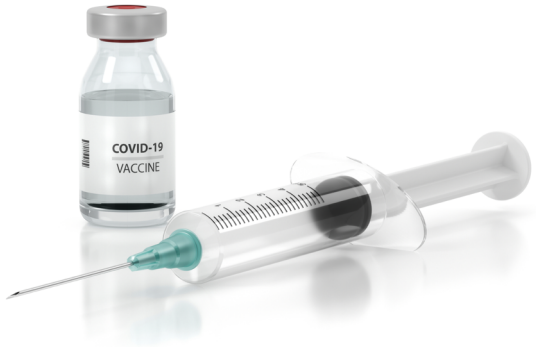
APPLICATION NOTE

Analysis and quality control of lipid-based formulations, *e.g.* mRNA vaccines, by HPTLC

A-138.1



Keywords

Lipids, liposomes, vaccines, cationic lipids, helper lipids, mRNA, LNP, phospholipids, cholesterol, COVID-19, SARS-CoV-2

Introduction

Lipid-based formulations enhance drug solubilization both by initially presenting the drug in a solubilized form and by preventing drug precipitation by altering the nature of both exogenous (formulation-derived) and endogenous solubilizing species in the intestinal milieu [1]. To protect fragile molecules (like mRNA) used for vaccination they are commonly encapsulated in liposomes or lipid nanoparticles. Otherwise, they would be digested/chopped before they reach their target.

Scope

The methodology shown herein describes how lipids and lipid-based formulations can be analyzed by HPTLC. Different developing solvents and derivatization reagents have been published and proven to be suitable for *e.g.* degradation studies and identity testing of diverse lipid classes [2,3]. Herein, we demonstrate as an example the separation and detection of lipids used for vaccine-production.

Required or recommended devices

HPTLC PRO Module APPLICATION or Automatic TLC Sampler 4, HPTLC PRO Module DEVELOPMENT or Automatic Developing Chamber ADC 2, optional: HPTLC PRO Module PLATE STORAGE (to run 5 plates autonomously), Derivatizer, TLC Plate Heater 3, TLC Visualizer 2, TLC Scanner 4, *visionCATS* 3.1

Sample

Samples are prepared in ethanol (or, if not soluble, in a mixture of dichloromethane and methanol), liquid formulations are directly applied or diluted with a suitable solvent.

Standards

Standards are prepared at different concentration levels, depending on the analytical task, *e.g.* for identity testing at a final concentration of 1 mg/mL in ethanol (or, for non-polar lipids, in a mixture of dichloromethane and methanol).

The universal HPTLC mix from [4] can be applied as System Suitability Test (SST) on each plate.

Chromatography

Stationary phase	HPTLC Si 60 F_{254}, 20 x 10 cm (Merck)
Sample application	Application as 8.0 mm bands with HPTLC PRO Module APPLICATION or ATS 4 (settings for ethanolic solutions), 15 tracks, track distance 11.4 mm, distance from left edge 20.0 mm, distance from lower edge 8.0 mm, application volume 2.0 µL for sample and standard solutions (for quantitation individual standards at different concentration levels between 0.1-1.0 mg/mL for primuline and concentration of the sample adjusted to the working range and analytical task, *e.g.* purity testing or degradation studies).
Developing solvent	Chloroform, methanol, water 65:25:4 (*V/V*) [5]
Development	In the HPTLC PRO Module DEVELOPMENT after activation at 0% relative humidity (molecular sieve) for 10 min and by using pre-conditioning for 300 s at 35% pump power). or: In the ADC 2 with chamber saturation (20 min with filter pad) and after conditioning at 33% relative humidity (saturated solution of magnesium chloride) for 10 min.
Developing distance	70 mm (from the lower edge)
Plate drying	Drying 5 min in the HPTLC PRO Module DEVELOPMENT or in the ADC 2
Documentation	With the TLC Visualizer 2 at UV 254 nm prior to derivatization and UV 366 nm after derivatization (for copper(II) sulfate also in white light after derivatization)

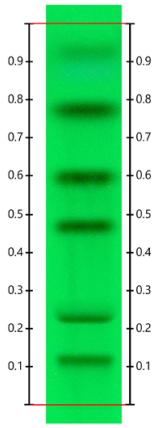
Densitometry	*For primuline reagent:* Densitometric analyses are performed in fluorescence mode with the TLC Scanner 4 at 366/>400 nm (mercury lamp), slit dimension 5.0 x 0.3 mm, scanning speed 20 mm/s, data resolution: 25 µm/step *For copper(II) sulfate reagent:* in absorbance mode at 330 nm (deuterium lamp), slit dimension 5.0 x 0.2 mm, scanning speed 20 mm/s, data resolution: 25 µm/step
Derivatization	Reagent name: Primuline reagent Reagent preparation: 10 mg of primuline are dissolved in 100 mL of 80% acetone. Reagent use: The plate is sprayed with 3.0 mL of the reagent with the Derivatizer, blue nozzle, spraying level 3 and heated for 2 min at 40°C on the TLC Plate Heater. *Alternative:* Reagent name: Copper(II) sulfate reagent Reagent preparation: 10% CuSO4 in 8% *o*-phosphoric acid Reagent use: The plate is sprayed with 3.0 mL of the reagent with the Derivatizer, red nozzle, spraying level 5 and heated for 10 min at 150°C on the TLC Plate Heater.

Results

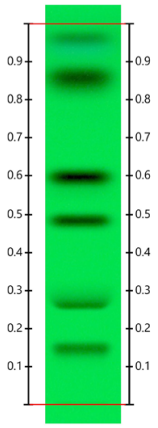
(Universal) SST:

HPTLC PRO: A quenching zone at R_F ~ 0.12, R_F ~ 0.23, R_F ~ 0.47, R_F ~ 0.60, and R_F ~ 0.77

ADC 2: A quenching zone at R_F ~ 0.14, R_F ~ 0.26, R_F ~ 0.48, R_F ~ 0.60, and R_F ~ 0.85



HPTLC PRO

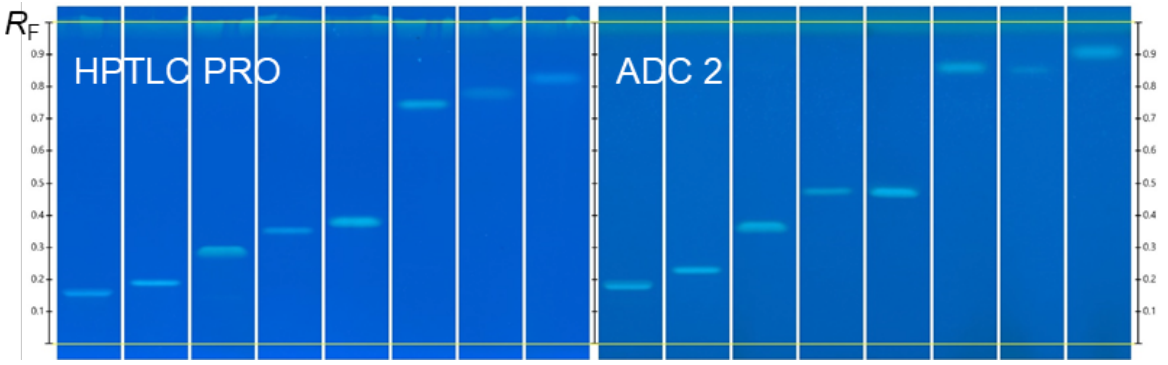


ADC 2

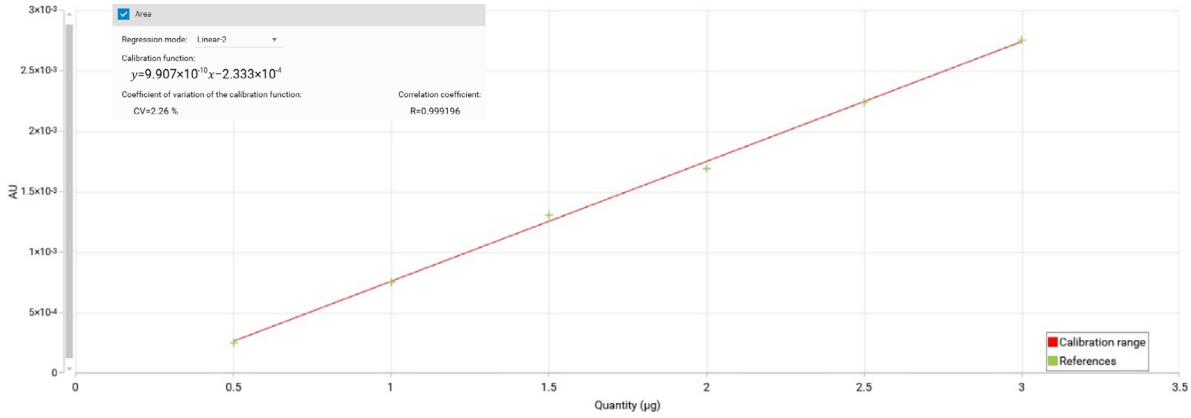
NOTE: The presented results are to be regarded as examples only!

Please contact CAMAG for more application notes and products!

Selected lipids to demonstrate the principle:

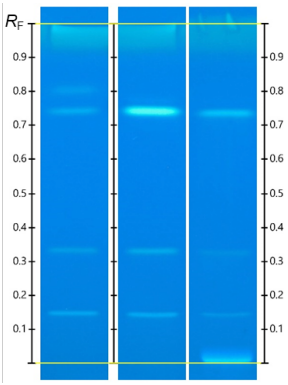


HPTLC chromatograms at UV 366 nm after derivatization with primuline reagent; From left: DOPS, lyso-DOPE, DOTAP, DOPE, DOTMA, DODMA, oleic acid, cholesterol



Calibration by volume to demonstrate the wide linear working range for detection in fluorescence mode after derivatization with primuline (for quantification, individual standards at different concentration levels and same application volumes of standard and sample solutions are recommended).

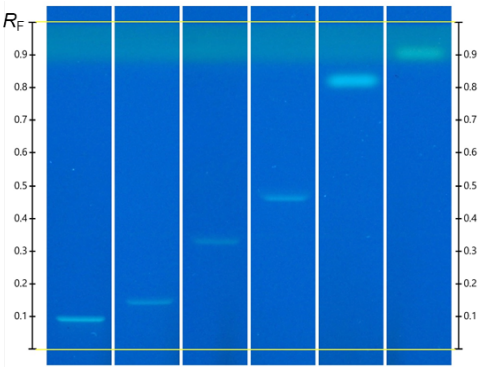
In the following chromatogram **a real case formulation** is shown. The samples (liposome solution and liposome-based formulation) were analyzed on a HPTLC plate by using the HPTLC PRO System.



HPTLC chromatograms at UV 366 nm after derivatization with primuline reagent; Track 1: mixture of DOPS, DOPE, DODMA, and cholesterol with increasing R_F; Track 2: solution with liposomes; Track 3: liposome-based formulation (sucrose and mRNA are not migrating with the selected developing solvent; front signals can be reduced by pre-washing of the plates prior to application)

Note: For the separation of the co-eluting lipids oleic acid and DODMA a 2-step development can be done with the HPTLC PRO Module DEVELOPMENT using two developing solvents of different selectivity.

First development with chloroform, methanol, water 65:25:4 (*V/V*) after activation at 0% relative humidity (molecular sieve) for 10 min, developing distance 30 mm, second development with cyclopentyl methyl ether, methanol, ammonia (35%) 65:30:5 (*V/V*) with pre-conditioning for 300 s at 35 % pump power, developing distance 70 mm.



Improved separation of oleic acid and DODMA (from left: DOPS, lyso-DOPE, DOPE, oleic acid, cholesterol, DODMA)

For further information send an e-mail to request@camag.com.

Literature

[1] Porter *et al.* Nature Reviews Drug Discovery (2007) 69:231–248
[2] Fuchs *et al.*, J Chromatogr A (2010) 1218(19):2754-74
[3] Cebolla *et al.* (2021) J Liq Chromatogr Relat (44) 148-170
[4] T. K. T. Do *et al.*, J Chromatogr A (2021) 1638
[5] https://avantilipids.com/tech-support/analytical-procedures/tlc-solvent-systems (access 06.08.2021)