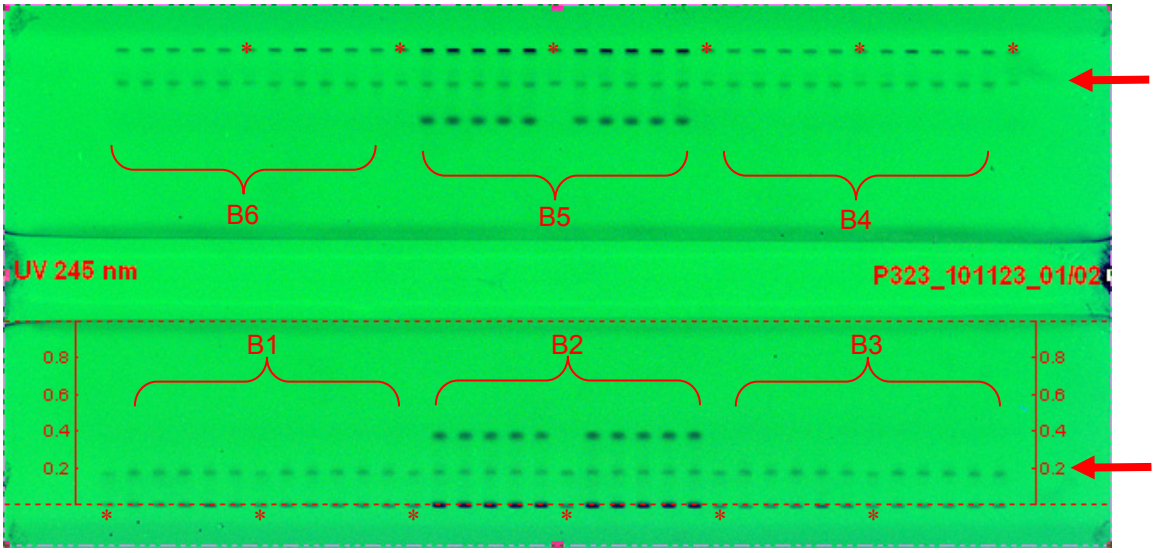

Fig. 1 60 samples (6 batches, B1-B6) and 12 standards are applied onto one HPTLC plate; Coenzyme Q10 is seen at Rf ≈ 0.20. In the Q10-batches on the center tracks vitamin B2 and vitamin E are also present (Rf ≈ 0 and 0.4); (NOTE: For better visualization in this figure the application volume was increased to 6 µL).*

NOTE: The presented results are to be regarded as examples only!

Please contact CAMAG for more application notes and products for analysis of herbals!

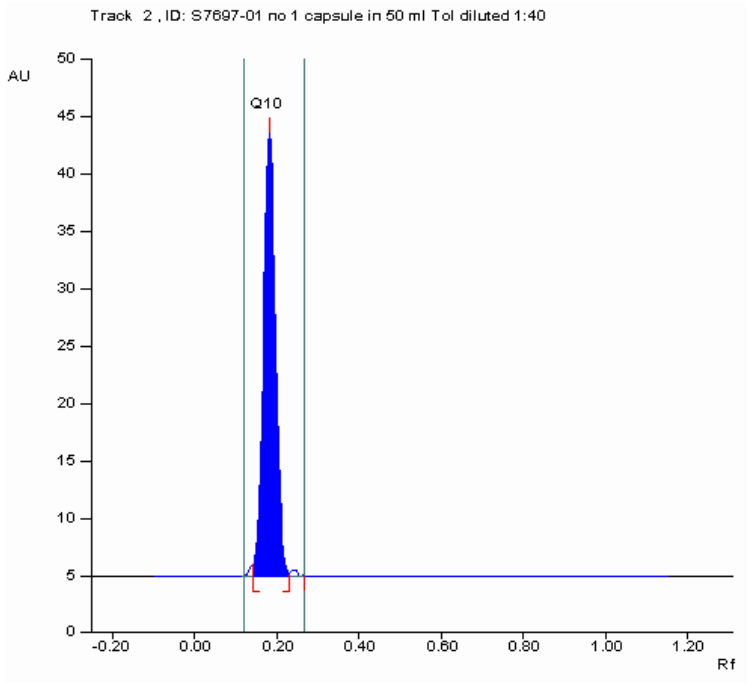

Fig. 2 Densitogram of a Q10 sample.

From the same raw data the content uniformity was calculated by:

a) single-level calibration
b) multi-level calibration using linear regression

a) Content uniformity test using single-level calibration (P323_101011_03)

Regression via height Y=0.000+2.047x

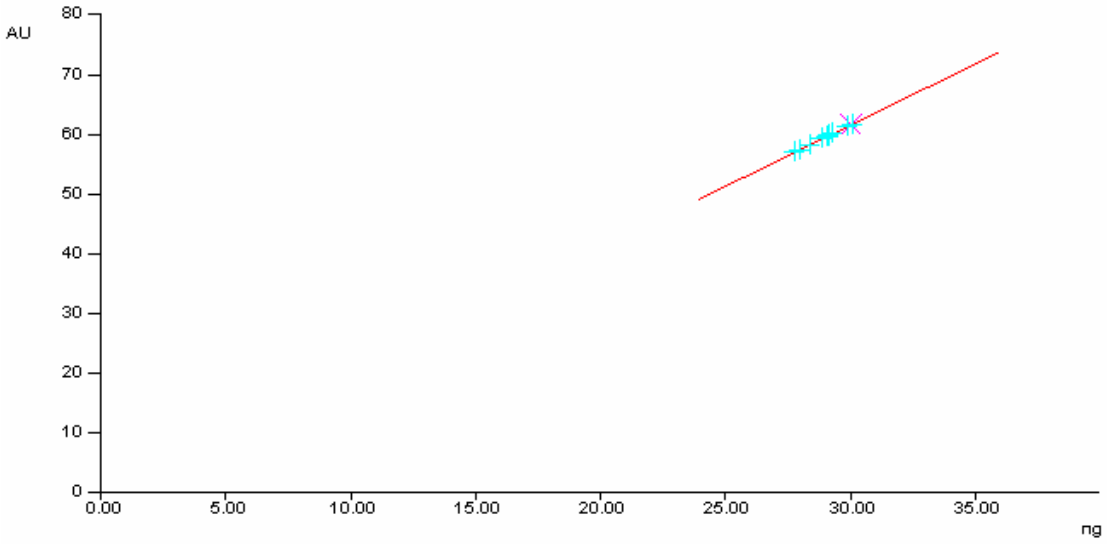

Fig. 3 Calibration function for Q10 at 282 nm. Measurement of 10 samples and 1 standard using single-level calibration.

b) Quantification of Coezyme Q10 in soft gel capsules with multi-level calibration

Regression via height Y=3.976+1.832x, r = 0.99849, sdv = 3.12 %

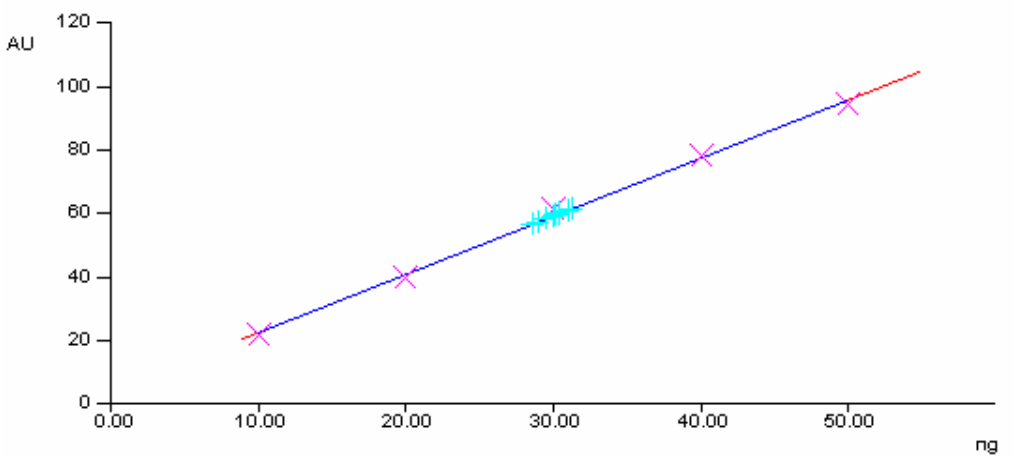

Fig. 4 Calibration function for Q10 at 282 nm. Measurement of 10 samples and 5 standards using linear calibration.

Time required for content uniformity test of 6 batches of 10 samples and 12 standards on one HPTLC plate

Steps	[min]
Application of 72 tracks with Automatic TLC Sampler (ATS 4)	64
Chromatography in Horizontal Developing Chamber (HDC)	6
Drying of the plate	10
Evaluation with TLC Scanner 4	6
Total time required	**86 min**

Calculation of Uniformity of Dosage Units according to USP 34 (<905>, s. 403-406)

Sample size (n = 10)

X(Average)		
29.53 ng		
X(Average)	X(Average)	X(Average)
31.28 ng	30.43 ng	28.64 ng
X(Average)	X(Average)	X(Average)
30.17 ng	30.99 ng	30.35 ng
X(Average)	X(Average)	X(Average)
30.05 ng	30.35 ng	28.97 ng

Target content T (label claim)	30 ng
Average (calculated)	30.08 ng
Mean of individual contents expressed as a percentage of the label claim $\overline{X}$ (calcul.)	$\overline{X}$ = 100.25
Sample standard deviation s (calcul.)	s = 2.76
Acceptability constant k (n=10)	k = 2.4
M (case 1), if 98.5 % ≤ $\overline{X}$ ≤ 101.5 %	M = $\overline{X}$
Acceptance value AV	AV = |M – $\overline{X}$ | + ks
AV (calcul.)	**AV = 6.6**
Max. allowed AV, L1 = 15.0	15.0

Literature

Agbaba D, Eric S, Markovic G, Nedeljkovic V, Veselinovic S and Vucetic M, The application of HPTLC in the quantitative analysis of drugs, J. Planar Chrom. 13, 2000, p. 333-336.