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Evaluation and Optimization of Method for Identification of Black Cohosh by HPTLC Fingerprint

1. Purpose

The method for identification of Black Cohosh published by the American Herbal Pharmacopoeia shall be evaluated prior to validation and, if necessary, optimized with particular respect to overall reproducibility.

2. Description of method

Preparation of test solutions
a) Plant acids
500 mg of each sample are mixed with 10 mL of ethanol-water 5:5, sonicated for 10 min, and then centrifuged. The supernatant is used as test solution.
b) Triterpenglycosides
500 mg of each sample are mixed with 10 mL of methanol, sonicated for 10 min, and then centrifuged. The supernatant is used as test solution.

Preparation of reference solutions
Botanical reference solution: 500 mg of sample are mixed with 10 mL of ethanol-water 5:5 or methanol respectively, sonicated for 10 min, and then centrifuged. The supernatant is used as test solution.

Chemical reference solutions: 1 mg each of actein, isoferulic acid, caffeic acid, and chlorogenic acid is dissolved in 10 mL methanol each.

Preparation of derivatizing reagent
Anisaldehyde-sulfuric acid reagent: 10 mL of sulfuric acid are carefully added to an ice-cooled mixture 170 mL methanol and 20 mL acetic acid. To this solution 1 mL anisaldehyde is added.

Stationary phase
10x10 cm (or 20x10 cm) glass plates HPTLC silica gel 60 F_{254} (Merck).

Sample application
2 µL of test solution and 2 µL of standard are applied each as 8 mm bands, at least 2 mm apart, 8 mm from the lower edge and at least 15 mm from left and right edges of the plate.

Temperature and Humidity
Record temperature and humidity in the laboratory.

Chromatography
Chamber type: 10x10 cm (or 20x10 cm) Twin Trough Chamber

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Configuration:	Saturated for 20 min (wetted filter paper in trough opposite to the plate)
Developing solvent:	Ethyl formiate, toluene, formic acid (30:50:20), 20 mL (respectively 10 mL) developing solvent per trough.
Developing distance:	70 mm from lower edge of plate (62 mm from application position)
Drying:	5 min with cold air (hair dryer)

Derivatization only for Triterpensaponins

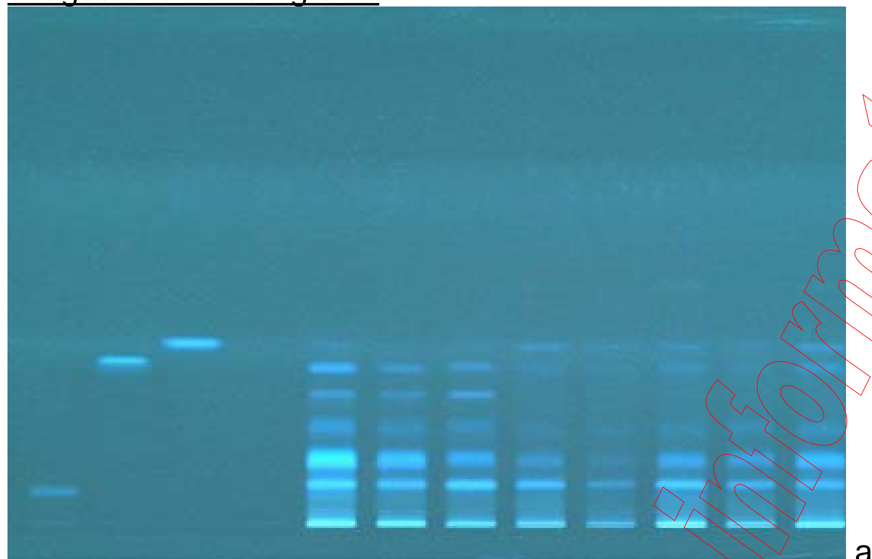
The plate is immersed into reagent for 1 s, then heated at 100°C for 5 min.

Documentation

a) prior to derivatization under UV 366 nm (for plant acids only)

b) After derivatization under white light

Images of chromatograms



a

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b

1: Chlorogenic acid
2: Caffeic acid
3: Isoferulic acid
4: Actein
5-12: Various samples of Black Cohosh (*Actaea racemosa*)

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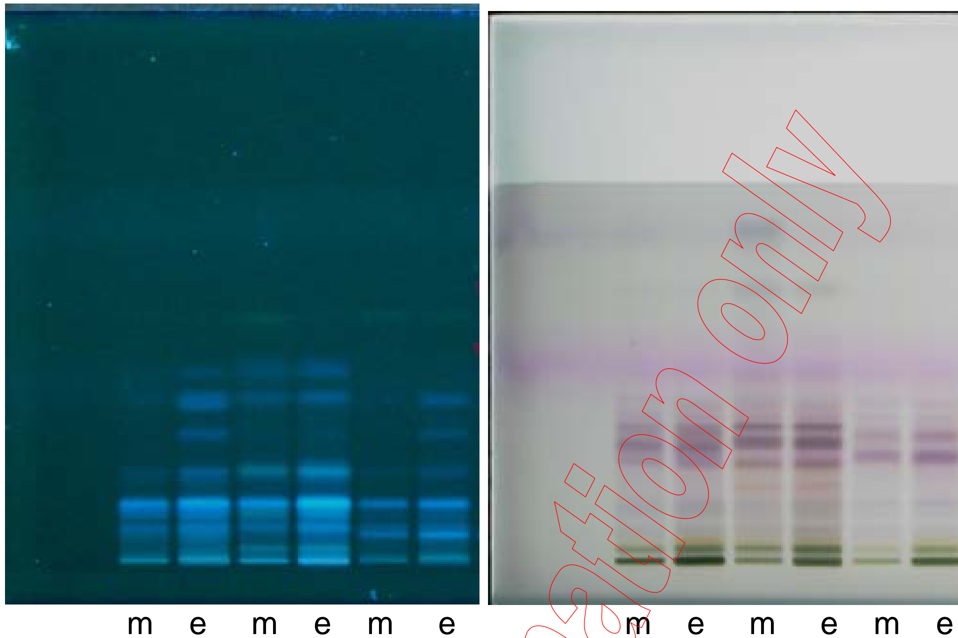
3. Materials

Name	Source / Lot	Authentication
Actaea racemosa (Black Cohosh root)	Removed - proprietary information	Yes (BRM)
Cimicifuga racemosa		Yes
Cimicifuga (wild USA)		No
Cimicifuga (cultivated Germany)		No
Cimicifuga (cultivated Switzerland)		No
Cimicifuga (cultivated Switzerland)		No
Cimicifuga (wild USA)		No
Cimicifugae rhizome conc.		No

4. Evaluation
4.1 Extraction

It was evaluated whether only one extraction can be used for both analyses.

Results:
UV 366 nm (no derivatization)   White light (derivatized plate)



m: extraction with methanol, e: extraction with ethanol-water (1:1)
While the extraction with ethanol-water (1:1) yield more zones in the plant acid profile (prior to derivatization, UV 366 nm), the methanol and the ethanol-water (1:1) extractions produce similar results in the triterpene profile (after derivatization).
The extraction with ethanol-water (1:1) will be used for both profiles.

4.2 Derivatizaiton

The use of anisaldehyde reagent yields pink-violet, yellow and brown-green zones. However, the plate background is commonly also very colored. A pink zone is typically seen across the plate after derivatization (arrow), which may interfere with the detection of some compounds of interest.
Sulfuric acid reagent shall be evaluated as alternative.

Results:
Anisaldehyde reagent Sulfuric acid reagent (different samples)

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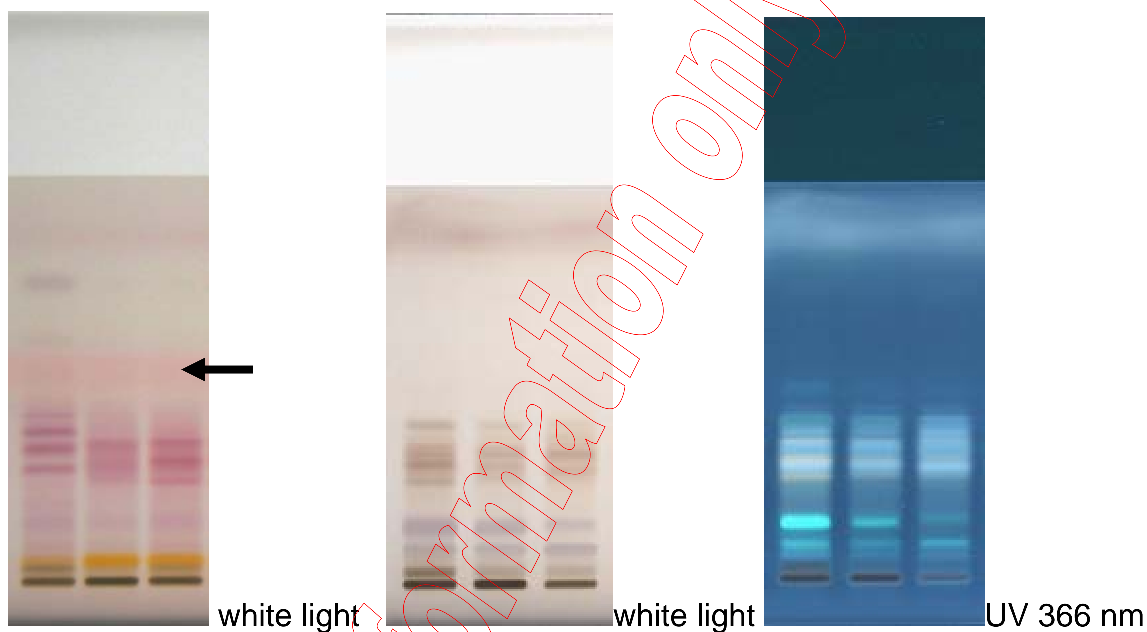

white light white light UV 366 nm

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Sulfuric acid reagent yields a very stable yet not as colorful derivatization. No disturbing zone across the plate is seen. This reagent also generates fluorescing zones under UV 366 nm, which can provide extra information.
Sulfuric acid reagent will be used for derivatization.

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4.3 Influence of humidity

It was noticed that the separation seemed to be influenced by the ambient relative humidity. When the plate was developed above 50%RH, the separation of the triterpenes was not achieved.
It was decided to condition all plates at the lowest possible humidity (about 5%RH). For more details on separation at other RH, see validation protocol.

Results:
Development at 50% RH   Development after conditioning at about 5% RH

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