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Date	Analyst	Project No.	Project Name
30.Sept.2003	Anne Schibli	P08	Kava

Development of a method for the identification of Kava

1. Analytical goal
Develop a method for the identification of Kava rhizome, which separates the six available kavalactones.

2. Materials used during method development
2.1 Samples

Sample Name	Source / Batch	Authentication	Notes
Piper methysticum chips	Removed - proprietary information	Yes	S285

2.2 Standards (marker compounds)

Name	Source / Batch
D/L-Kavain	ChromaDex 01-11300-101
Dihydrokavain	ChromaDex 01-04476-101
Methysticin	ChromaDex 01-13860-101
Dihydromethysticin	ChromaDex 01-04477-101
Yangonin	ChromaDex 01-25010-101
Desmethoxyyangonin	ChromaDex 01-04236-101

2.3 Plates

Type	Source	Batch
HPTLC 10X10 F254 Si 60	Merck	none
Caffeine 20X10 F254s Si 60	Merck	45212327
Lichrospher 20X10 F254s Si 60	Merck	740259362

3. Method development
3.1 Selection of extraction solvent and derivatization reagent
Results (meaningful images from worksheet) of development with
not evaluated.

1) Toluene, ethyl acetate (95:5) / Anisaldehyde reagent
2) Chloroform, methanol, water (70:30:4) / Anisaldehyde reagent
3) Ethyl acetate, acetic acid, formic acid, water (100:11:11:27) / NP reagent
4) Acetonitrile, water, formic acid (30:8:2) / Ninhydrin reagent
5) 1-Butanol, acetic acid, water (7:1:2) / Anisaldehyde reagent
Conclusions: -
Most suitable extraction solvent:-
Most suitable solvent system:-
Most suitable derivatization:-

3.2 Selection of application volume and extraction solvent
Results:
The kind of extraction solvent does not affect the extraction. The lactones are extracted equally well with methanol, acetone, or dichloromethane.

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Extraction was performed by sonicating of 1 g of Kava for 10 min with 10 mL of solvent.
Images:
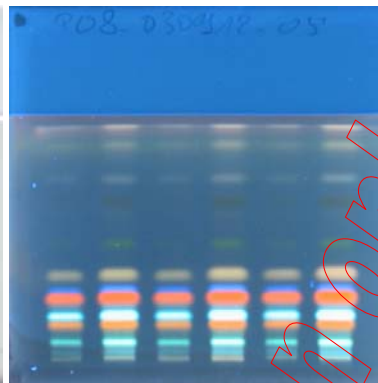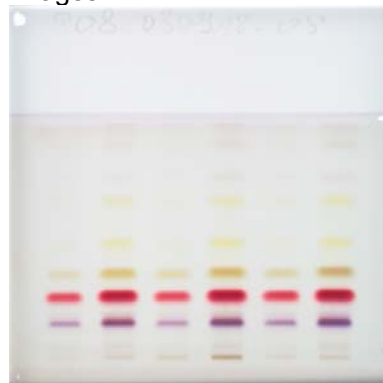
1: acetone, 2µL
2: acetone, 5µL
3: methanol, 2µL
4: methanol, 5µL
5: dichloromethane, 2µL
6: dichloromethane, 5µL

Conclusion: the extraction will be performed with methanol. This solvent is more convenient to use for chromatography.
Application volume: 2µL

3.3   Optimization of mobile phase
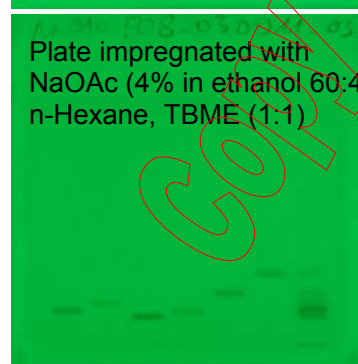
Summary of results:

Best results on silica gel stationary phase were obtained with t-butyl methyl ether and hexane as mobile phase. However, the separation of the six kavalactones was insufficient.
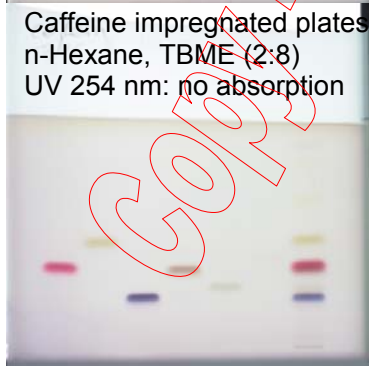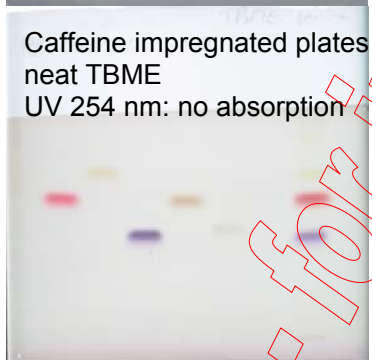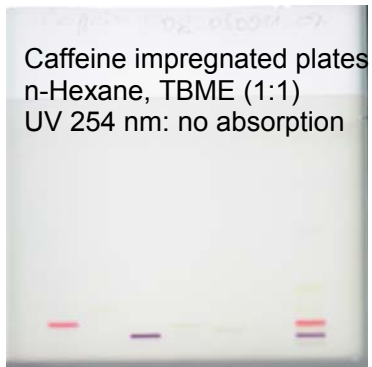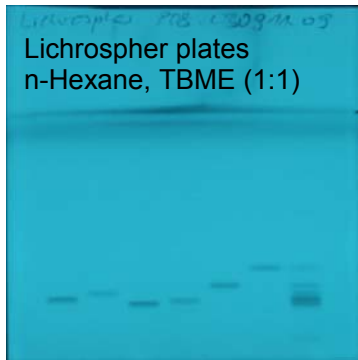
Different stationary phases were tried. The use of Alox plates was discontinued due to the availability as conventional TLC plates only. All plates were developed in unsaturated chambers.
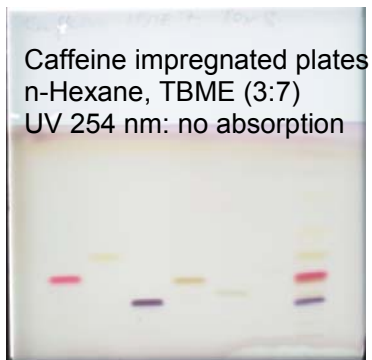
Images:

Silica gel
n-Hexane, TBME (1:1)

Plate impregnated with
NaOAc (4% in ethanol 60:40),
n-Hexane, TBME (1:1)

1: D/L-Kavain
2: Dihydrokavain
3: Methysticin
4: Dihydromethysticin
5: Yangonin
6: Desmethoxyyangonin
7: Kava

Lichrospher plates
n-Hexane, TBME (1:1)

Caffeine impregnated plates
n-Hexane, TBME (1:1)
UV 254 nm: no absorption

Caffeine impregnated plates
neat TBME
UV 254 nm: no absorption

Caffeine impregnated plates
n-Hexane, TBME (2:8)
UV 254 nm: no absorption

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Caffeine impregnated plates
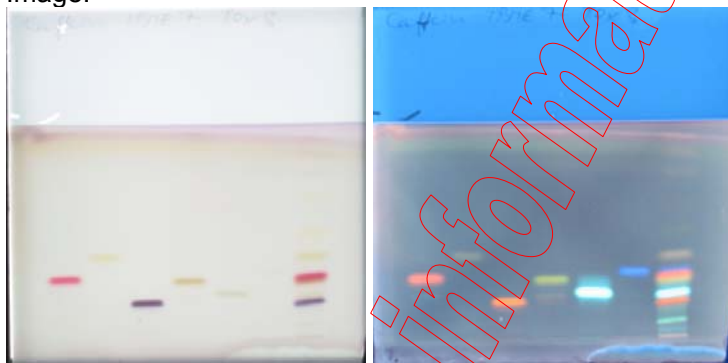n-Hexane, TBME (3:7)
UV 254 nm: no absorption

Conclusion: The caffeine impregnated plates show the best separation.
Selected developing solvent: n-Hexane, TBME (3:7), no chamber saturation.

3.4 Optimization of detection and fine-tuning of method
Results:
Detection is best under UV 366 nm. All substances appear.
Image:

Conclusion: the plate should be documented under UV 366 nm after derivatization.
Steps optimized: the detection after derivatization

3.5 Final method
Results:
See 3.4.
Image:

Conclusion: check one
X Analytical goals are achieved: → continue with section 4
... Analytical goals are not achieved: → restart with section 3.3

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4. Evaluation of stability (pre-validation)
4.1 Stability of analyte during chromatography
Result:
No zone is located aside of the diagonal. The sample is stable during chromatography.
Images: P08_030915_2D

Acceptance criteria:
The sample is stable during chromatography if all zones are located on the diagonal connecting the application position with the intersection of the two solvent fronts.
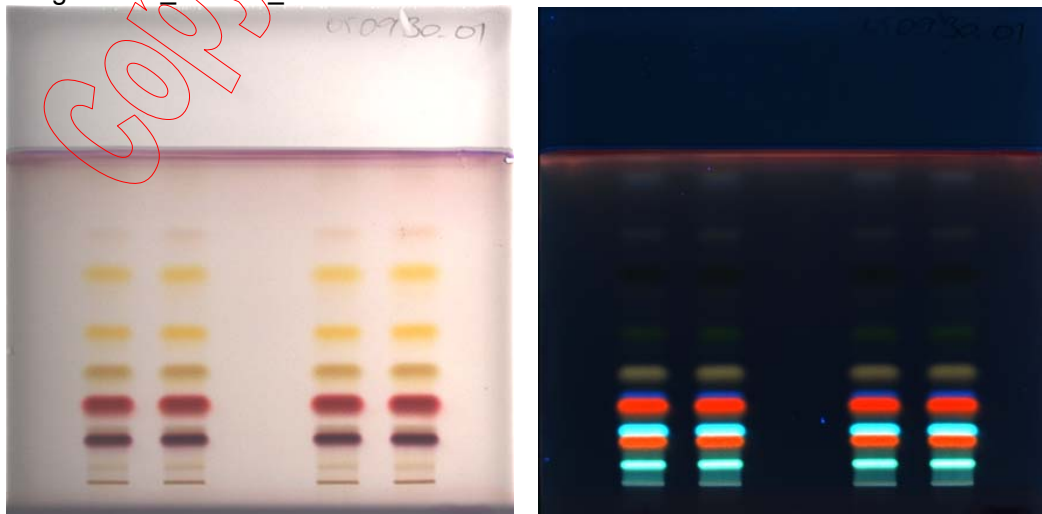
Pass: Yes

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4.2 Stability of analyte in solution and on the plate
Result:
No differences are seen between the tracks. The sample is stable for 3 hours in solution and 3 hours on the plate.
Images: P08_050930_01

Acceptance criteria:

The sample is stable for at least 3 hours in solution and 3 hours on the plate prior to chromatography if no differences are seen between the four tracks.
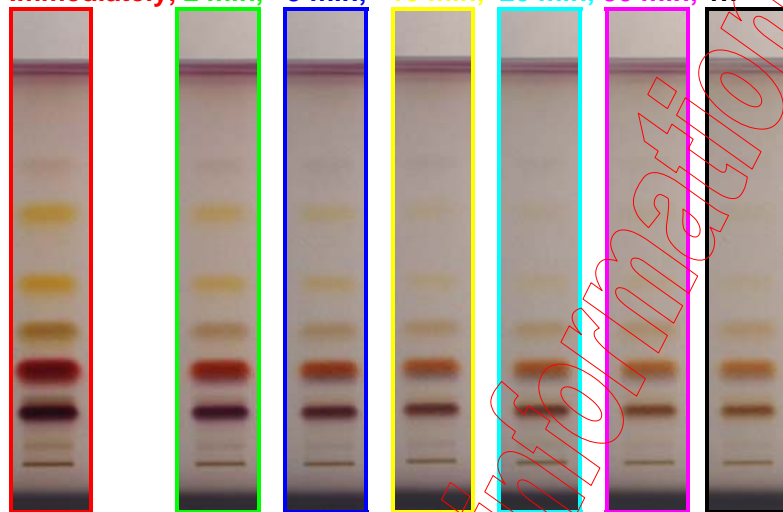
Pass: Yes

4.3   Stability of result (for documentation)

Result:

The result of the derivatized plate is not stable. Significant changes in the coloration of bands are seen within 2 min after derivatization, particularly under white light. The plate should be looked at/documented immediately after heating, while still hot.

Image under white light: P08_050930_01

Immediately, 2 min, 5 min, 10 min, 20 min, 30 min, 1h
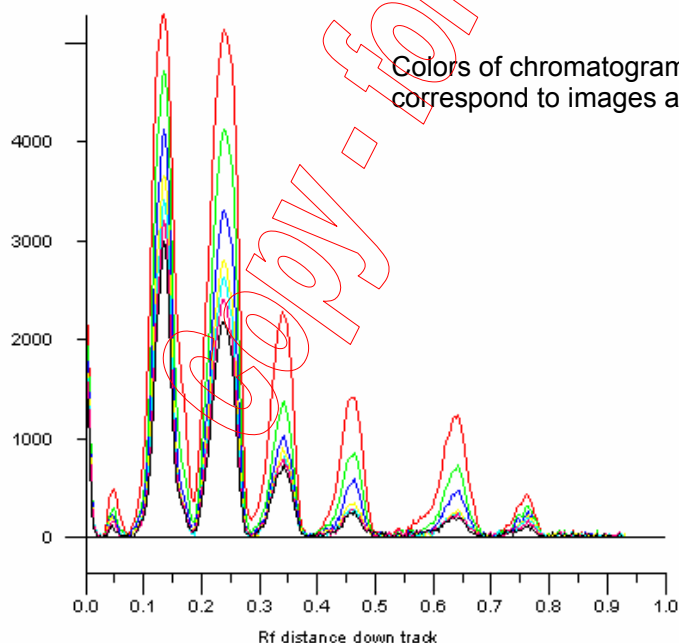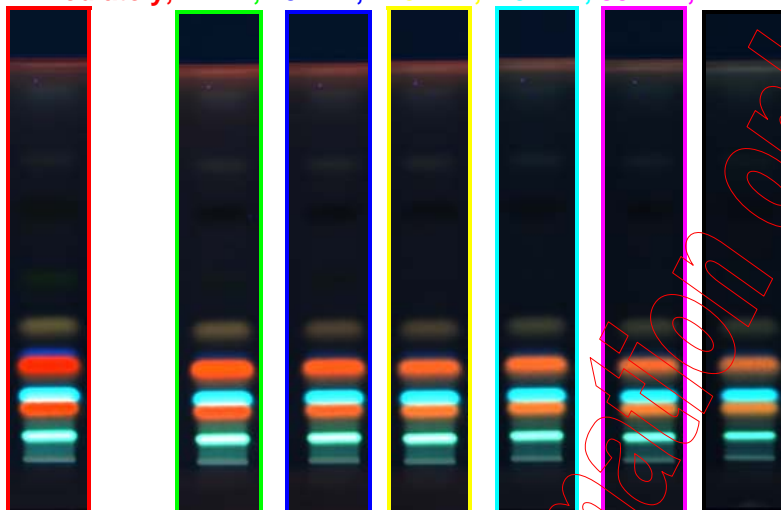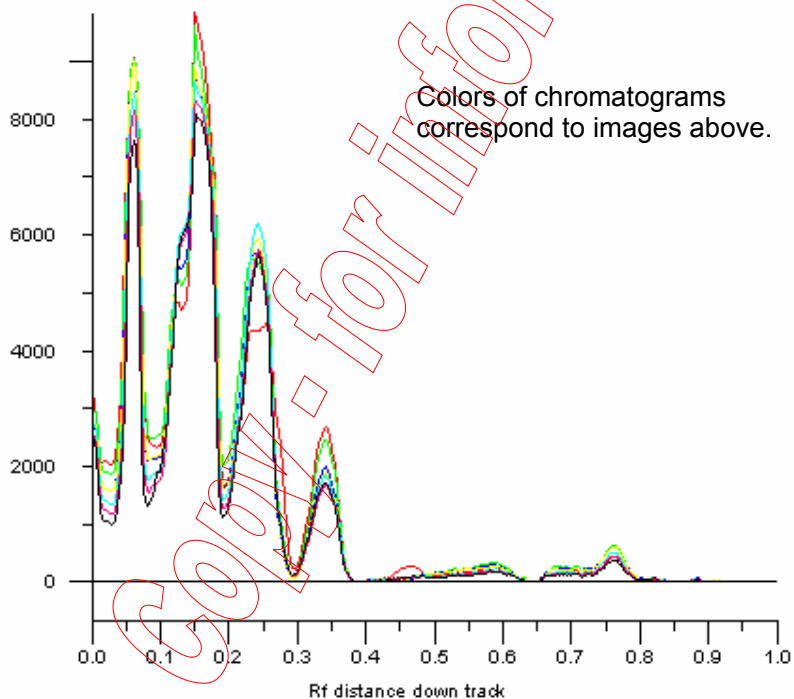
Densitograms:

Profile height

Colors of chromatograms correspond to images above.

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Image under UV 366 nm:
Immediately, 2 min, 5 min, 10 min, 20 min, 30 min, 1h

Densitograms:

Colors of chromatograms correspond to images above.

Acceptance criteria:
The derivatization/visualization yields a stable result, if there is no significant change in the image within 30 min.

Pass: YES, with the following restriction: the plate must be documented immediately after derivatization, while still hot.

4.4   Conclusion

X Stability tests passed → Use FO 70.002.02 "Method to be validated" for method write up, then validate method according to SOP 70.002.01 "Evaluation, development, optimization, and validation of methods for identification of medicinal plants and products thereof". If the method is not intended to be validated, use FO 70.002.06 "Application Note" for method write up.

…Stability tests failed → restart with section 3.3/3.4 or refer to SOP 70.002.01 "Evaluation, development, optimization, and validation of methods for identification of medicinal plants and products thereof".

Printed
Date: Signed:

Date of review: Name: Title: Signature: