

Date	Analyst	Project No.	Project Name
21.Mar.2002	Anne Schibli	P08	Kava

Evaluation and optimization of methods for identification of Kava

1. Analytical goal:

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

2. Paper review of methods from literature:

Literature (see appendix)	Scope	Mobile phase / Stationary phase / derivatization	Refer to Figure # below
Wagner and Bladt	Kawain	n-Hexane – Ethyl acetate (70:30) / double development on Alox plate / anisaldehyde reagent	1a
Pharmacopoeial forum 28(1)	Kawain	n-Hexane – Ether (30:70) / double development on Alox plate / anisaldehyde reagent	1b

3. Experimental evaluation of selected methods

3.1 Materials

3.1.1 Samples

Sample name	Source / Batch	Authentication	Notes
Kava Kava root	Removed proprietary information	No	old sample

3.1.2 Standards (marker compounds)

Name	Source
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

3.1.3 TLC plates

Name, Type	Source
TLC Plate 20x20, Alox	Unknown



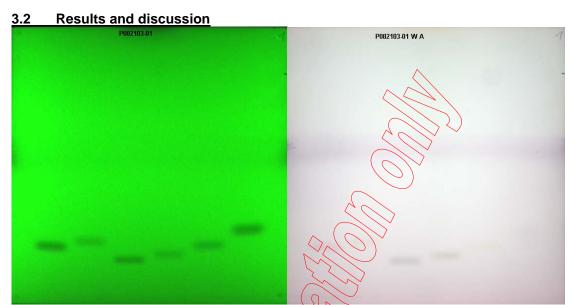


Figure 1a: Wagner [only single development, 2h dev. time]



Figure 1a: USP-PF

3.3 Conclusions:

Check one

- ... Method from literature is suitable \rightarrow continue with section 5
- X Method "USP-PF" needs optimization → continue with section 4
- \dots No suitable method is found \rightarrow refer to SOP 70.002.01 "Evaluation, development, optimization, and validation of methods for identification of medicinal plants and products thereof".

4. Method optimization

4.1 Sample preparation

4.2 HPTLC methodology

4.3 **Derivatization**



4.4 Mobile phase

Substitution of ether with TBME, and increasing of the ether ratio.

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

n-Hexane - TBME (1:1) double development.

The separation is improved but still not sufficient.

4.5 Method including all optimized parameters

4.6 Conclusions

Check one

- ... Analytical goals achieved > continue with section 5
- X Analytical goals not achieved refer to SOP 70.002.01 "Evaluation, development, optimization, and validation of methods for identification of medicinal plants and products thereof".
- 5. Evaluation of stability (pre-validation)
- 5.1 Stability of analyte during chromatography
- 5.2 Stability of analyte in solution and on the plate
- 5.3 Stability of result/(for documentation)
- 5.4 Conclusion
- ... 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.
- \dots Stability tests failed \rightarrow restart with section 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: