Determination of the flavonoid rutin in *Ginkgo biloba* dry extract by HPTLC

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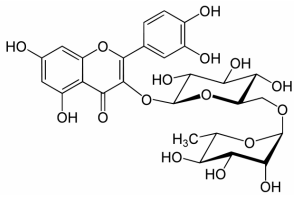


Key words:

HPTLC, densitometry, flavonoids, rutin *Ginkgo biloba* extract

Scope:

This method is suitable for the quantification of rutin in *Ginkgo biloba* dry extract. For additional visual evaluation of the HPTLC fingerprint the plate can be derivatized with natural products reagent.

Required or recommended CAMAG devices:

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

Sample:

0.1 g of dry extract is sonicated with 10 mL of methanol for 10 min and filtered. The supernatant is used as test solution.

Standards:

A standard solution containing 0.1 mg/mL rutin in methanol.

Derivatization reagent (optional):

Natural Products reagent (NP reagent): 1 g of diphenylborinic acid aminoethylester is dissolved in 200 mL of ethyl acetate.

Macrogol reagent: 10 g of polyethylene glycol 400 (macrogol) are dissolved in 200 mL of dichloromethane.

Chromatography:

Stationary phase:	HPTLC Si 60 F_{254}, 20 x 10 cm (Merck).
Sample application:	2-5 µL each of test solution and 2, 4, 6, and 8 µL of standard are applied as 8 mm bands, min. 2 mm apart, 8 mm from lower edge of plate.
Developing solvent:	Ethyl acetate, glacial acetic acid, formic acid, water (100:11:11:27)
Development:	ADC2 or 20 x 10 cm Twin Trough Chamber, saturated for 20 min.
Developing distance:	70 mm from lower edge of plate.
Plate drying:	5 min in a stream of cold air.
Derivatization (optional):	the plate is heated at 100 °C for 3 min, dipped while still hot in NP reagent, dried in a stream of cold air and dipped in Macrogol reagent.
Detection:	a) UV 254 nm b) UV 366 nm c) (optional) UV 366 nm, derivatized with NP/Macrogol reagent

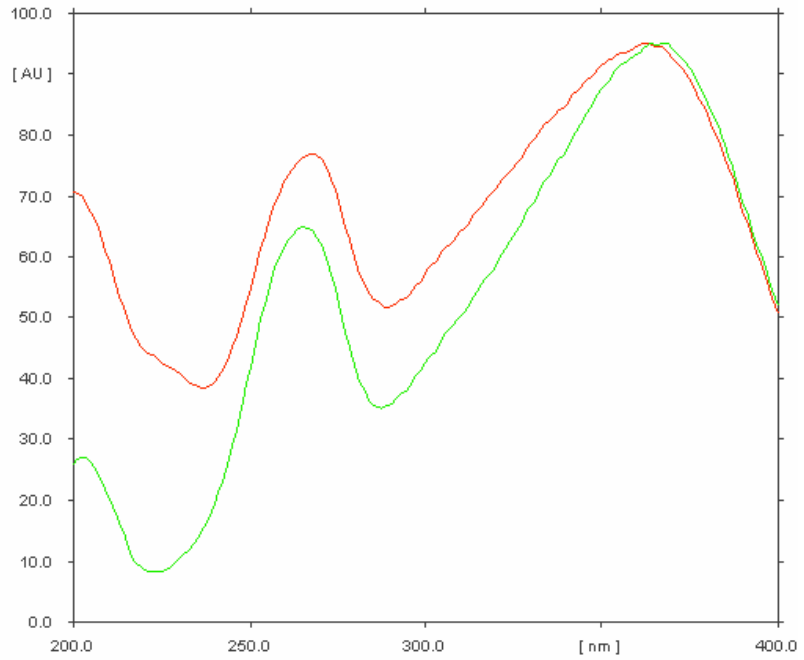
Densitometry:

With CAMAG TLC Scanner and winCATS software in absorption mode at 360 nm (prior to optional derivatization) using a D2 lamp; evaluation via peak height, polynomial regression.

Results:

Fig. 1 UV spectra of rutin standard (green) and corresponding zone in sample (red)

Based on the UV spectrum of rutin the UV_{max} of 360 nm was selected as measurement wavelength for quantification.



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 Linear calibration function of rutin in samples measured at 360 nm

Y = -15.65 + 0.3664x -8.598e-005x^2 r = 0.99975 sdv = 1.96%

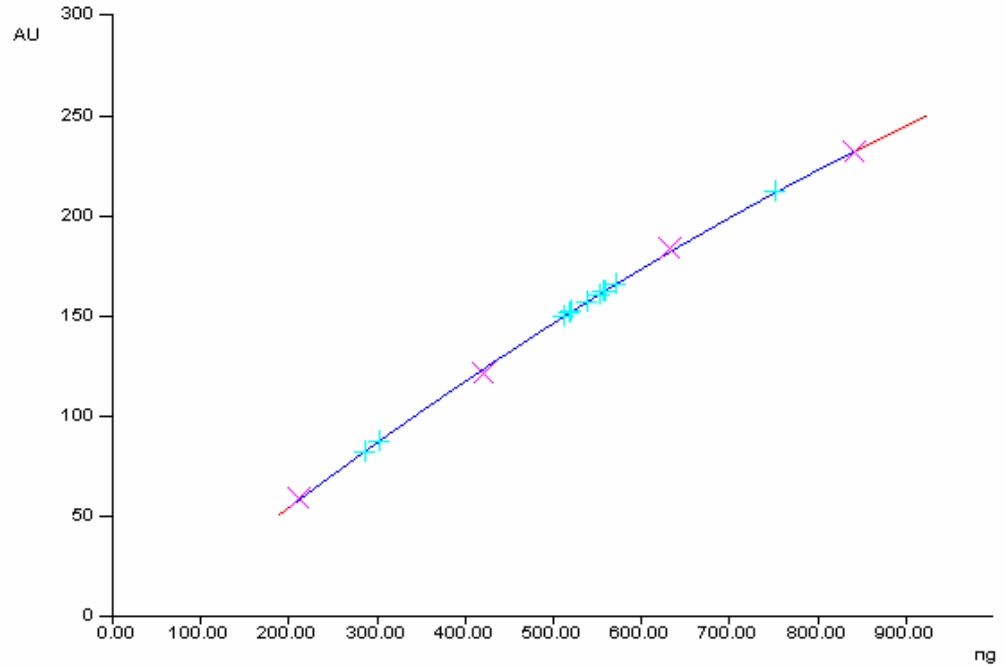


Fig. 3 Densitograms of standard (left) and a Ginkgo dry extract sample (right)

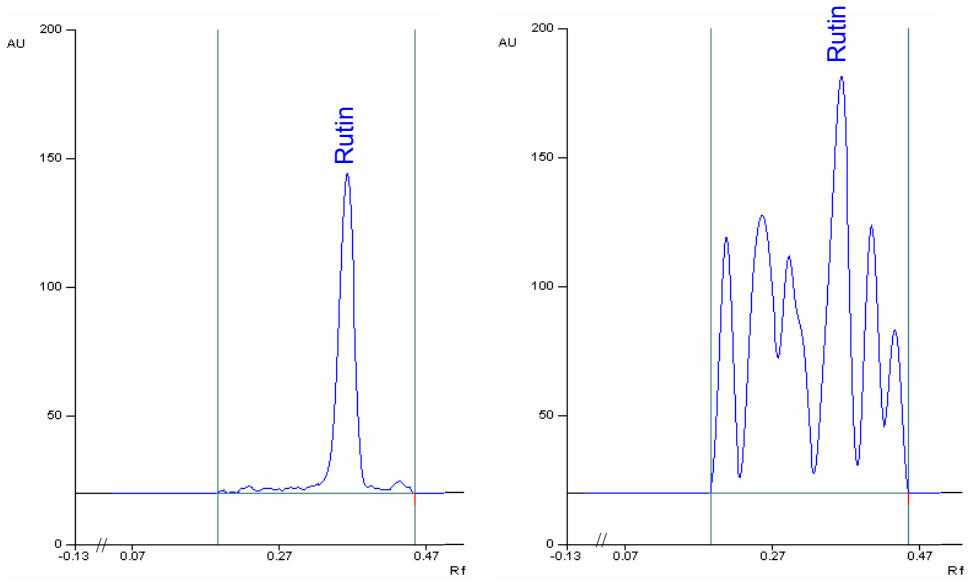
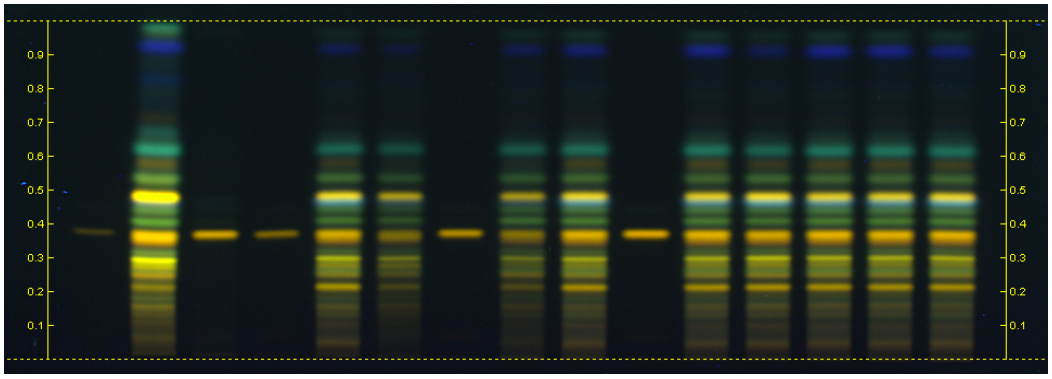


Fig. 4 Image under UV 366 nm, after derivatization



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Rutin	9	4 µL	Ginkgo dry extract #6
2	2 µL	Ginkgo dry extract #1	10	8 µL	Rutin
3	2 µL	Ginkgo dry extract #2	11	4 µL	Ginkgo dry extract #7
4	4 µL	Rutin	12	7 µL	Ginkgo dry extract #3
5	7 µL	Ginkgo dry extract #3	13	4 µL	Ginkgo dry extract #7
6	5 µL	Ginkgo dry extract #4	14	4 µL	Ginkgo dry extract #7
7	6 µL	Rutin	15	4 µL	Ginkgo dry extract #6
8	6 µL	Ginkgo dry extract #5			

Literature

Based on the HPTLC method for identification of flavonoids in Ginkgo, American Herbal Pharmacopoeia, 2003.