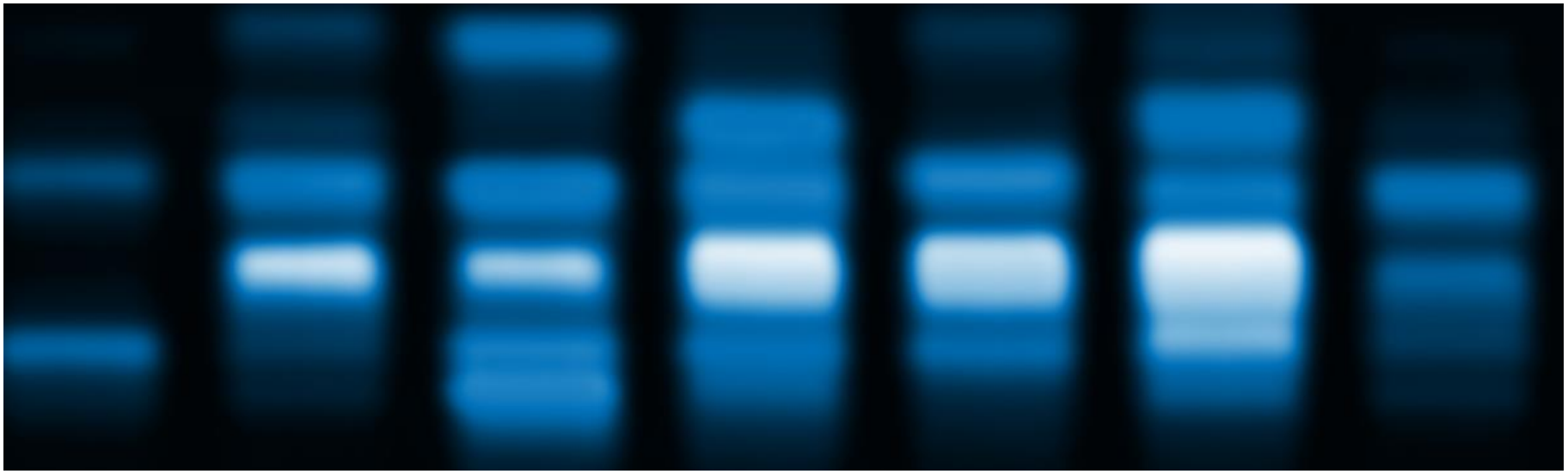

One HPTLC Method for Identification of Seven Different Resin Species

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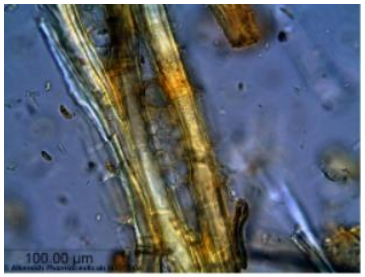
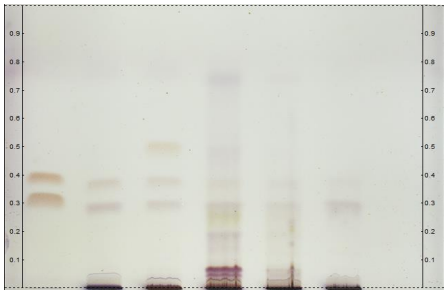
Introduction

- TLC was one of the first methods for identification of plants based on their chemical constituents.
- These methods are part of monographs in the Pharmacopoeias.
- In this context TLC methods are part of a suite of tests that describes the quality of plants used as medicines.

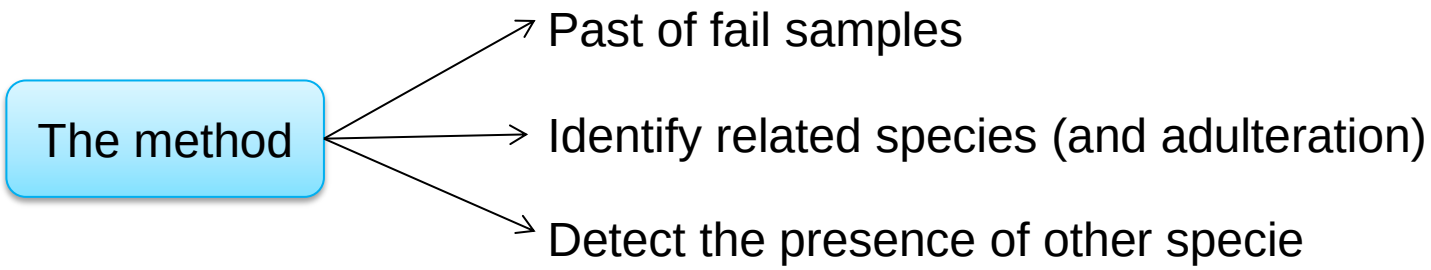





- Related plants often have different monographs include different TLC methods

Introduction

- Same plant can also be regulated as spice, food, ingredient for cosmetics, and dietary supplement, the pharmacopoeia monograph does not apply.
- In the absence of monographs, specificity of the ID method, as a stand-alone test must ensure quality and discriminate falsification.
- Related species should be analyzed using the same highly specific method.



Existent TLC monographs for the identification of Resins

Resin	Monograph published in
Benzoe sumatra (Styrax benzoin)	European Pharmacopoeia, Vol. 8, Nr. 1814
Benzoe sumatra (Styrax benzoin) tincture	European Pharmacopoeia, Vol. 8, Nr. 1813
Benzoe tonkinensis (Styrax tonkinensis)	European Pharmacopoeia, Nr. 2158
Benzoin tonkinensis (Styrax tonkinensis) tincture	European Pharmacopoeia, Nr. 2157
Benzoinum (Styrax tonkinensis)	Pharmacopoeia of the People's Republic of China, 2010, Vol. 1
Dammar gum	US Pharmacopoeia-DSC 2009-2010
Guggul (Commiphora wightii)	US Pharmacopoeia 34
Indian Frankincense (Boswellia serrata)	Quality Control of Indian Medicinal plants, volume 2
Indian Frankincense (Boswellia serrata)	European Pharmacopoeia, Vol. 8, Nr. 2310
Indian Frankincense (Boswellia serrata)	US Pharmacopoeia, DSC, 2011
Indian Frankincense (Boswellia serrata) extract	US Pharmacopoeia, DSC, 2011
Myrrh (Commiphora molmol)	European Pharmacopoeia, Nr. 1349
Myrrh (Commiphora molmol) tincture	European Pharmacopoeia, Nr. 1877
Myrrh (Commiphora molmol)	Pharmacopoeia of the People's Republic of China, 2010, Vol. 1
Myrrh (Commiphora molmol)	US Pharmacopoeia 34
Tolu Balsam	European Pharmacopoeia, Nr. 1596

Total = 16 ID Monographs for 7 resins, from 4 different sources

13 different methods to analyze 7 resins types

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Resin	Monograph	Sample preparation	Mobile phase
Benzoe sumatra; Benzoe tonkinensis	Ph.Eur., Nr. 1814 Ph.Eur., Nr. 2158	Sonicate 0.2 g of the powdered herbal drug in 5 mL of ethanol (96 per cent)	Acetic acid, di-isopropyl ether, hexane (10:40:60)
Benzoe sum. tinct; Benzoe tonk. tinct.	Ph.Eur., Nr.1813 Ph.Eur., Nr. 2157	1 part of the drug and 5 parts of ethanol (75-96 %) by a suitable procedure.	Acetic acid, di-isopropyl ether, hexane (10:40:60)
Benzoinum (Styrax tonkinensis)	PPRC 2010, Vol.1	1.0 g of the powdered sonicated with 2 mL of methanol for 5 min.	Pet.-ether 60-90°C, hexane, EtOAc, acetic acid (6:4:3:0.5)
Dammar gum	USP-DSC 2010	100 mg/mL of chloroform	Diethyl ether, heptane (30:25)
Guggul	US 34 DSC	0.5 g of the drug in 25 mL of CAN, heat in a water bath for 10 – 15 minutes while shaking.	Hexane, ethyl acetate (6:4)
Indian Frankincense (*Boswellia serrata*)	QCIMP, Volume 2	1.0 g of the powdered shaken with (3 x 25 mL) of Chloroform for 30 min. Evaporate the solution and dilute to 25 mL (chloroform)	Toluene, ethyl acetate, methanol (8:2:1)
Indian Frankincense	Ph.Eur., Nr. 2310	1.0 g of the powdered sonicated with 90 mL of methanol for 10 min. Dilute to 100 mL (MeOH)	Formic acid, heptane, ethyl acetate, toluene (3:10:20:80)
Indian Frankin. Indian Frank. ext.	USP, DSC, 2011 USP, DSC, 2011	30 mg/mL of methanol, dissolve the powder with gentle heating	Hexane, ethyl acetate (6:4)
Myrrh	Ph.Eur., Nr. 1349	To 0.5 g of the powder add 5.0 mL of ethanol (96 %) and warm the mixture for 2-3 min.	Ethyl acetate, toluene (2:98).
Myrrh tincture	Ph.Eur., Nr. 1877	1 part of the drug and 5 parts of ethanol (90%). Dilute 5 mL of the tincture to 10 mL with alcohol.	Ethyl acetate, toluene (2:98).
Myrrh	PPRC 2010, Vol.1	To 2 g of the drug add 2 mL and heat for 2.5 h.	Cyclohexane, ether (4:1)
Myrrh	USP 34	To 0.5 g of add 5 mL of alcohol, and warm the mixture in a water bath for 2 to 3 minutes	Toluene, ethyl acetate (98 : 2)
Tolu Balsam	Ph.Eur., Nr. 1596	Stir 0.40 g of the fragmented drug with 10 mL of methylene chloride R for 5 min and filter.	Light petroleum, toluene (5:95)

Our goal

- Create a single harmonized method capable of distinguishing all seven resins species

How to achieve the goal?

- Evaluate existent TLC method and transfer to HPTLC
- Evaluate the sample preparation/obtain a simple extraction method
- Compare different mobile phases (specificity of the method)
- Set parameters

Evaluate existent TLC method / transfer to HPTLC

- Among the 13 methods, 3 of them were previously tested:

Tolu Balsam	Ph.Eur., Nr. 1596	Stir 0.40 g of the fragmented drug with 10 mL of methylene chloride R for 5 min and filter.	Light petroleum, toluene (5:95)
Indian Frankincense	Ph.Eur., Nr. 2310	1.0 g of the powdered sonicated with 90 mL of methanol for 10 min. Dilute to 100 mL (MeOH)	Formic acid, heptane, ethyl acetate, toluene (3:10:20:80)
Benzoe sumatra; Benzoe tonkinensis	Ph.Eur., Nr. 1814 Ph.Eur., Nr. 2158	Sonicate 0.2 g of the powdered herbal drug in 5 mL of ethanol (96 per cent)	Acetic acid, di-isopropyl ether, hexane (10:40:60)

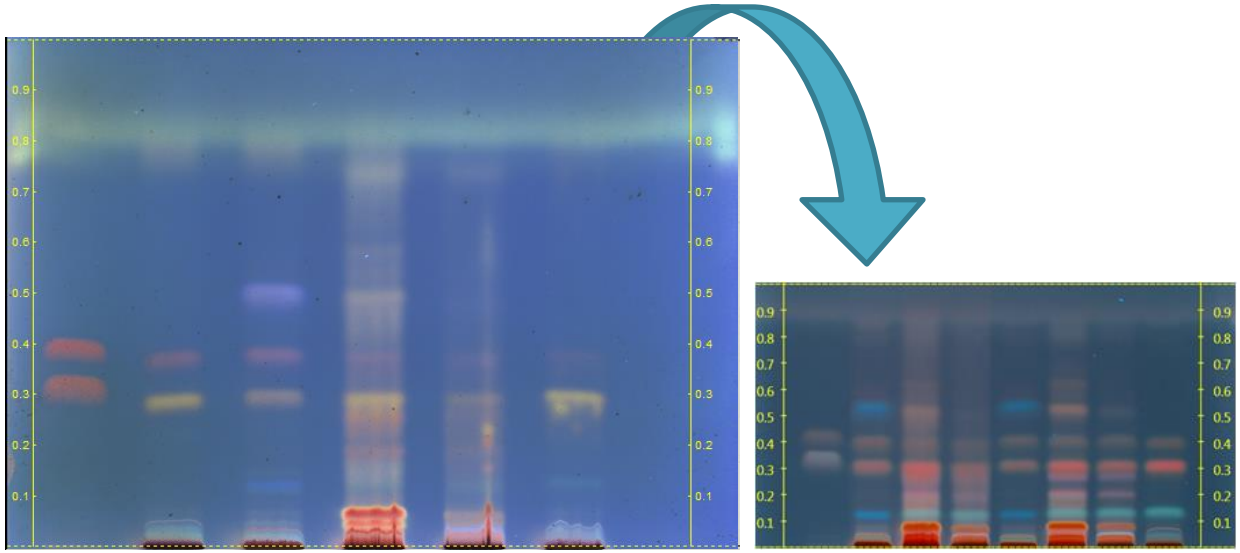
In general, the following standard HPTLC conditions* were adopted:

- The application volume was reduced 5 times
- The samples were applied as bands (8 mm width)
- The plate used was TLC *silica gel* F_{254} (particle size: 2-10 µm)
- The chamber configuration was saturated (20 minutes with filter paper)
- The plate was activated for 10 min at Rh 33% (with $MgCl_2$)
- The plate was developed over a path of 6 cm

* <203> PF 40(3)

M1: Identification of Tolu balsam

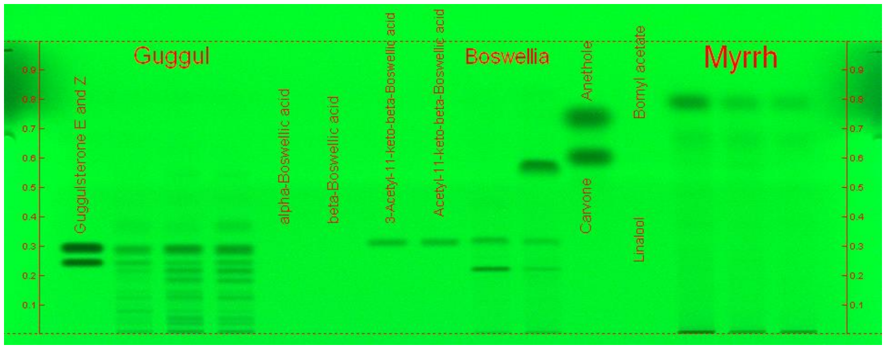
- Optimization of an existent TLC method: converting into HPTLC method

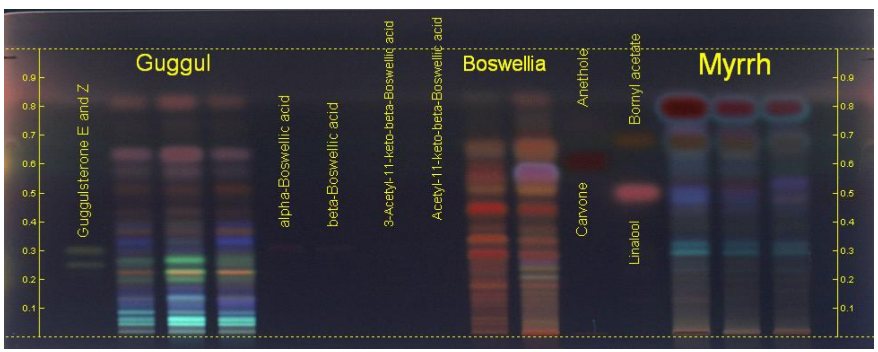


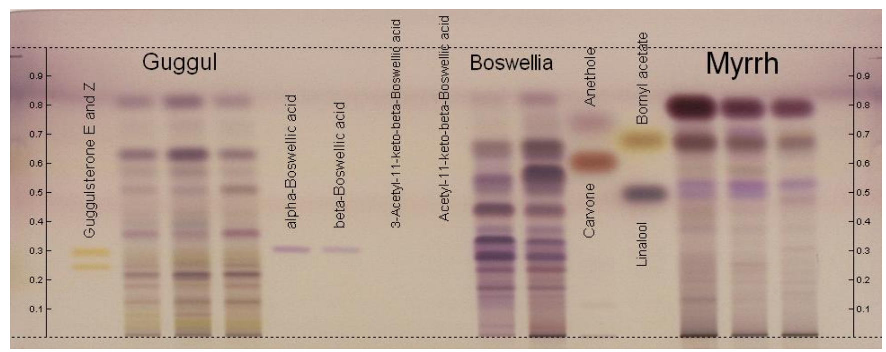
Sample preparation	Mobile phase	Derivatization reagent	App. vol.
Stir 0.40 g of the fragmented drug with 10 mL of methylene chloride R for 5 min and filter.	Light petroleum, toluene (5:95)	Anisaldehyde	20 µL

Sample preparation	Mobile phase	Derivatization reagent	App. vol.
To 0.2 g of the powdered drug add 4 mL of ethanol 96%, sonicate for 10 min, centrifuge, and use the supernatant	Cyclohexane, toluene (5:95)	Anisaldehyde	4 µL

M2: Identification of Indian Frankincense

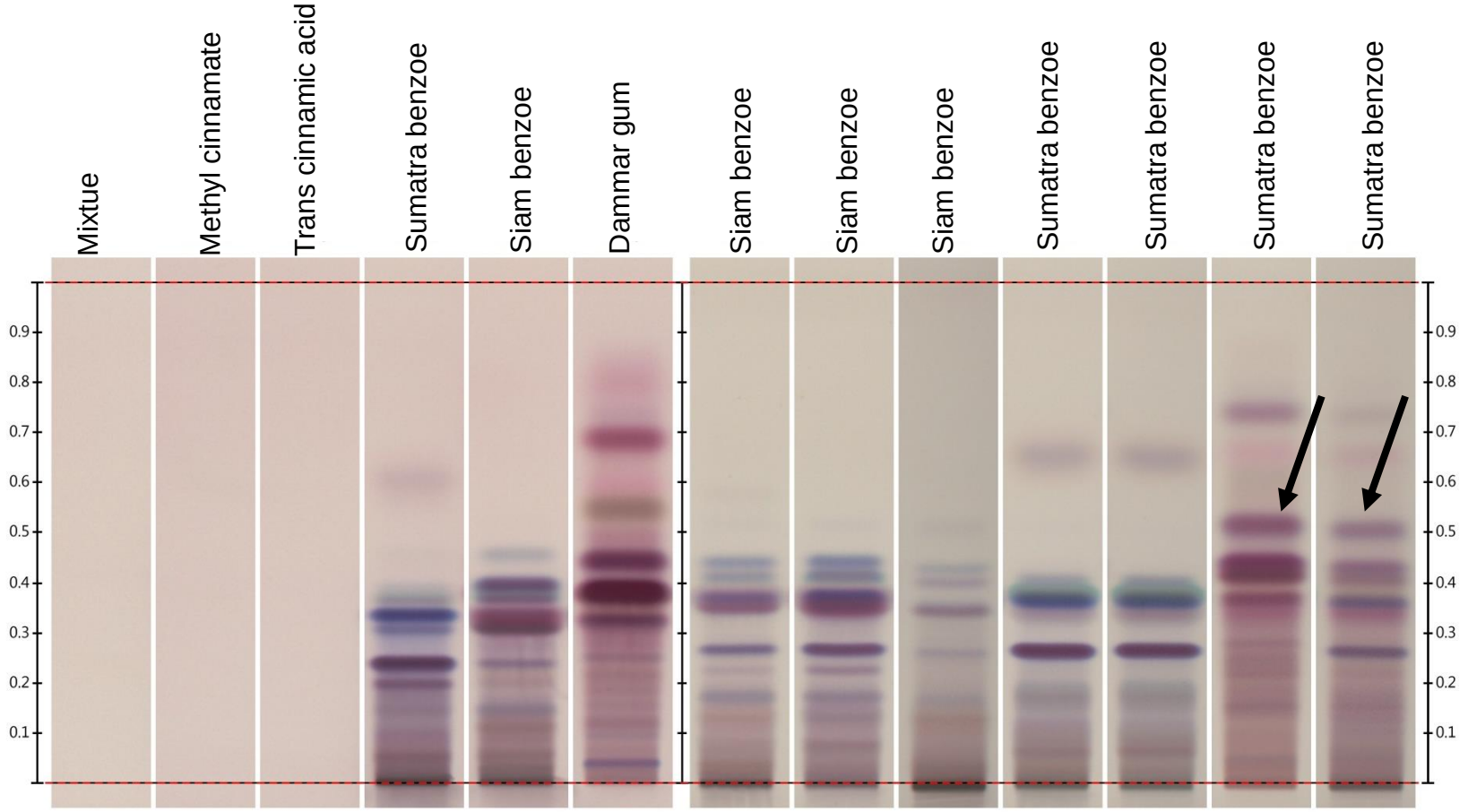






Sample preparation	Mobile phase	Derivatization reagent	App. vol.
0.5 g of the powdered sonicated with 10 mL of methanol for 10 min.	Formic acid, heptane, ethyl acetate, toluene (3:10:20:80)	Anisaldehyde	2 µL

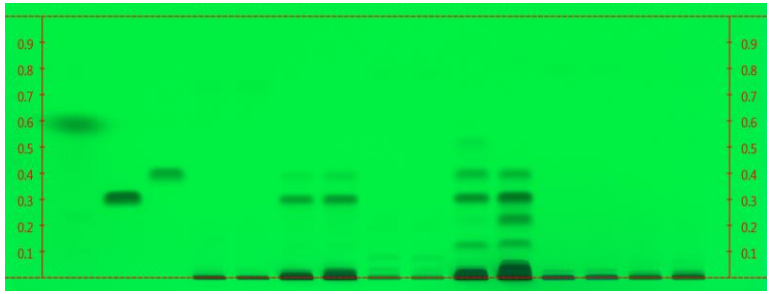
M3: Identification of Benzoe

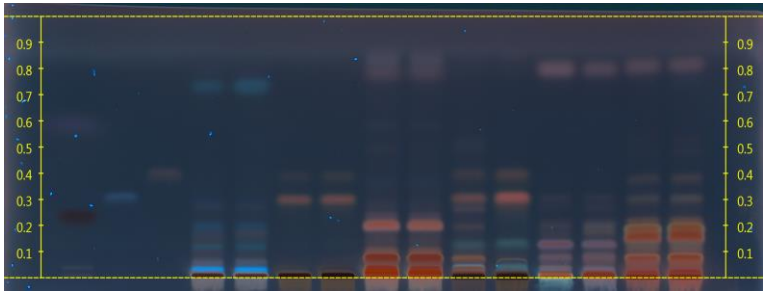


Sample preparation	Mobile phase	Derivatization reagent	App. vol.
Sonicate 0.2 g of the powdered herbal drug in 5 mL of ethanol (96 per cent)	Acetic acid, di-isopropyl ether, cyclohexane (10:40:60)	Anisaldehyde	2 µL

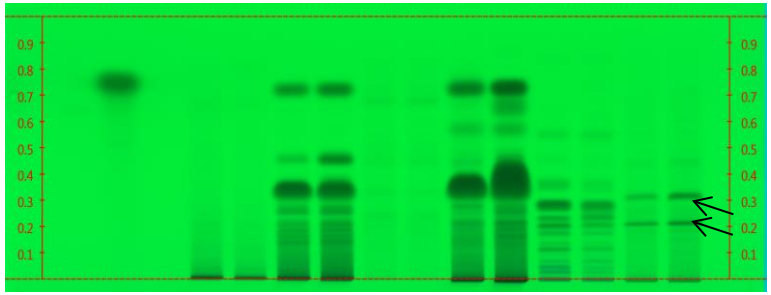
What about a single method?

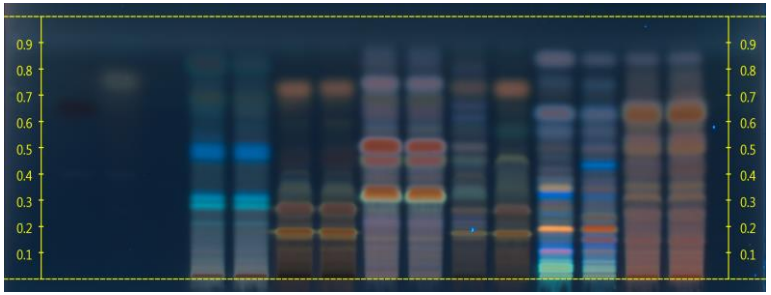
Evaluation of the mobile phase



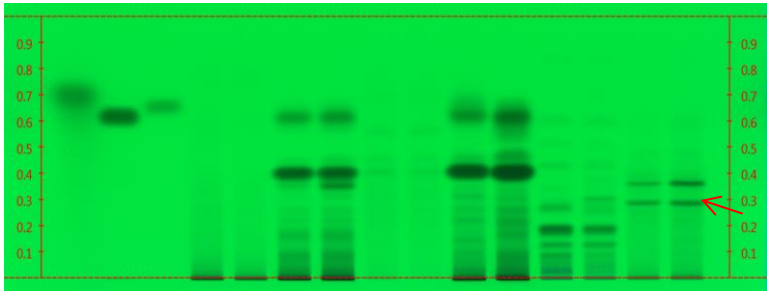


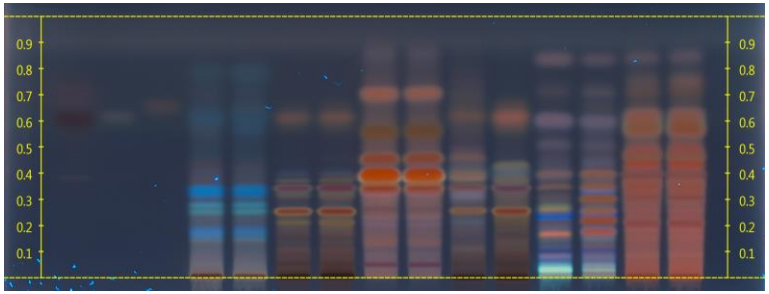
Cyclohexane, toluene (5:95)





Formic acid, heptane, ethyl acetate, toluene (3:10:20:80)





Acetic acid, di-isopropyl ether, cyclohexane (10:40:60)

Evaluation of the sample prep. / Set parameters

Sample preparation:

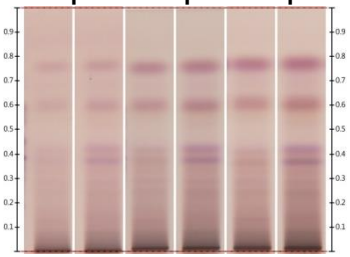
200 mg / 4 mL → in ethanol 96%

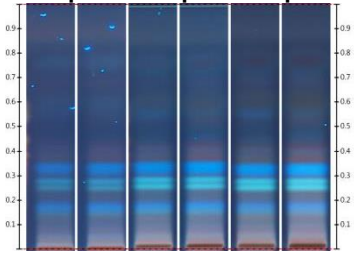
in methanol

brighter zones

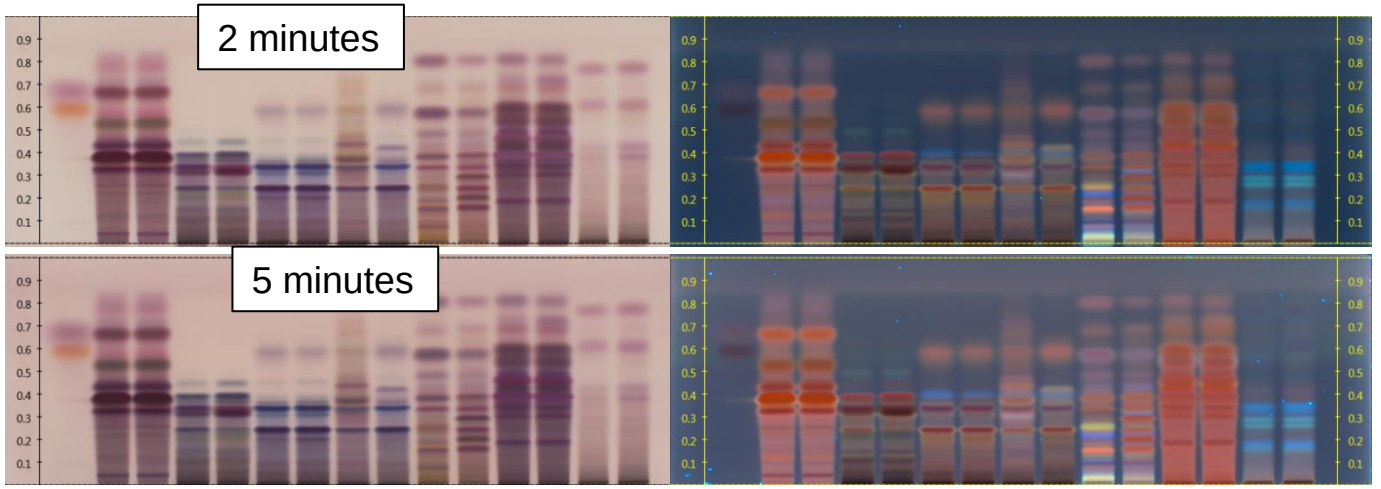
Similar results

Application volume:





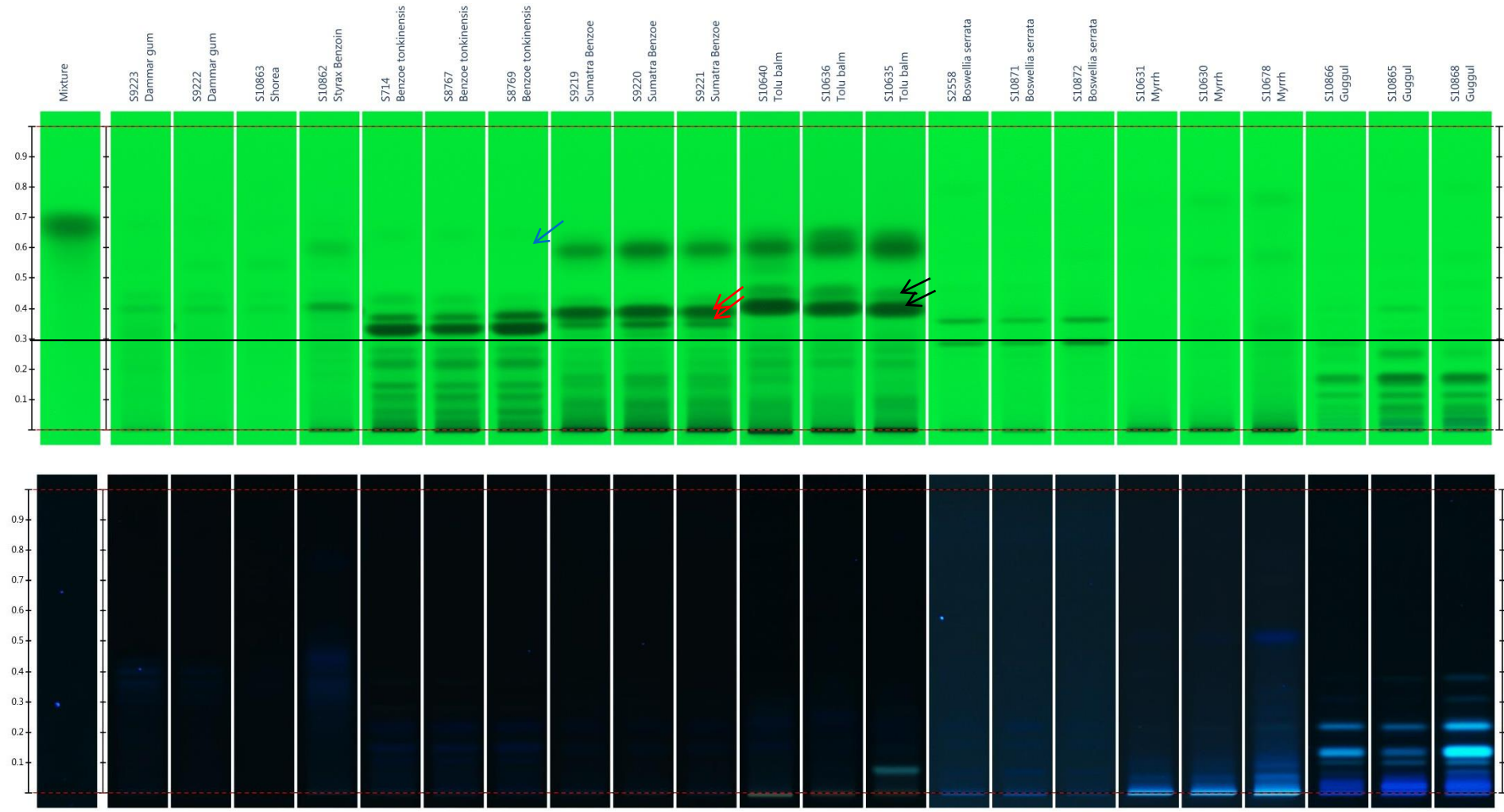
Heating time (deriv.):



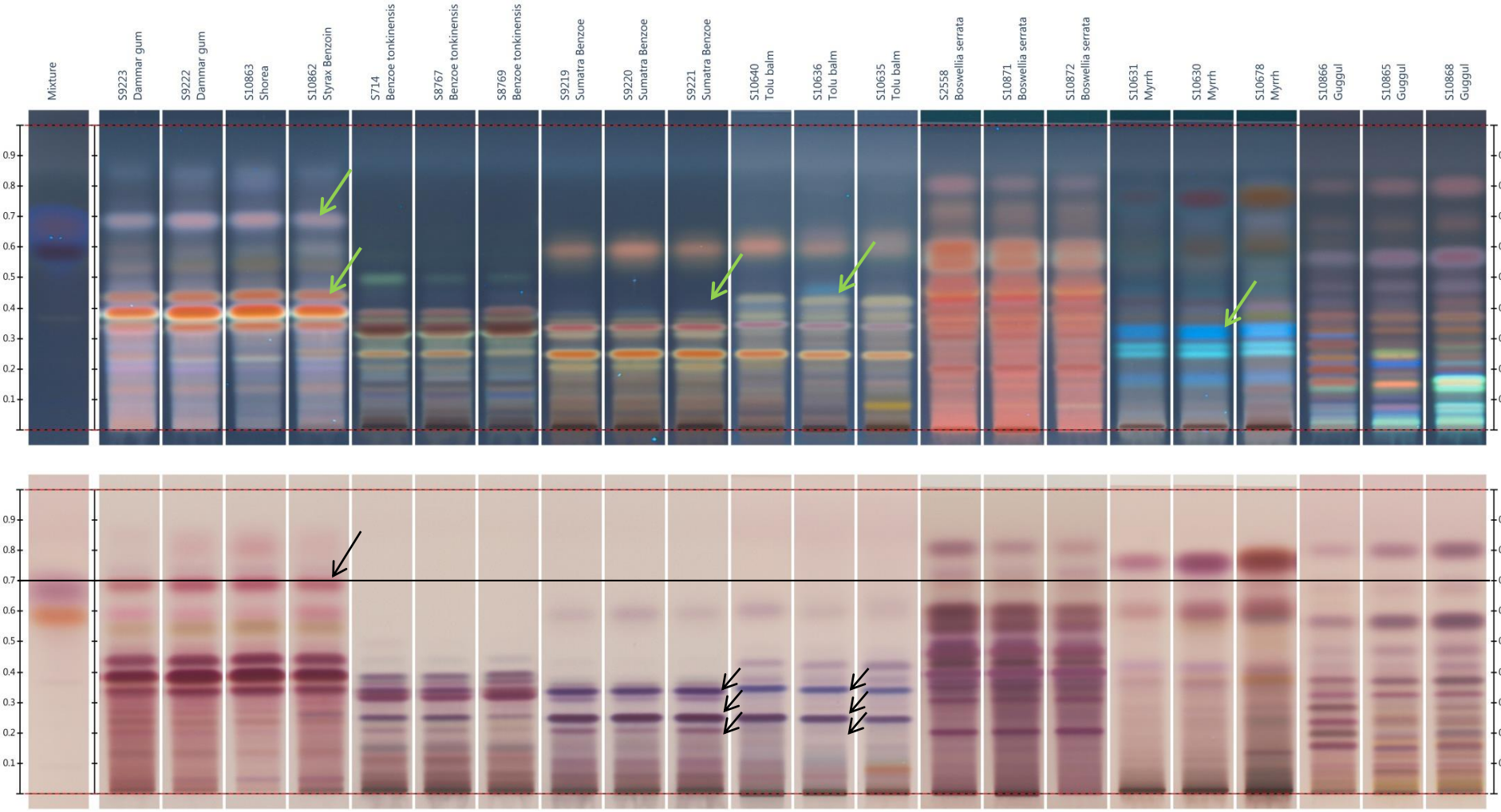
Harmonized method for identification of resins

Sample preparation	Mix 0.2 g of powdered sample with 4 mL of ethanol 96% and sonicate for 10 minutes, then centrifuge or filter the solution and use the supernatant / filtrate as test solution.
Reference substances	According to each sample
Stationary phase	HPTLC Si 60 F254
Application	2 µL of references and test solutions. For myrrh samples apply 5 µL
Mobile phase	Cyclohexane, diisopropyl ether, acetic acid 60:40:10 (v/v/v)
Development:	- Saturated chamber - Developing distance 70 mm from lower edge - Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent. Preparation: 10 mL of sulfuric acid are carefully added to an ice-cooled mixture of 170 mL of methanol and 20 mL of acetic acid. To this solution, 1 mL of p-anisaldehyde is added. Dip (speed: 5, time: 0), heat at 100°C for 2 min
Documentation	1) UV 254 nm 2) 2) UV 366 nm 3) Anisaldehyde reagent, white RT 4) Anisaldehyde reagent, UV 366 nm

Harmonized method for ID of resins



Harmonized method for ID of resins



Conclusion

- A single method to identify 7 different types of resins was developed
- Presence and identity of other species in a sample can be detected
- A simple and harmonized sample preparation method was established
- The analysis time was reduced from 4,5 h (3 HPTLC methods) to 1,5 h, including sample preparation.
- The amount of solvent was reduced from 150 mL (3 HPTLC methods) to 50 mL

Thanks for your attention

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