HPTLC method for the determination of apolar lipids from human skin

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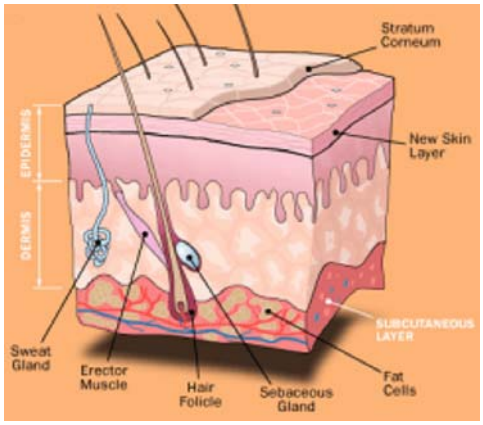


Key words:

HPTLC, densitometry, apolar lipids, neutral lipids, human skin

Introduction:

Skin care and protection is of high interest in our society, therefore the cosmetic industry is investigating new and innovative products. Their active ingredients show an influence on the composition of skin lipids. Quantitative analysis of these lipids is a promising way to investigate the effect of these products.

Scope:

This method is suitable for the screening and quantification of apolar lipids in samples taken from human skin.

Required or recommended CAMAG devices:

Automatic TLC Sampler 4 or Linomat 5, Automatic Developing Chamber ADC2 with humidity control 33 % r.H. or Twin Trough Chamber 20 x 10 cm, TLC Scanner and winCATS software, Immersion device III and plate heater.

Derivatization reagent:

Copper(II)sulfate: Dissolve 20 g of copper sulfate pentahydrate in 200 mL of methanol at less than 20°C. Under cooling with ice add 8 mL of sulfuric acid 98 % and 8 mL of ortho-phosphoric acid 85 %.

Sample from human skin:

Press the opening of a 300 mL Erlenmeyer flask (d = 3 cm) containing 5 mL of ethanol against the inner forearm. Let the solvent make contact with the skin for one minute. Repeat this procedure with 5 mL of fresh ethanol on an area next to the first. Combine the two extracts and evaporate the solvent. Dissolve the residue in 250 µL of chloroform, methanol (2:1).

Standards:

Stock solution: Dissolve 2.0 mg of each, squalene, triolein, palmitic acid, 1,2-dipalmitoyl-sn-glycerol, stearyl palmitate, cholesteryl palmitate, and cholesterol in 10 mL of chloroform, methanol (1:1).
Working standards: Dilute 1 mL of stock solution with 4 mL of chloroform, methanol (1:1).

Chromatography:

Stationary phase: HPTLC LiChrospher Silica gel 60 F_{254}, 20 x 10 cm (Merck), pre-washed by development with methanol and dried during 2 h at 120 °C in an oven.

Sample application: 2-30 µL of test solution and 2.5-10 µL of standard solution are applied as 8 mm bands, min. 2 mm apart, 8 mm from lower edge and ≥ 26 mm from the right and left edges of the plate.

Developing solvent: 1st development: toluene.
2nd development: n-hexane, tert-butyl methyl ether, glacial acetic acid (80:20:1)

Development: Both developments in the ADC2 with humidity control at 33 % relative humidity (using a saturated solution of $MgCl_2$), chamber saturated for 20 min (using filter paper), 10 mL developing solvent per trough.

Developing distance: 1st development: 80 mm from lower edge of plate
2nd development: 45 mm from lower edge of plate

Plate drying: 5 min in a stream of cold air after each development steps

Derivatization: Copper(II) sulfate: after second development immerse the plate into the reagent for 6 s. Dry the plate during 30 s with cold air and heat it at 140 °C for 30 min using a plate heater.

Evaluation: Examination in white light

Densitometry:

With CAMAG TLC Scanner 4 and winCATS software in absorption mode at 350 nm using a deuterium lamp; evaluation via peak height, linear regression (see above) or polynomial regression.

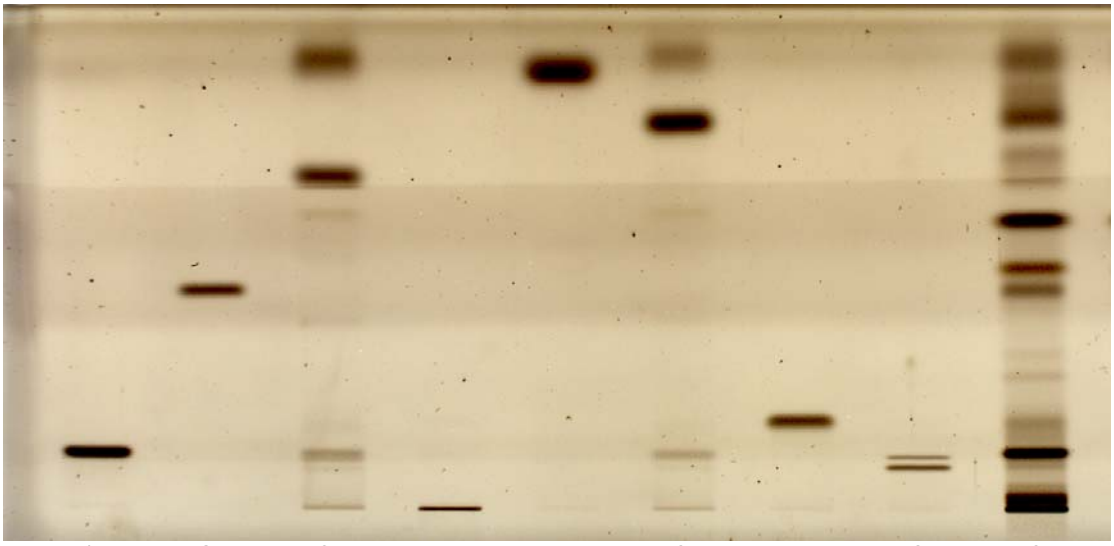
Results:

Tab. 1: Linear range, LOD und LOQ

Compounds	Linear range [ng]	LOD[ng]	LOQ[ng]
Squalene	107-362	48	206
Cholesteryl palmitate	109-369	44	198
Stearyl palmitate	100-340	17	76
Triolein	128-357	22	101
Palmitic acid	102-345	50	223
Cholesterol	41-165	22	95
1,2 Dipalmitoyl-sn-glycerol	102-285	42	174

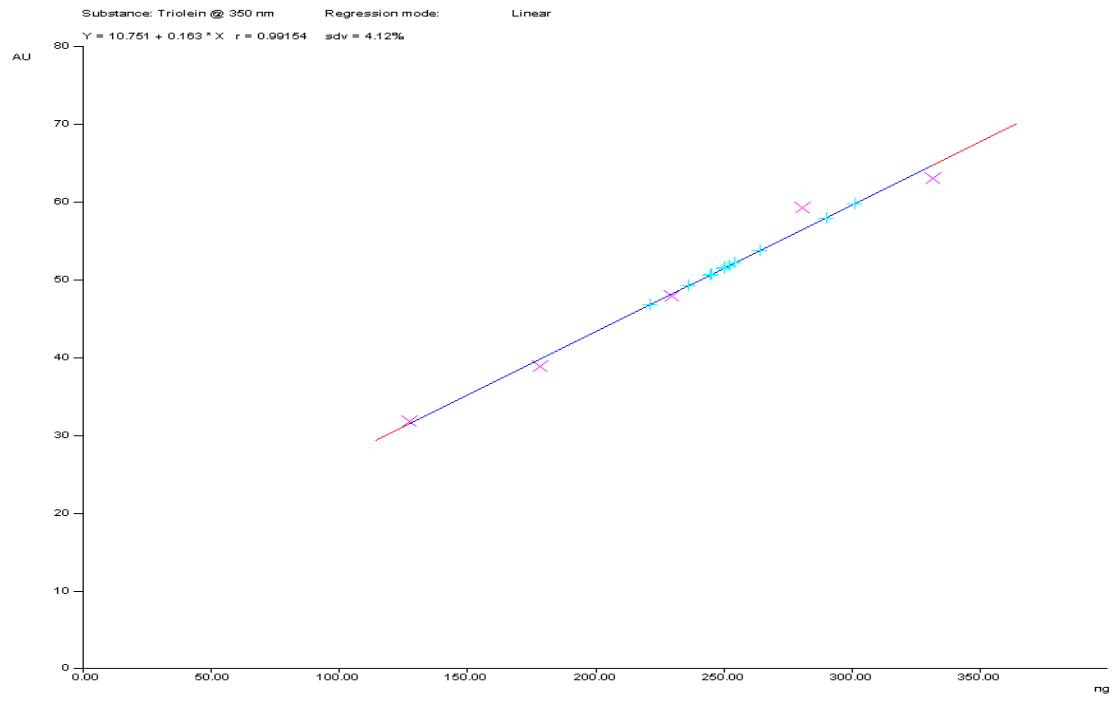
NOTE: The presented results are to be regarded as examples only!

Fig. 1 Standards and samples of human skin on HPTLC plate after derivatization, evaluation under white light (P258_090220_02)



1. Cholesterol, 2. Triolein, 3. Stearyl palmitate, 4. Ceramid VI, 5. Squalene, 6. Cholesteryl palmitate, 7. Palmitic acid, 8. 1,2 Dipalmitoyl-sn-glycerol, 9. Skin lipids extracted with ethanol

Fig. 2 Calibration function for triolein measured at 350 nm (P258_090406_02)



Literature

K. Rothenbühler (2009) HPTLC zur qualitativen und quantitativen Analyse von Hautlipiden. Master thesis, University of Basel, Department of Pharmaceutical Sciences, Institute of Pharmaceutical Biology.