

Comparison of conventional TLC and HPTLC for identity testing of herbal medicinal extracts



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WALA Heilmittel GmbH has been manufacturing anthroposophical and homoeopathic medicines based on medicinal plants as well as on substances of animal and mineral origin since 1935. The Analytical Development/Research Department is responsible for the development of qualitative and quantitative analytical methods and the establishment of the quality requirements for about 900 different medicinal products, their raw materials, and active ingredients. TLC is the most frequently used method for qualitative analysis of herbal components. In phytochemical analysis it is particularly advantageous, as heat sensitive plant components are not affected and analytes devoid of chromophores can be analyzed after postchromatographic derivatization. TLC has been widely used for identity testing of herbal active substances, although increasingly, it is HPTLC that is generally accepted in pharmacopoeias.

Introduction

In research laboratories, HPTLC is preferred in lieu of TLC [1] due to its improved efficiency, reproducibility and automation of relevant steps in the procedure. Furthermore, it saves time and is economical in solvent consumption. The new European Pharmacopoeia (Ph. Eur. 9.0) HPTLC chapter 2.8.25 to become valid in 2017, will include herbal drug-specific monographs such as *birch leaves* (01/2017:1174) illustrating the increasing relevance of HPTLC on a pharmacopoeia level. The method trans-

fer from TLC to HPTLC is at the same time connected with the contemporary conversion from manual sample application and derivatization to automated operations.

For pharmaceutical manufacturers like WALA Heilmittel GmbH, method changes require extensive consideration of not only regulatory issues, e. g. cGMP, but also marketing authorization and financial aspects. Therefore, these changes can only be implemented after thorough investigations. The Analytical Development/Research Department is currently evaluating selected medicinal products and their active ingredients whether a change from TLC to HPTLC is possible with a universal adaptation utilizing HPTLC plates with fluorescence indicator, same mobile phase composition, migration distances (MD) and application volumes. The overall aim is to change the method for identity testing from TLC to HPTLC without affecting the specified zones of the chromatographic fingerprint.

Chromatogram layer

HPTLC plates silica gel 60 F₂₅₄ (Merck), 10 × 10 cm *versus* TLC plates silica gel 60 (Merck), 20 × 20 cm

Standard solutions

Methanolic solutions of quinine hydrochloride, hyperoside, caffeic acid and rutin (each 1 mg/mL), fructose and caffeic acid (each 2 mg/mL) and nescapine hydrochloride (4 mg/mL)

Sample preparation

Four products were selected: (1) An aqueous-ethanolic extract of kidney vetch was treated by liquid-liquid extraction with ethyl acetate, evaporated to dryness and dissolved in 70% ethanol. (2) Suppositories containing caraway extract were melted, mixed with water and degreased by alternation of centrifugation and cooling. Subsequently, the sample was extracted with ethyl acetate, evaporated to dryness and dissolved in methanol. Both aqueously fermented root extracts of (3) barberry and (4) Solomon's seal were directly applied.

Sample application

TLC: manual application with band length, distance from the left and lower edge of the plate 15 mm each, track distance 25 mm, application volumes for reference solutions 10 μ L and for sample extracts (1) 15 μ L, (2) 50 μ L, (3) 20 μ L and (4) 30 μ L

HPTLC: bandwise with Automatic TLC Sampler (ATS 4), band length 8 mm, distance from lower edge 10 mm, distance from left edge 16 mm, track distance 11 mm, application volumes 20 % of those of TLC

Chromatography

TLC: in twin trough chamber 20 x 20 cm after saturation for 30 min (1) with chloroform – methanol – water 70:30:5 up to 165 mm after 3-h preconditioning at a relative humidity of 75 % (saturated sodium chloride solution); (2) with ethyl acetate – anhydrous formic acid – water 84:8:8 up to 115 mm; (3) with ethyl acetate – anhydrous formic acid – water 80:10:10 up to 95 mm and (4) with chloroform – methanol – water 50:42:8 up to 165 mm

HPTLC: in twin trough chamber 10 x 10 cm after saturation for 10 min with the respective solvent system up to 70 mm (from lower plate edge)

Postchromatographic derivatization

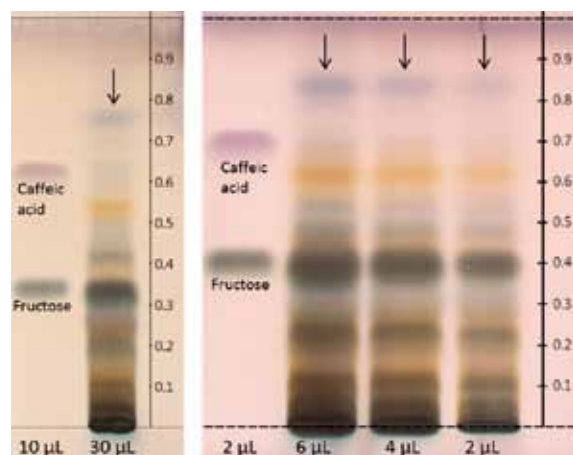
Plates were sprayed manually with (1) a solution of 20 % antimony(III) chloride in chloroform and heated at 105 °C for 30 min; (2) with a 1 % methanolic solution of diphenylboric acid aminoethyl ester (natural product reagent), followed by a 5 % methanolic polyethylene glycol (macrogol) 400 solution and detection at UV 366 nm after 30 min; (3) with a bismuthate reagent (mixture of 0.85 g alkaline bismuth nitrate, 40 mL water, 10 mL acetic acid (99 %), and 20 mL potassium iodide solution (400 g/L), glacial acetic acid and water, 1:2:10); (4) with the Derivatizer and 4 mL of a 1:1 mixture of 5 % sulphuric acid in ethanol and 2 % vanillin solution in ethanol (yellow nozzle, spray level 3) and heated for 15 min at 105 °C.

Documentation

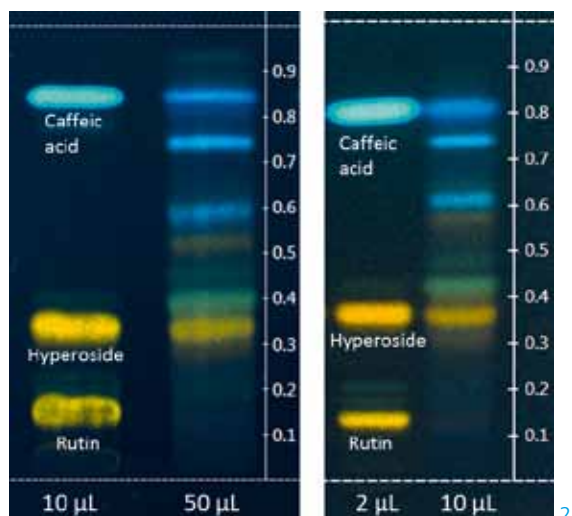
With TLC Visualizer and *visionCATS*

Results and discussion

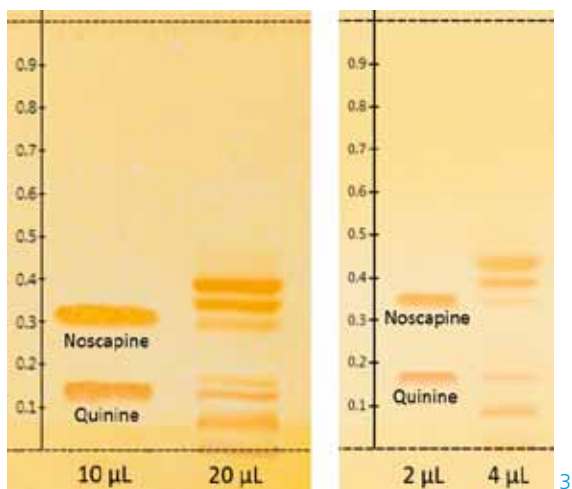
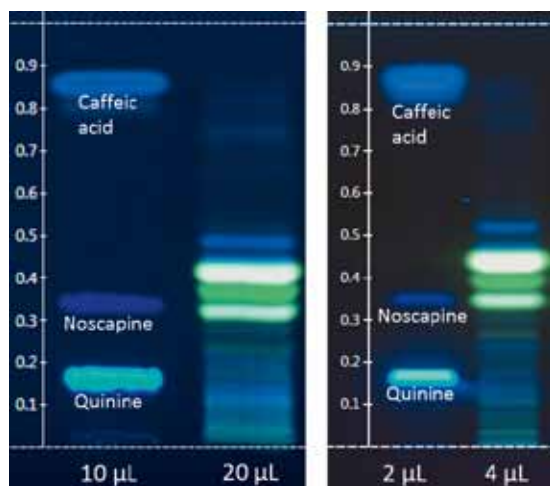
Some effects related to the change from TLC to HPTLC were observed. Referring to Solomon's seal extract, the reduced application volumes (20 % of those of TLC) led to similarly blurred zones on HPTLC plates, but the overloading was necessary for the detection of the specific weak blue zone at hR_F 85. Developing times were substantially decreased from 70 min TLC to 20 min HPTLC. Whereas these were roughly the same for suppository and barberry extracts. Only a moderate shift in hR_F values was observed, most pronounced for the Solomon's seal extract.



TLC (left) versus HPTLC (right) chromatograms of Solomon's seal extract under white light after derivatization with sulphuric acid vanillin reagent (normalized on MD)



TLC (left) versus HPTLC (right) chromatograms of suppository extracts under UV 366 nm after derivatization with natural product reagent and polyethylene glycol (normalized on MD)



TLC (left) versus HPTLC (right) chromatograms of barberry extract under UV 366 nm, and after derivatization with bismuthate reagent under white light (normalized on MD)



CAMAG Derivatizer

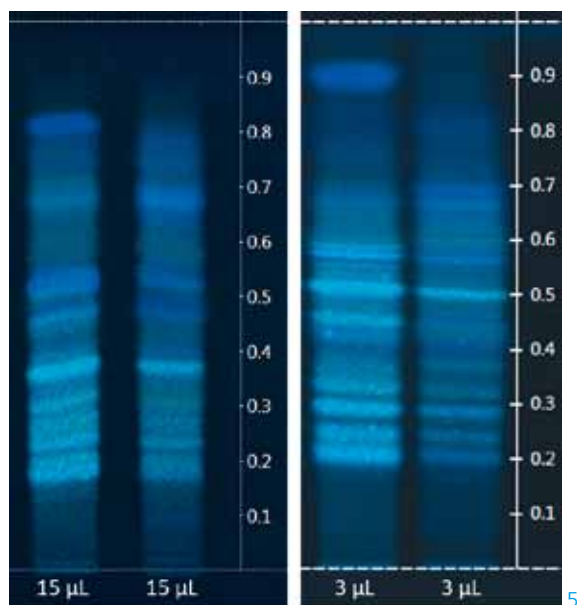
The Derivatizer is used for automated reagent transfer in the derivatization of thin-layer chromatograms. Thanks to its unique "micro droplet" spraying technology the Derivatizer ensures homogeneity and reproducibility in applying derivatization reagents, using the most common reagents.

To meet the diverging physicochemical properties of different reagents, e. g. acidity, viscosity, four different color-coded spray nozzles are employed with six spraying modes available to the user.

In addition to the significantly increased homogeneous reagent distribution, the Derivatizer offers other advantages compared to manual spraying:

- Environmentally friendly safe handling through a closed system
- Intuitive handling and easy cleaning
- Low reagent consumption through efficient operation (4 mL for 20 × 20 cm and 2 mL for 20 × 10 cm plates), which is particularly beneficial when expensive reagents are used
- Reproducible and user-independent results

The chromatograms of kidney vetch extract demonstrated the higher separation power of HPTLC, as more zones were separated (hR_F 20–70). Also separation times were different (TLC 60 min versus HPTLC 15 min).



TLC (left) versus HPTLC (right) chromatograms of kidney vetch extract under UV 366 nm after derivatization with antimony(III)-chloride reagent (normalized on MD)

In summary, the change from TLC to HPTLC by a universal adaptation appears promising. Zones were sharper in HPTLC chromatograms compared to TLC. Specified zones are evaluated in relation to a reference. Hence, a moderate shift of hR_F values is no limitation for the method transfer. As demonstrated by the kidney vetch extract, adaptations may be necessary in individual cases. Impact on specified zones as well as changes in composition of the mobile phase should be minor to avoid validations for batches of different harvest years. Such regulatory and expensive efforts are compensated by the advantages of HPTLC. The increasing relevance of HPTLC in pharmacopoeiae encourages the Analytical Development/Research Department to proceed with the method transfer from TLC to HPTLC. Other requirements, such as a system-specific suitability test or intensity markers, must be addressed as well.

Note: According to the general chapter 2.8.25 the standard HPTLC plate format is 20 × 10 cm. For the sample application, the distance from left edge should be 20 mm and from the lower edge 8 mm. In a non-humidity controlled lab the HPTLC plate is conditioned at 33 % relative humidity until an equilibrium is reached. Time for chamber saturation is 20 min. CAMAG recommends the development in the Automatic Developing Chamber (ADC 2).

[1] Morlock, G. E., Schwack, W., J. Chromatogr. A 1217 (2010) 6600–6609

Further information is available on request from the authors.

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