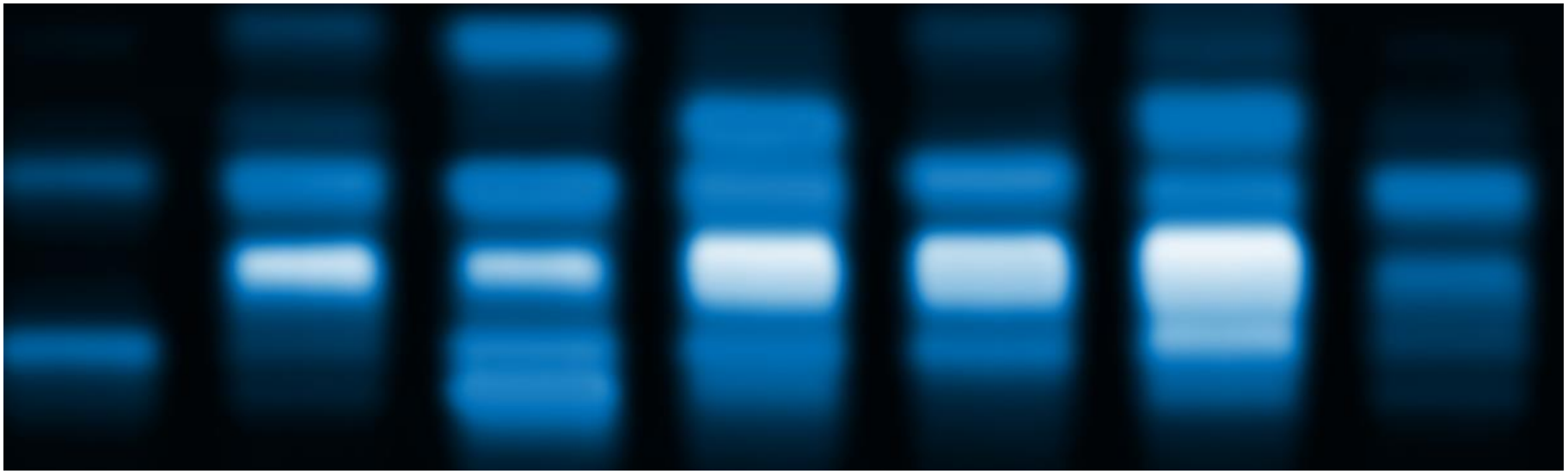

Standardized and internationally harmonized HPTLC methods for describing the quality of reference material for *Angelica*, *Ligusticum* and related species

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Introduction

- Under cGMP (medicinal) plants as raw material require proper identification → 100% ID rule
- Global supply chains and international trade need methods that can give reliable and reproducible results, regardless of the laboratory applying them.
- Specifications for reference material must be set.
- Agreement with specifications must be determined.
- HPTLC has great potential to become the method of choice because of its principal fitness for this purpose.

The FHHM concept of RMPM

- Forum for the Harmonization of Herbal Medicines organized by the WHO Regional Office for the Western Pacific.
- Objective: promoting public health by recognizing and developing standards and technical guidelines aiming to improve quality, safety and efficacy of herbal medicines.
- A protocol for establishing RMPM was adopted in March of 2014
- HPTLC is accepted as primary tool for identification.
- Work in progress to establish limit test for content.

HPTLC – methodology*

- HPTLC glass plates Si 60 F_{254} 20x10 cm
- 15 tracks, 8 mm bands, 8 mm from lower edge
- Conditioning to 33% rel. humidity (sat. $MgCl_2$)
- 20 min chamber saturation (with filter paper)
- Development to 70 mm from lower edge of plate

*** USP GC <203> PF 40 (3)**

PhEur* method for identification of *Angelica* species

Test solution

Mix 1.0 g of powdered sample with 5 mL of methanol and shake for 10 min, then centrifuge and use the supernatant as test solution.

Reference solution

1 mg each of isoimperatorin, imperatorin, osthole and 10 µL of Z-ligustilide in 1 mL of methanol.

Application volume: 4 µL

*** Modified from PhEur Monograph 2556: Angelica dahurica root**

Method cont.

Mobile phase

Toluene, ethyl acetate, acetic acid 90:10:1 (v/v/v)

Derivatization

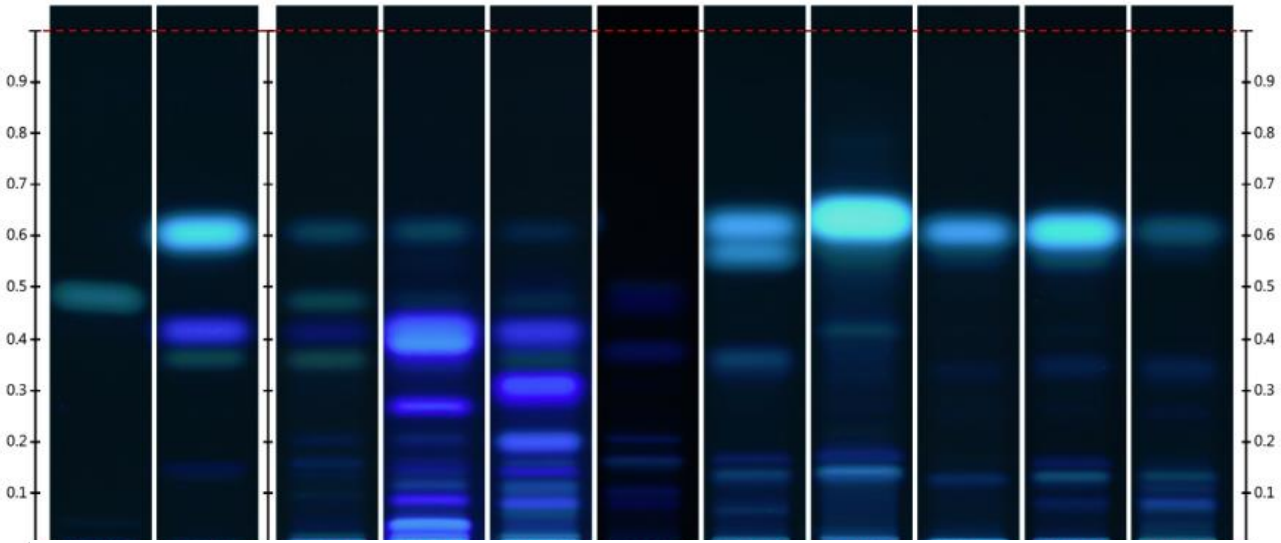
Dipping into 20% sulfuric acid in methanol; heating at 100°C for 3 min.

Evaluation

1) UV 366 nm, 254 nm after development

2) white light RT, UV 366 nm after derivatization

HPTLC for ID of *Angelica* and related species (PhEur)



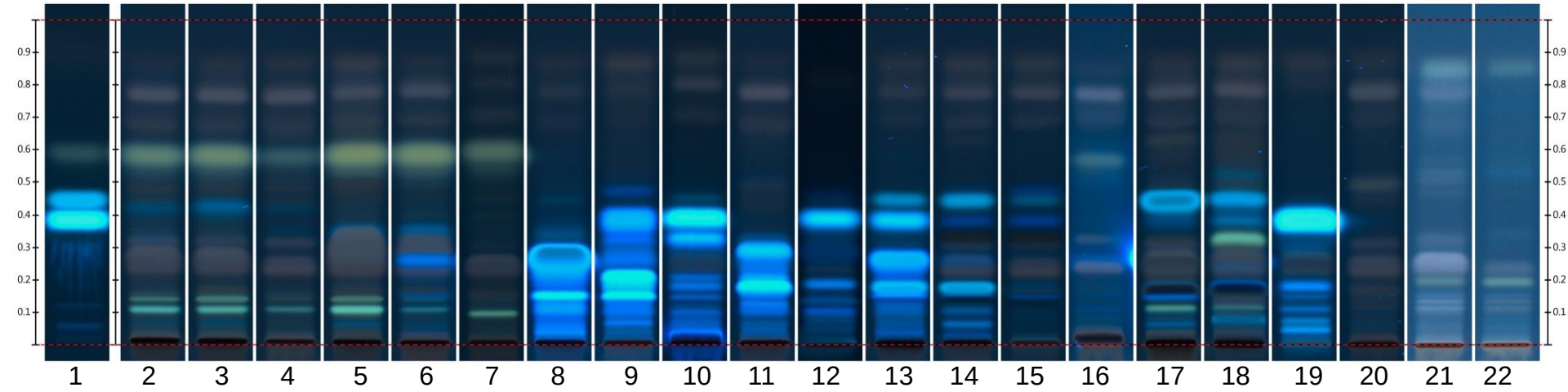
One method for all related species

→ Assessment of variability

→ Setting suitable acceptance criteria

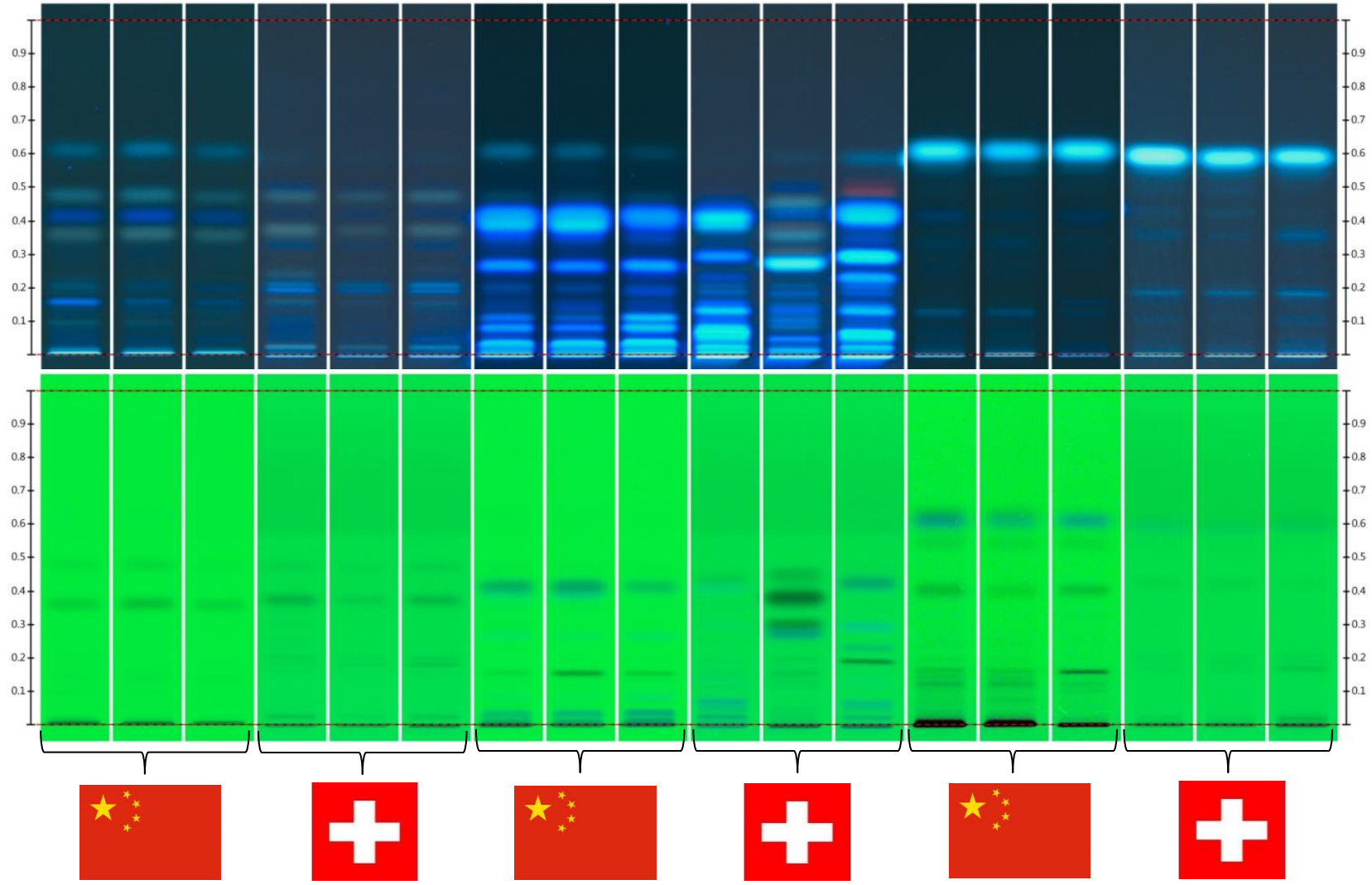
→ International collaboration

EP method for ID of 21 related species



Track	Samples	Track	Samples
1	*Imperatorin, osthole, Isoimperatorin, Ligustilide***	12	*Angelica archangelica*
2	*Ligusticum chuanxiong*	13	*Ostericum koreanum*
3	*Ligusticum sinensis*	14	*Angelica dahurica*
4	*Cnidium officinalis*	15	*Angelica dahurica var. formosana*
5	*Ligusticum jeholense*	16	*Angelica acutiloba*
6	*Ligusticum tenuissimum*	17	*Notopteryguii forbesii*
7	*Angelica sinensis*	18	*Notopteryguii incisum*
8	*Angelica gigas*	19	*Cnidium monnierii fruit*
9	*Angelica decursiva*	20	*Glehnia littoralis*
10	*Angelica pubescens*	21	*Bupleurum falcatum*
11	*Peucedanum praeruptorum*	22	*Bupleurum scorzonerifolium*

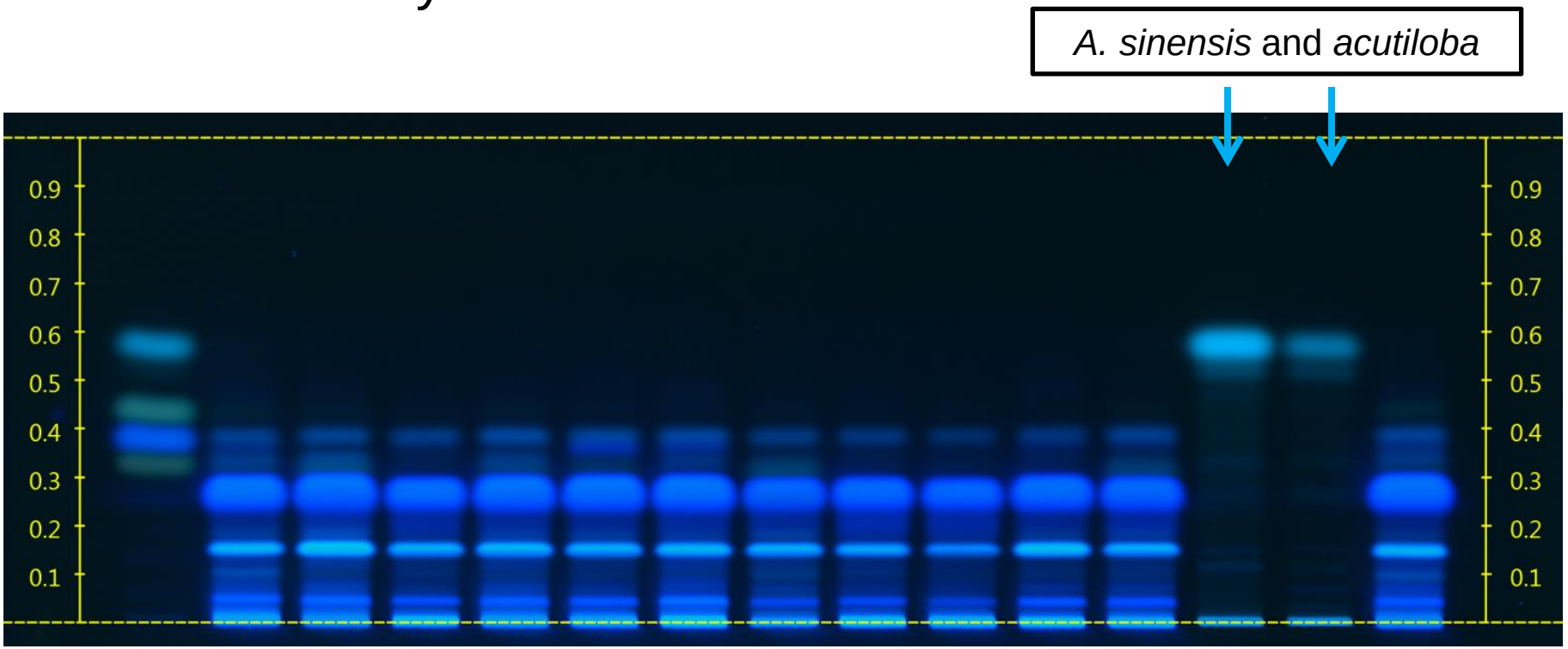
Inter-laboratory collaboration



Angelica dahurica *Angelica pubescens* *Angelica sinensis*

Batches of RMPM for *Angelica gigas*

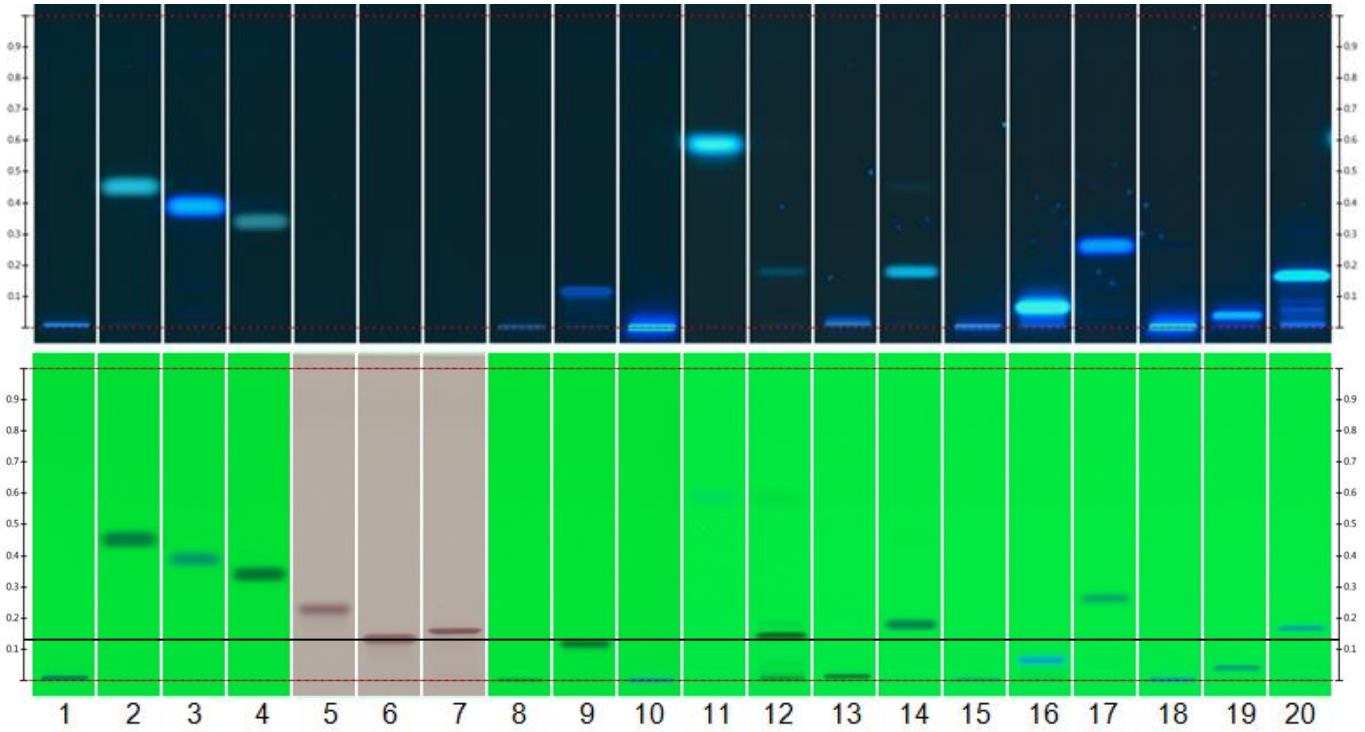
Natural variability



→ Identification and discrimination from other species is ok!

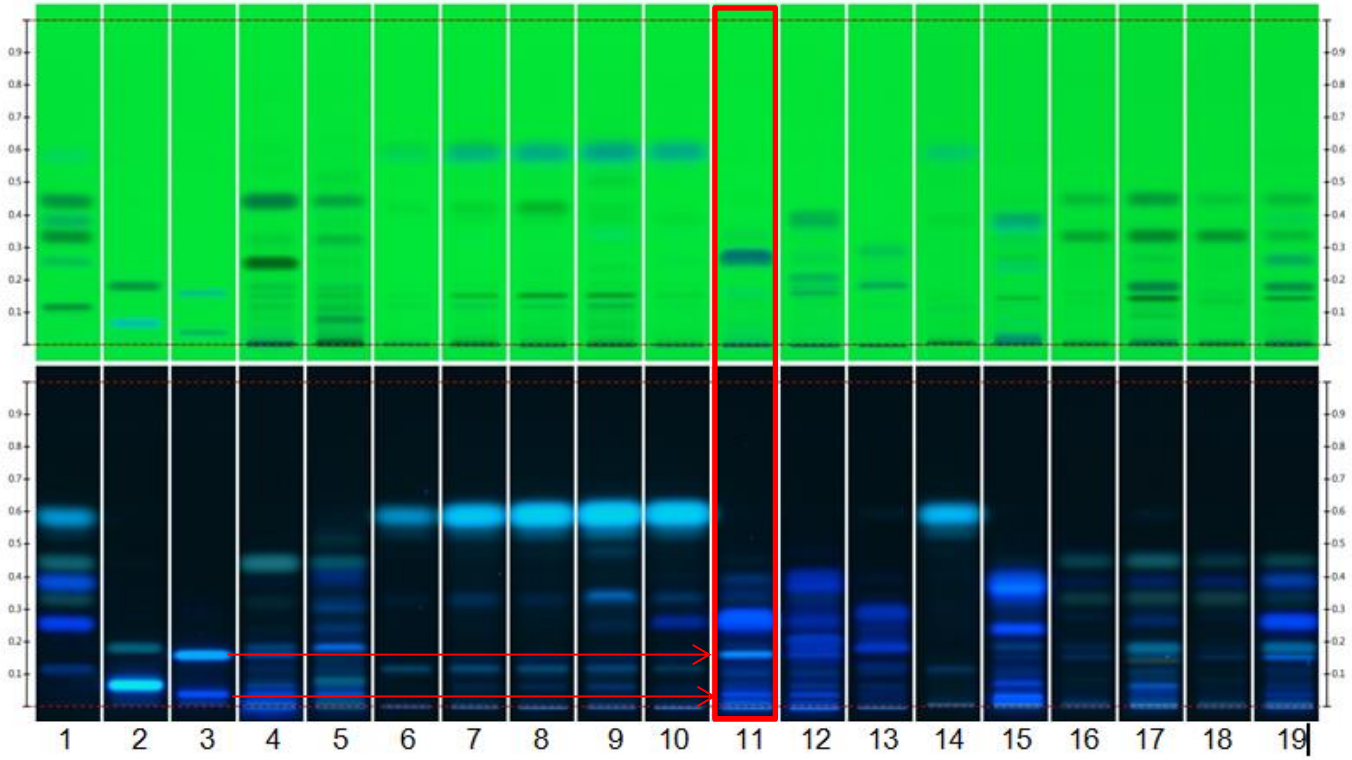
Reference compounds for *Umbelliferae*

Evaluation of chromatographic behavior



Track	Sample	Track	Sample	Track	Sample	Track	Sample
1	Prim-O-glucopyransoyl-cimifugin	6	Pregnenolone	11	Z- ligustilide	16	Scopoletin
2	Isoimperatorin	7	Oleanolic acid	12	Bisabolangelone	17	Decursin
3	Osthole	8	Chlorogenic acid	13	β-Yakangelicin	18	Nodakenin
4	Imperatorin	9	Ferulic acid	14	Oxypeucedanin	19	Decursinol
5	B-sitosterol	10	Marmesinin	15	1-O-b-D-glucopyranosyl-(2`S, 3`R)-3`-hydroxy marmesin	20	Demethylsuberosine

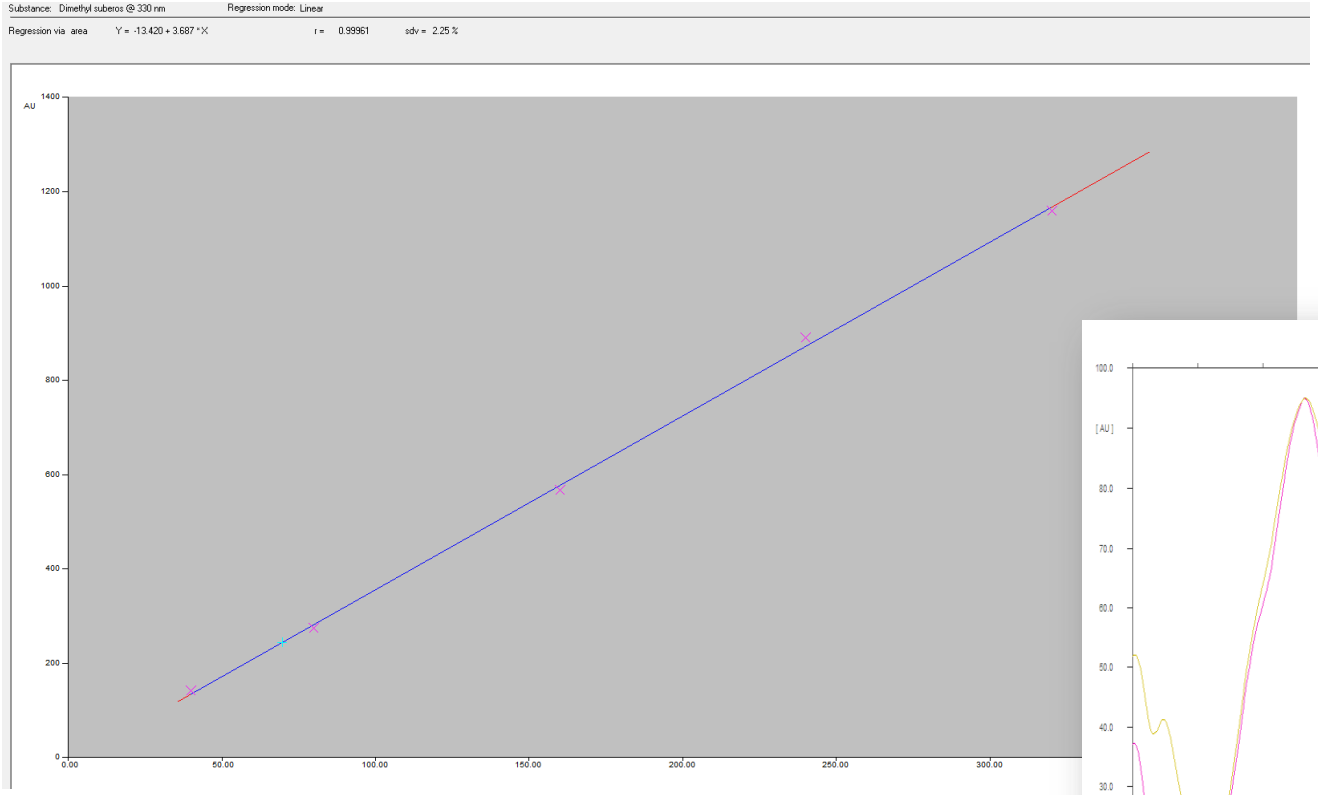
Determination of spectral properties for markers

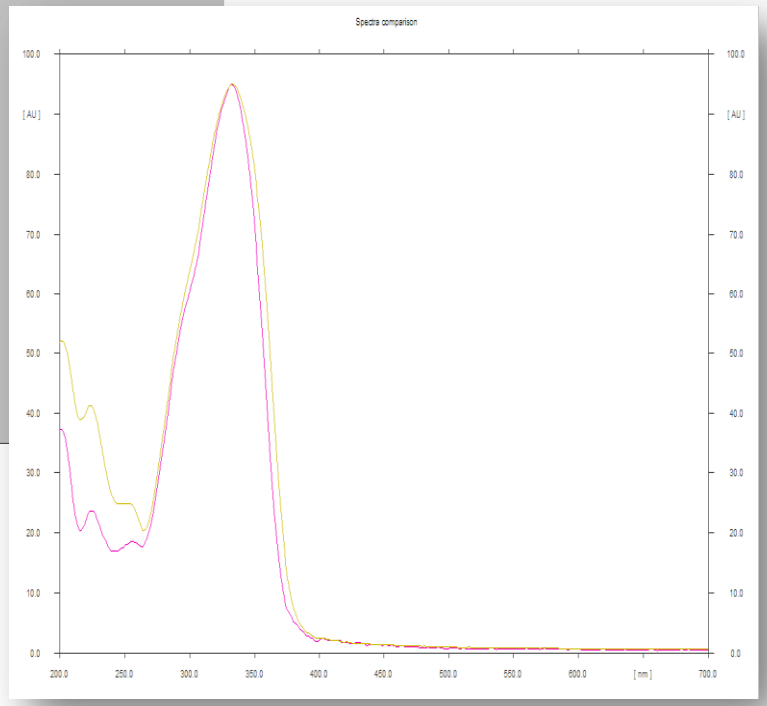


Track	Sample	Track	Sample	Track	Sample
1	Z- ligustilide (Rf ~ 0.58)	3	Decursinol (Rf ~ 0.04)	12	*Angelica decursiva*
1	Isoimperatorin (Rf ~ 0.44)	4	*Notopteryguii forbesii*	13	*Peucedanum praeruptorum*
1	Osthole (Rf ~ 0.38)	5	*Notopteryguii incisum*	14	*Angelica sinensis* (CAMAG)
1	Imperatorin (Rf ~ 0.33)	6	*Cnidium officinalis*	15	*Angelica pubescens* (CAMAG)
1	Decursin (Rf ~ 0.25)	7	*Ligusticum chuanxiong*	16	*Angelica dahurica* (CAMAG)
1	Ferulic acid (Rf ~ 0.11)	8	*Ligusticum sinensis*	17	*A. dahurica* var. *formosana*
2	Oxypeucedanin (Rf ~ 0.17)	9	*Ligusticum jeholense*	18	*Angelica dahurica*
2	Scopoletin (Rf ~ 0.07)	10	*Ligusticum tenuissimum*	19	*Ostericum koreanum*
3	Demethylsuberosine (Rf ~ 0.16)	11	*Angelica gigas*		

Quantitative investigation of *Angelica gigas* (1:100)

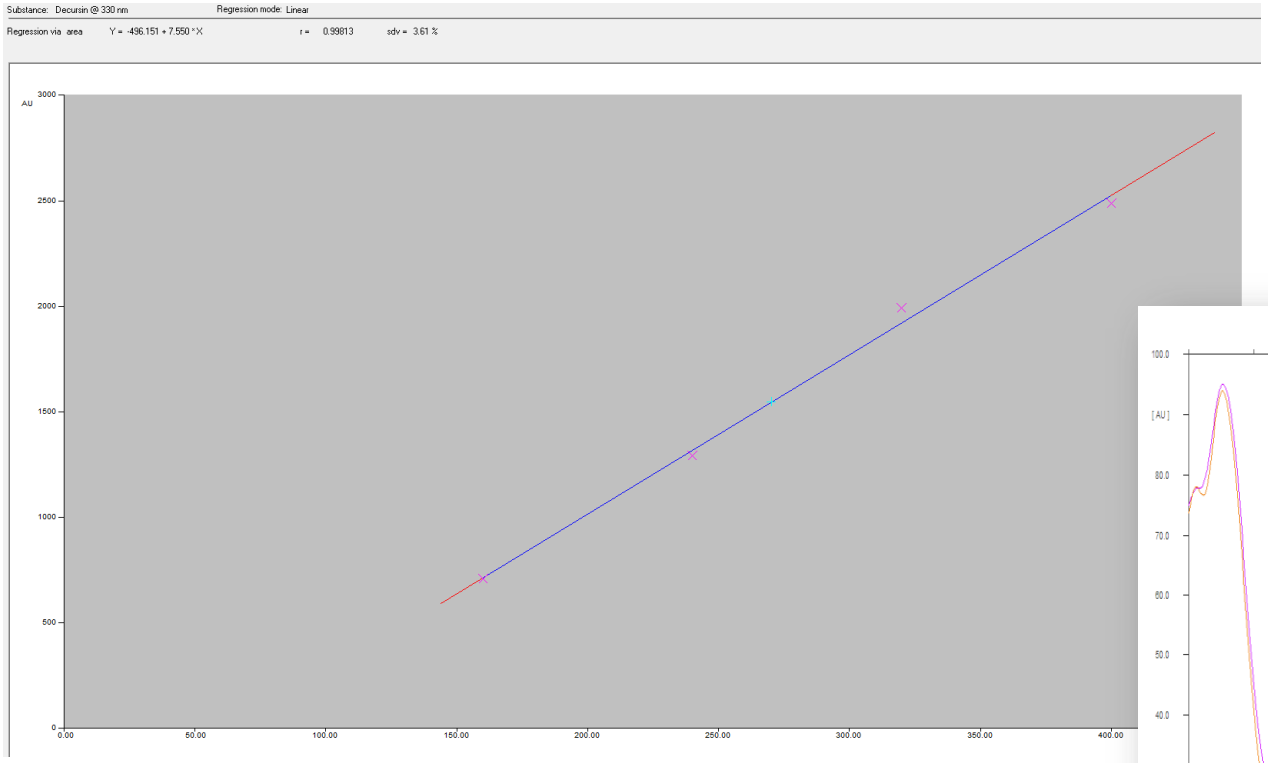
Demethylsuberosin (area, 330 nm)

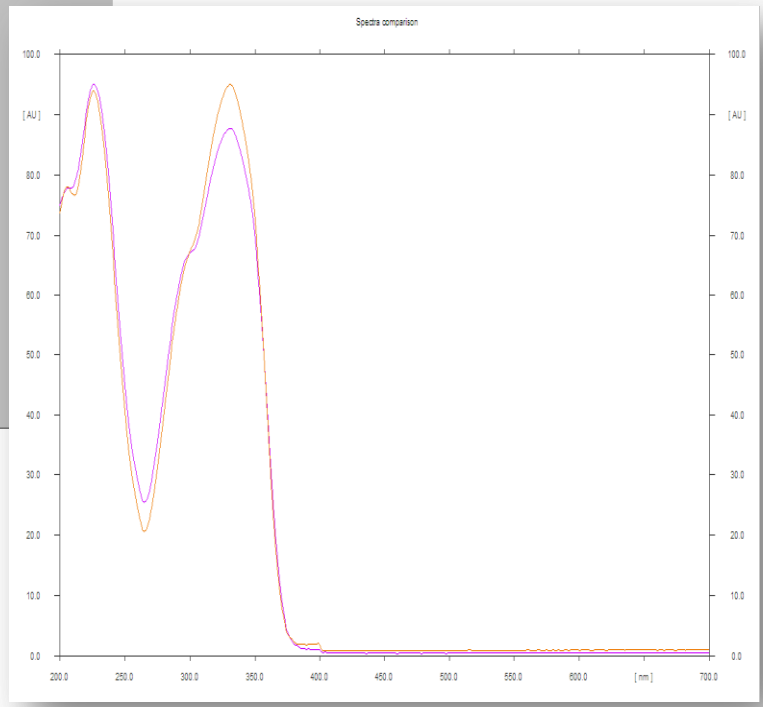




Quantitative investigation of *Angelica gigas* 1:500

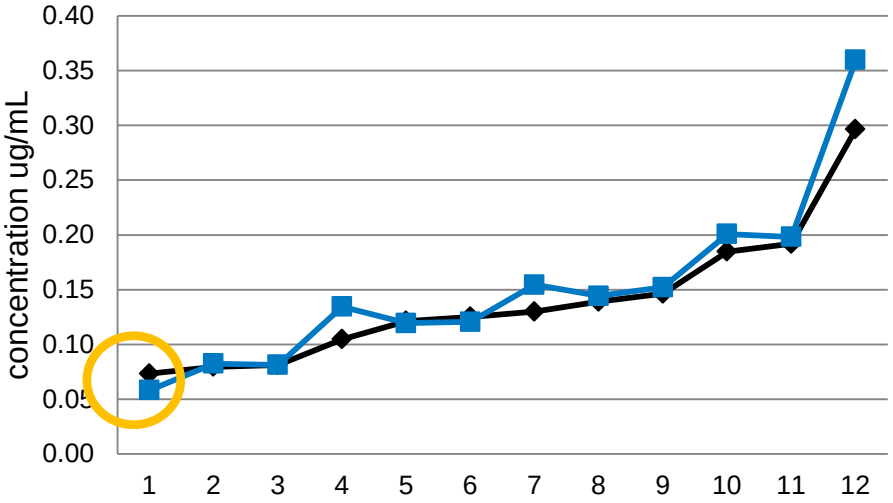
Decursin (area, 330 nm)



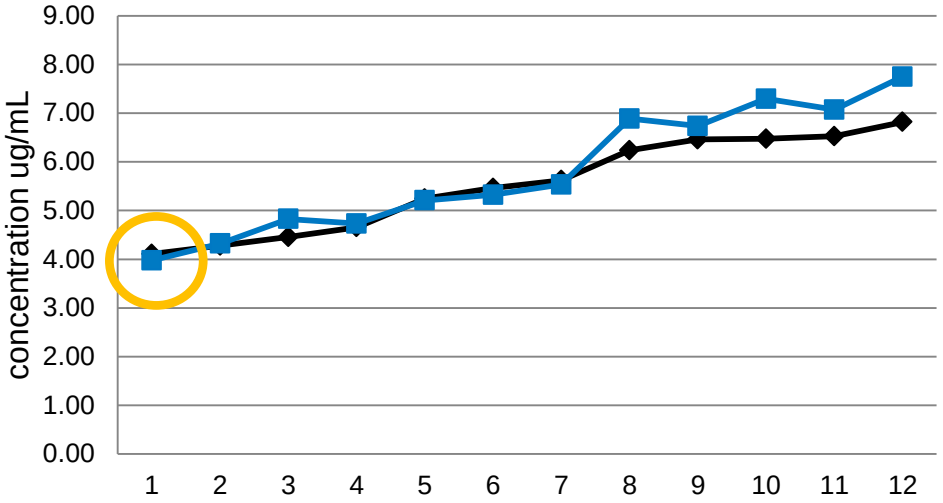


Marker content (12 samples) of *Angelica gigas*

Correlation Image (black) / Scann (blue) for DMS



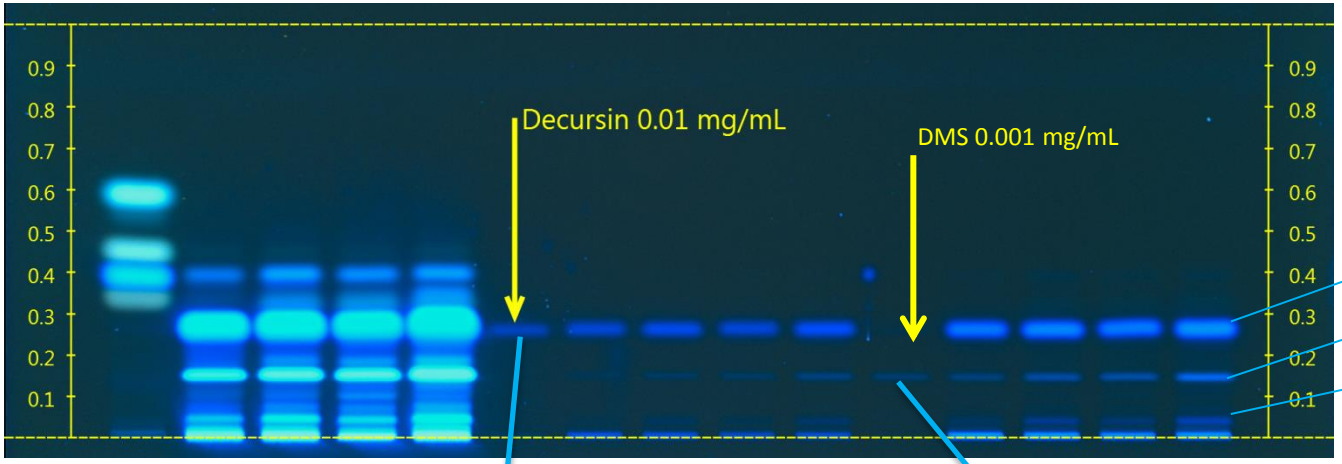
Correlation Image (black) / Scann (blue) for Decursin

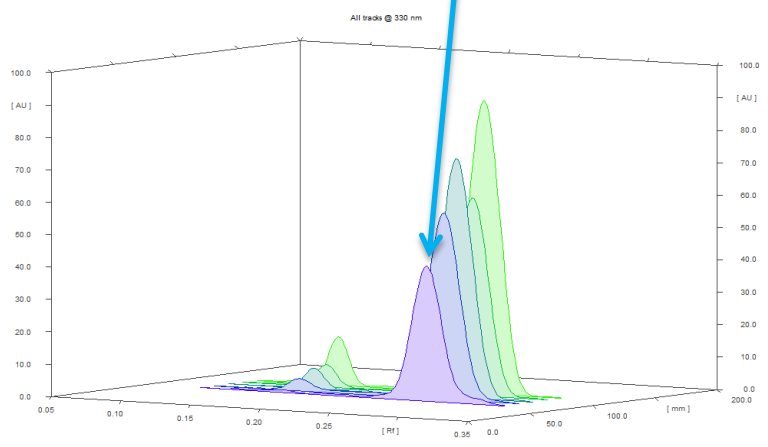


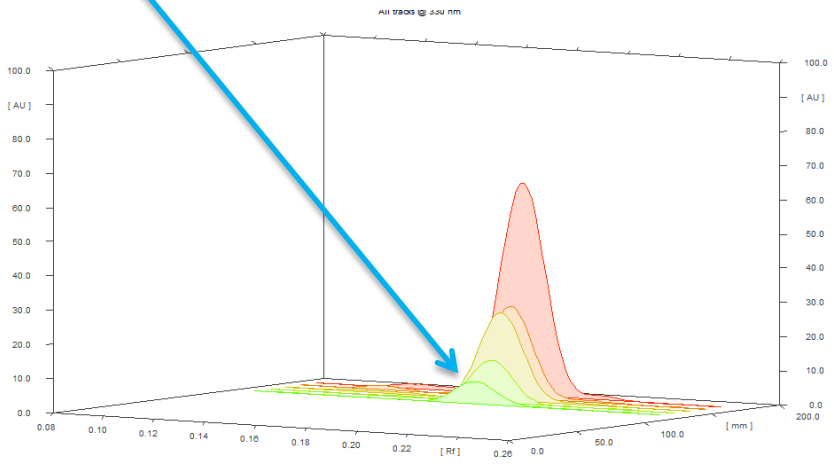
Minimum content of both standards is based on the lower concentration obtained of each standard in the sample.

5% below the lowest concentration of both RMPM

Limit test


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Decursin 0.01 mg/mL
DMS 0.001 mg/mL
Decursin
Demethyl suberosine
Decursinol





Conclusion

- Proof of concept for use of a single method for ID and assessment of content in a visual limit test
- Protocol for collaborative trial is under development
- Further validation of method characteristic are in progress

- In principal method can be used for true quantitation

→ Stability study for RMPM

Many thanks to my team!