

# Retour sur le Symposium de LJUBJANA



HPTLC'20-22

# OUR MEETINGS

(Interlaken 1997 \_ 70)

Lyon 2003 \_ 120

Berlin 2006 \_ 140

Helsinki 2008 \_ 130

Basel 2011 \_ 300 (incl Camag invited distributors)

Lyon 2014 \_ 210

Berlin 2017 \_ 200

Bangkok 2018 \_ 160

Boulder 2019 (> Princeton \_ 20 ? + course)

Ljubjana 2022 \_ 80

en même temps que l'ISSS (< HPLC & ISC)  
80 participants (SLO 18, D 13, Thai 6, F5 H5)  
17KL(PBS) 4OP (JMR) 6YS 37Posters





HPTLC & what else?

# SCIENCE & FRIENDSHIP

For the future of a wonderful  
chromatographic technique.

WITH

US

ALL





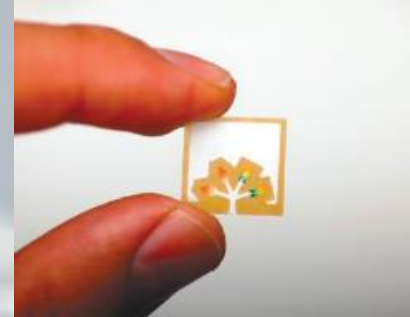
trends:

US

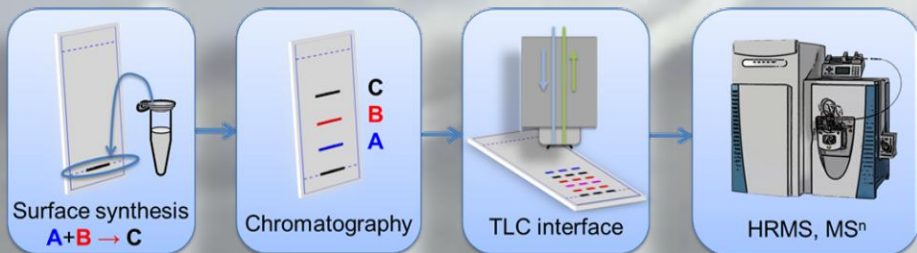
&

THEM

2D-LC  
techno MS  
NEW apps  
smartphones

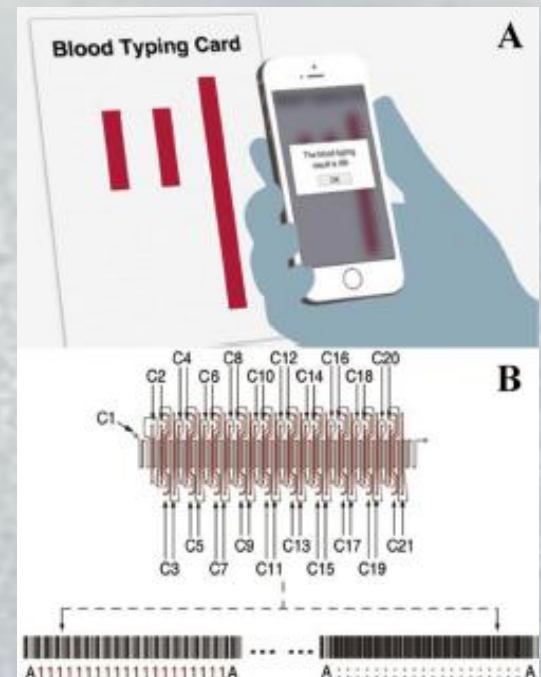
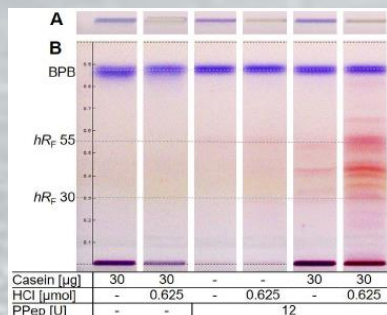


*Anal. Chem.* 2010, 82, 3–10



*Symposium HPTLC'17 O7-Yüce, Morlock : Targeted combinatorial on-plate synthesis as new tool for structure elucidation*

more green  
more fun



M. Rezazadeh et al. / Trends in Analytical Chemistry 118 (2019) 548e555

Analytical chemistry goes closer to the final users  
Manufacturers investments is a real issue/ there is a gap

# Some unforgettable memories

BAYER Klaus BURGER

(PBS invitations to our French HPTLC CLUB and HPTLC until 2003)



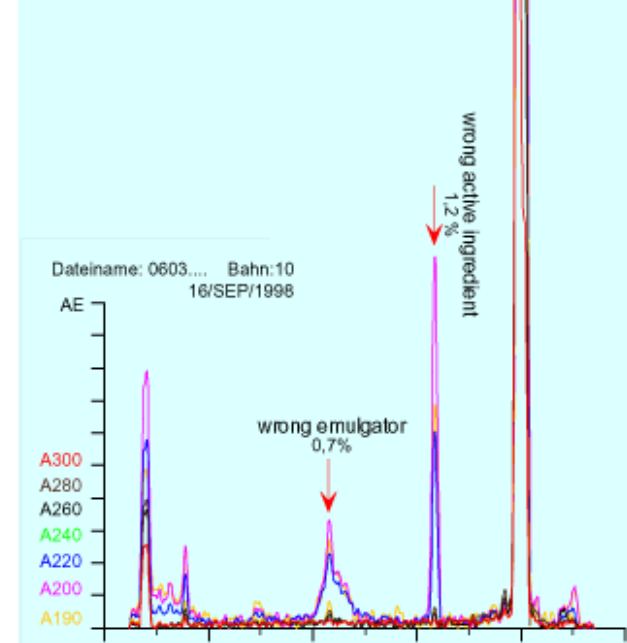
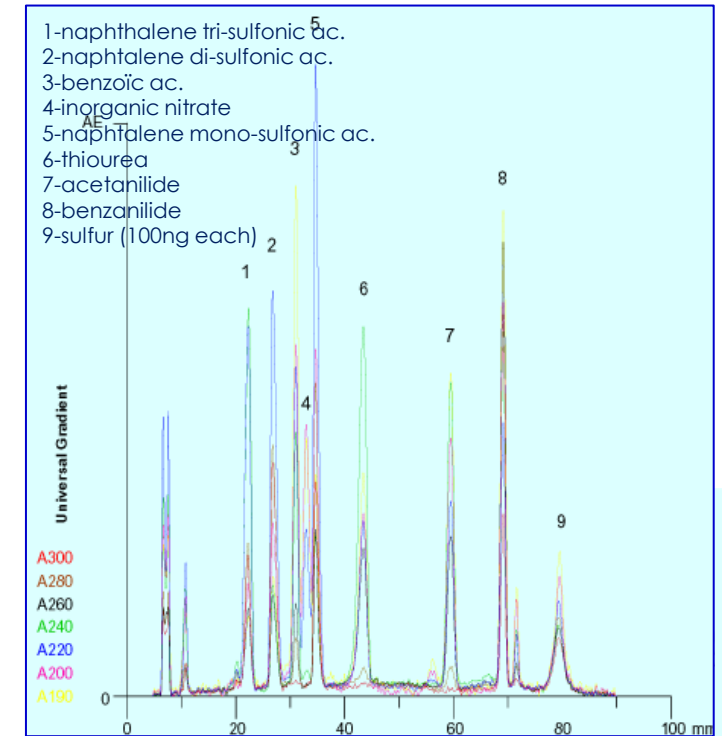
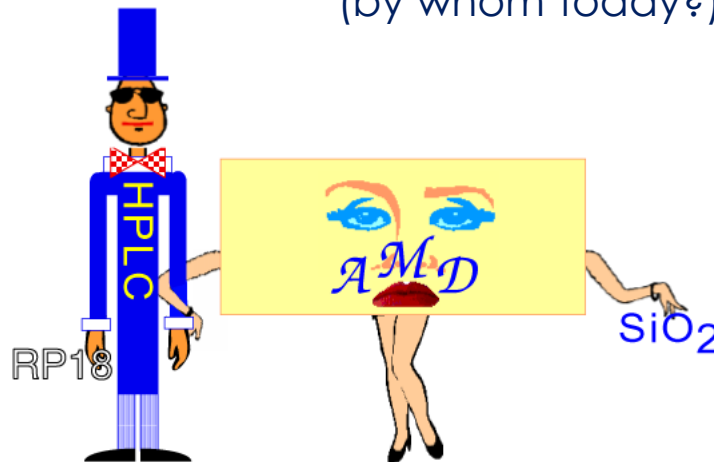
A lot of humor

Working clean is a must

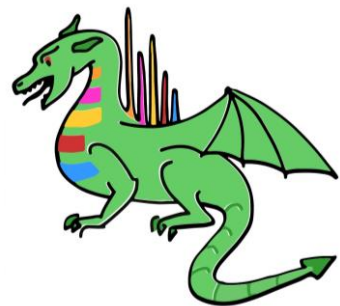
Column > Plate

High class baseline separations

(by whom today?)



25th HPTLC 2022



LJUBLJANA

25<sup>th</sup> International Symposium for High-Performance Thin-Layer Chromatography

June 28 - July 1, 2022, Ljubljana, Slovenia



# HPTLC and DNA Profiling of Thai *Cannabis* Strains and Hybrids

Wanchai De-Eknamkul, Ph.D.

Department of Pharmacognosy and Pharmaceutical Botany  
Faculty of Pharmaceutical Sciences, Chulalongkorn University  
Bangkok, Thailand

Ljubljana, Slovenia

June 29, 2022



## This study

### Sample preparation:

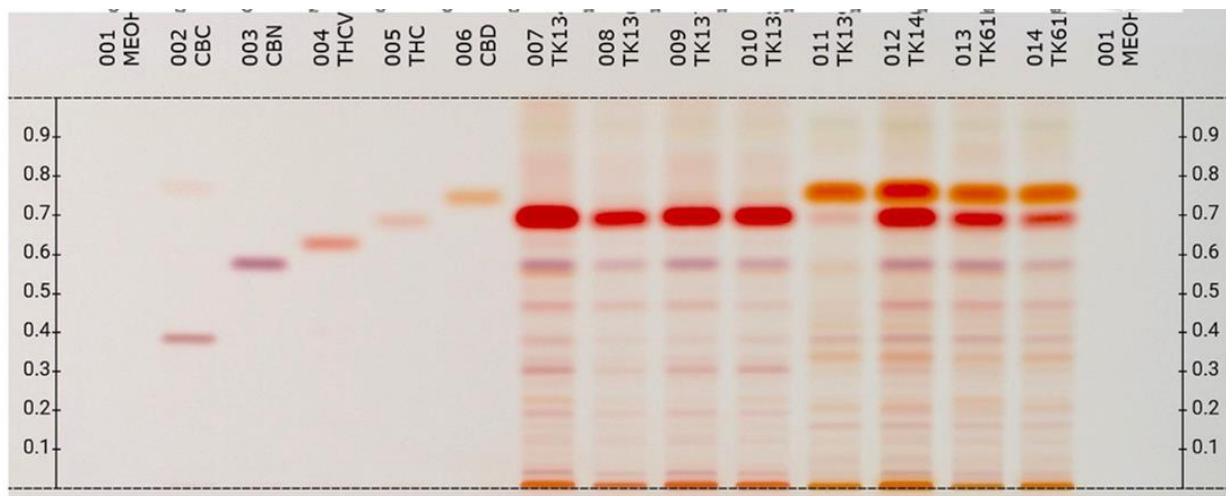
- 20 mg of powered sample extracted in 1 ml dichloromethane with 30-min sonication

### HPTLC system:

- 5 µl application/sample
- Silica gel 60 F<sub>254</sub> 20 × 10 cm plate
- Mobile phase : diethylamine-toluene

### Derivatization:

- Spraying with 0.2 % Fast Blue Salt B



## CAMAG Application Note A-108.1 (2017)

### Sample preparation:

- 500 mg of powered sample extracted with 5 ml methanol-hexane 9:1 (v/v) with 15-min sonication

### HPTLC system:

- 2 µl application/sample
- Silica gel 60 F<sub>254</sub> 20 × 10 cm plate
- Mobile phase : *n*-heptane-diethyl ether-formic acid 75:25:0.3

### Derivatization:

- Spraying with 0.5 % Fast Blue Salt B solution

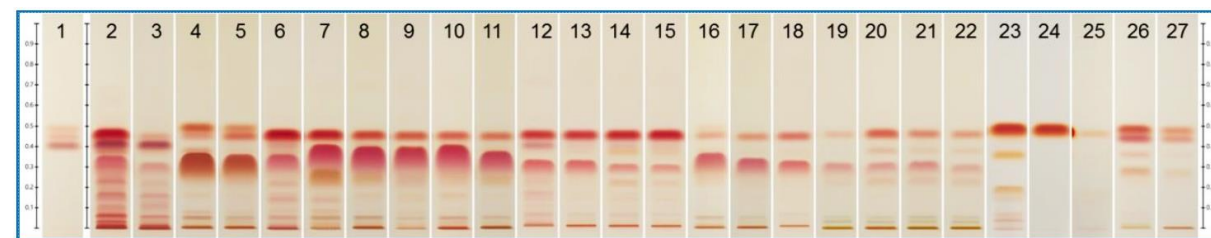
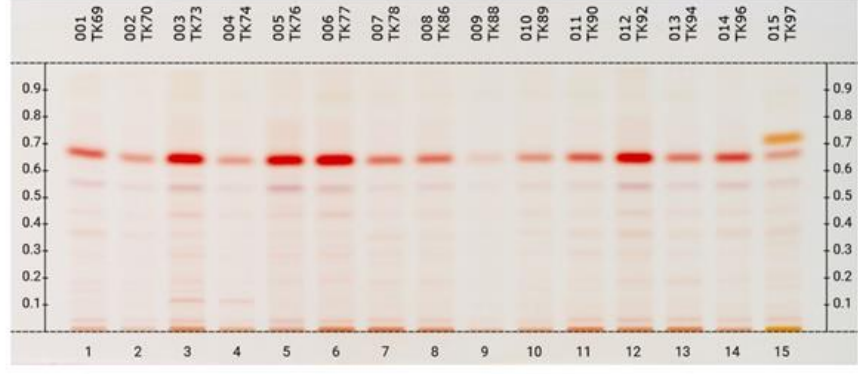
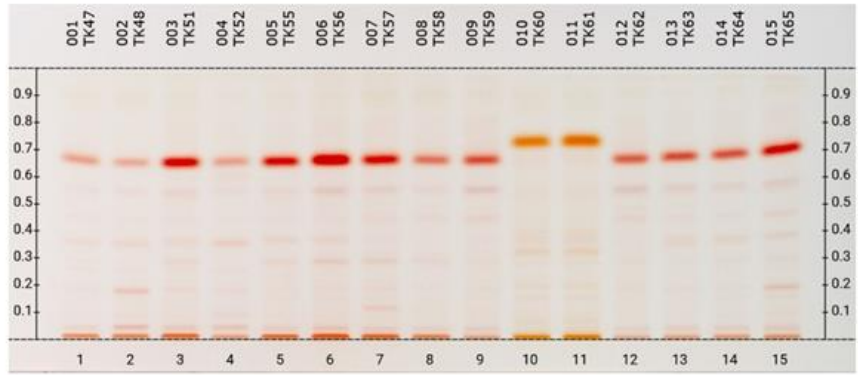
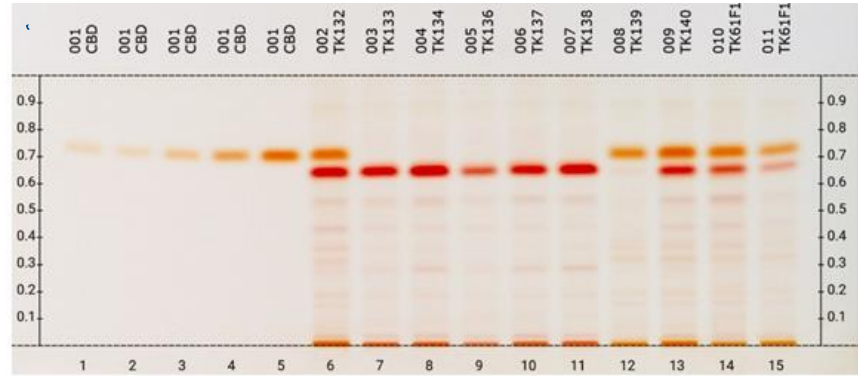
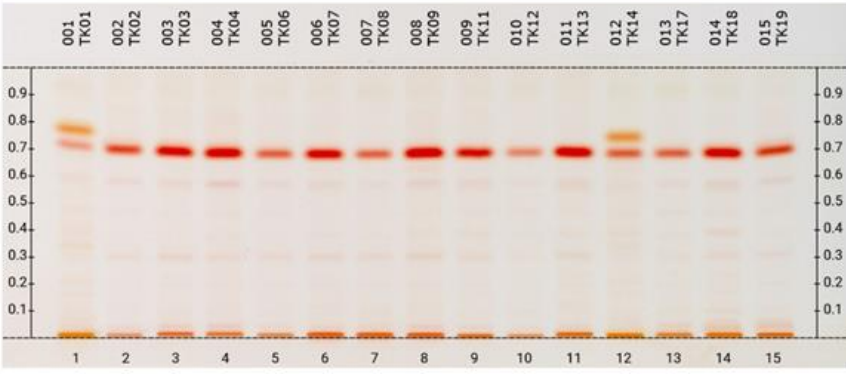
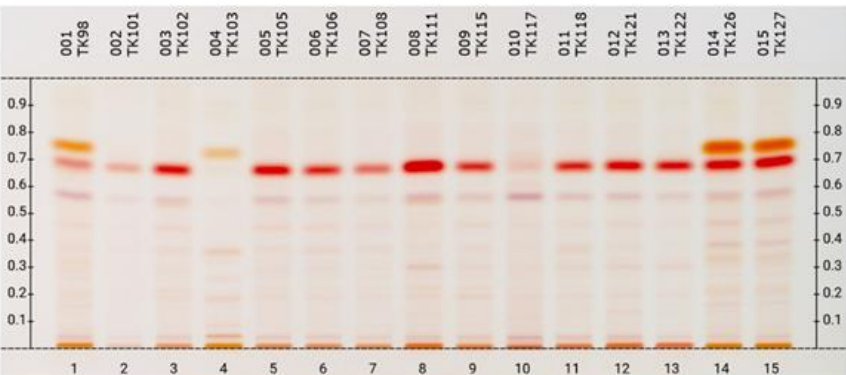
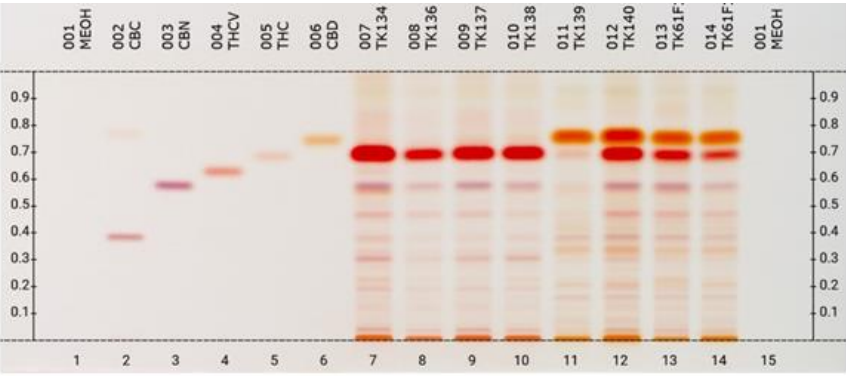


Fig. 1 Comparison of HPTLC fingerprints (chromatograms) under white light after derivatization with FBS reagent (method 1); track 1: SST (CBN, THC, and CBD with increasing RF), track 2: hashish sample, tracks 3-18: *C. sativa* samples, tracks 19-22: *C. sativa* samples (young plants), track 23: intermediate from CBD extraction (mother liquor, 1:10 diluted), track 24: CO<sub>2</sub>-extracted CBD (1:10 diluted), track 25: CBD cream, track 26: Cannabis oil (1:20 diluted), track 27: Cannabis tincture (1:10 diluted);



# Results: HPTLC of 80 Thai Cannabis strains and hybrids



| No. | Code         | % CBD (Avg) | % THC (Avg) | Log (%THC/%CBD) |
|-----|--------------|-------------|-------------|-----------------|
| 41  | TK61         | 2.960       | 0.07        | -1.61           |
| 40  | TK60         | 2.382       | 0.07        | -1.56           |
| 83  | TK139        | 2.776       | 0.14        | -1.29           |
| 65  | TK103        | 0.861       | 0.07        | -1.09           |
| 1   | TK01         | 2.823       | 0.74        | -0.58           |
| 85  | TK61F1-CO-SP | 1.658       | 0.51        | -0.51           |
| 29  | TK45         | 1.341       | 0.54        | -0.40           |
| 62  | TK98         | 1.332       | 0.54        | -0.40           |
| 42  | TK61F1-CO-2G | 3.388       | 1.38        | -0.39           |
| 84  | TK140        | 6.495       | 2.68        | -0.39           |
| 61  | TK97         | 1.309       | 0.54        | -0.38           |
| 12  | TK14         | 3.053       | 1.34        | -0.36           |
| 75  | TK126        | 3.371       | 1.52        | -0.35           |
| 76  | TK127        | 3.039       | 1.69        | -0.25           |
| 77  | TK132        | 4.598       | 3.68        | -0.10           |
| 71  | TK117        | 0.018       | 0.11        | 0.76            |
| 9   | TK11         | 0.141       | 1.05        | 0.87            |
| 14  | TK18         | 0.140       | 1.47        | 1.02            |
| 3   | TK03         | 0.155       | 1.66        | 1.03            |
| 7   | TK08         | 0.077       | 0.95        | 1.09            |
| 18  | TK28         | 0.097       | 1.21        | 1.10            |
| 11  | TK13         | 0.120       | 1.55        | 1.11            |
| 6   | TK07         | 0.138       | 1.80        | 1.11            |
| 4   | TK04         | 0.144       | 2.08        | 1.16            |
| 10  | TK12         | 0.034       | 0.50        | 1.17            |
| 2   | TK02         | 0.062       | 0.93        | 1.17            |
| 23  | TK37         | 0.106       | 1.69        | 1.20            |
| 69  | TK111        | 0.132       | 2.14        | 1.21            |
| 64  | TK102        | 0.056       | 0.91        | 1.21            |
| 24  | TK40         | 0.048       | 0.85        | 1.25            |
| 13  | TK17         | 0.035       | 0.61        | 1.25            |
| 17  | TK25         | 0.116       | 2.05        | 1.25            |
| 16  | TK20         | 0.120       | 2.17        | 1.26            |
| 54  | TK86         | 0.042       | 0.82        | 1.29            |
| 82  | TK138        | 0.143       | 2.80        | 1.29            |
| 43  | TK62         | 0.039       | 0.78        | 1.31            |
| 45  | TK64         | 0.041       | 0.84        | 1.31            |
| 19  | TK29         | 0.080       | 1.66        | 1.31            |
| 63  | TK101        | 0.015       | 0.31        | 1.32            |
| 74  | TK122        | 0.055       | 1.18        | 1.33            |
| 8   | TK09         | 0.077       | 1.97        | 1.41            |
| 81  | TK137        | 0.078       | 2.03        | 1.41            |
| 39  | TK59         | 0.035       | 0.91        | 1.42            |
| 80  | TK136        | 0.043       | 1.18        | 1.44            |
| 5   | TK06         | 0.030       | 0.83        | 1.44            |
| 60  | TK96         | 0.032       | 0.87        | 1.44            |
| 57  | TK90         | 0.033       | 0.98        | 1.47            |
| 30  | TK46         | 0.062       | 1.88        | 1.48            |
| 21  | TK34         | 0.043       | 1.32        | 1.49            |
| 79  | TK134        | 0.100       | 3.12        | 1.49            |
| 78  | TK133        | 0.068       | 2.24        | 1.52            |
| 32  | TK48         | 0.011       | 0.37        | 1.55            |
| 27  | TK43         | 0.048       | 1.72        | 1.55            |
| 70  | TK115        | 0.025       | 0.88        | 1.55            |
| 66  | TK105        | 0.023       | 0.85        | 1.56            |
| 33  | TK51         | 0.051       | 1.91        | 1.57            |
| 20  | TK33         | 0.030       | 1.14        | 1.57            |
| 52  | TK77         | 0.052       | 2.04        | 1.59            |
| 22  | TK36         | 0.053       | 2.18        | 1.62            |
| 58  | TK92         | 0.069       | 2.86        | 1.62            |
| 38  | TK58         | 0.018       | 0.78        | 1.63            |
| 28  | TK44         | 0.044       | 1.88        | 1.63            |
| 51  | TK76         | 0.038       | 1.66        | 1.64            |
| 49  | TK73         | 0.029       | 1.25        | 1.64            |
| 37  | TK57         | 0.034       | 1.52        | 1.65            |
| 36  | TK56         | 0.056       | 2.48        | 1.65            |
| 35  | TK55         | 0.037       | 1.64        | 1.65            |
| 15  | TK19         | 0.024       | 1.21        | 1.71            |
| 67  | TK106        | 0.017       | 0.88        | 1.72            |
| 25  | TK41         | 0.023       | 1.19        | 1.72            |
| 31  | TK47         | 0.008       | 0.45        | 1.72            |
| 72  | TK118        | 0.015       | 0.92        | 1.79            |
| 73  | TK121        | 0.016       | 1.11        | 1.83            |
| 26  | TK42         | 0.014       | 1.02        | 1.85            |
| 44  | TK63         | 0.010       | 0.85        | 1.92            |
| 34  | TK52         | 0.002       | 0.49        | 2.30            |
| 68  | TK108        | 0.002       | 0.50        | 2.31            |

Log (%THC/%CBD)

< -1.0  
Hemp-type

> -1.0 - < 1.0  
Intermediate

> 1.0 - < 1.5  
Drug-type

> 1.5  
Drug-type

# À côté Quantification of the Non-Eluting Biopolymer Lignin by Densitometry and Multivariate Calibration

Christian Schuster, Hajar Khalilayan, Ivan Sumerskii, Matthias Guggenberger, Josua T. Oberlechner, Thomas Rosenau, Antje Potthast, Stefan Böhmendorfer

University of Natural Resources and Life Sciences (BOKU), Vienna, Austria  
Institute of Chemistry of Renewable Resources



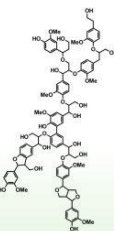
DCh  
Department of  
Chemistry



**Biorefineries** produce chemical feedstocks from plant biomass.

**Liquors**, the intermediary products, are demanding mixtures of carbohydrates, oils, extractives, salts and lignin.

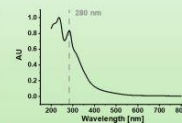
A biobased economy must make use of all **biomass components**.



**Lignin** is

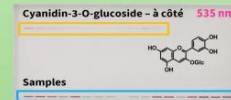
- a substantial plant component,
- a polymer
- from several phenolic monomers
- with several possible linkages and thus
- with an indeterminate structure.

Traditionally, lignin is **quantified** by precipitation or photometry at the local maximum of **280 nm**.

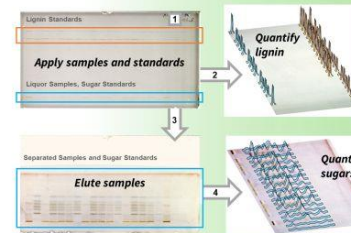
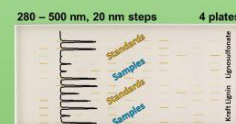


Extractives **interfere** at this wavelength.

**À côté calibration** – densitometry before development – allows quantitation of non-eluting compounds, as demonstrated with anthocyanins. [1]



A **multivariate (Partial Least Squares) calibration** was necessary to quantify lignin in the presence of interfering liquor components.



**Non-eluting components are quantified in the presence of interferences by a multivariate calibration.**

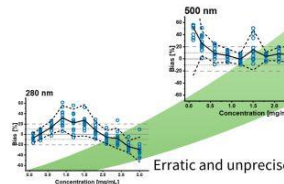
**With one analysis, both fermentable sugars and non-eluting lignin are determined with minimal sample pretreatment.**

Take a look at the full paper!

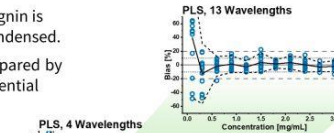


During kraft pulping, lignin is depolymerized and condensed.

Pure standards are prepared by precipitation and sequential extraction.



**Perfect accuracy and good precision!**



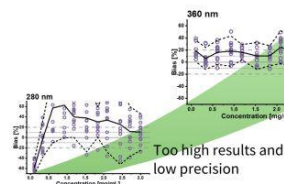
**Good accuracy and good precision!**

Validation performed according to Société Française des Sciences et Techniques Pharmaceutiques.

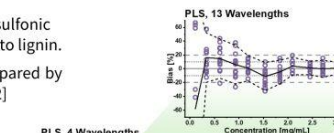


During sulfite pulping, permanently charged sulfonic acid groups are added to lignin.

Pure standards are prepared by adsorption to a resin.[2]



**Acceptable accuracy and precision**



**Good accuracy and serviceable precision**

## References

- [1] Oberlechner, J. T.; Fuchs, C.; Guggenberger, M.; Böhmendorfer, S. A Côté Calibration – Making Optimal Use of Time and Space in Quantitative High Performance Thin Layer Chromatography. *Journal of Chromatography A* **2018**, 1531, 193–198.
- [2] Sumerskii, I.; Kottner, P.; Zimovny, G.; Rosenau, T.; Potthast, A. Fast Track for Quantitative Isolation of Lignosulfonates from Spent Sulfite Liquors. *RSC Advances* **2018**, 8 (312), 92732–92742.
- [3] Oberlechner, J. T.; Böhmendorfer, S.; Rosenau, T.; Potthast, A. A Matrix-Resistant HPTLC Method to Quantify Monosaccharides in Wood-Based Lignocellulose Biorefinery Streams. *Holzforschung* **2018**, 72 (8), 645–652.





ChRR

Institute of Chemistry  
of Renewable  
Resources

# Exploring HPTLC Data with Self-Organizing Maps

Matthias Guggenberger, Josua T. Oberlerchner,  
Heinrich Grausgruber, Thomas Rosenau, Stefan Böhmdorfer

Institute of Chemistry of Renewable Resources, Institute of Plant Breeding  
University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

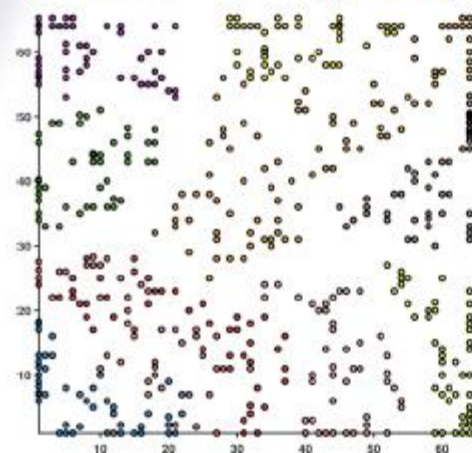
Guggenberger, M.; Oberlerchner, J. T.; Grausgruber, H.; Rosenau, T.; Böhmdorfer, S.  
Self-Organising Maps for the Exploration and Classification of Thin-Layer Chromatograms.  
*Talanta* 2021, 233, 122460. <https://doi.org/10.1016/j.talanta.2021.122460>



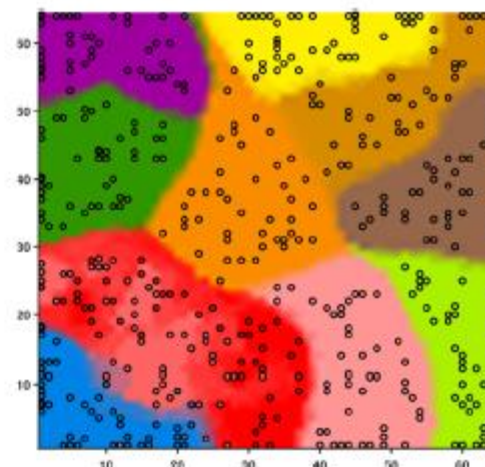


## Sample position

(Best Matching Unit)

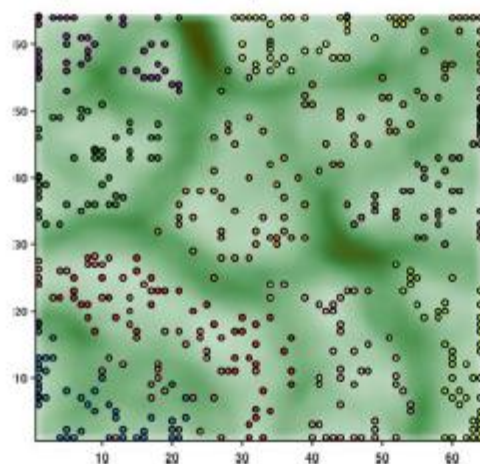


## Classes



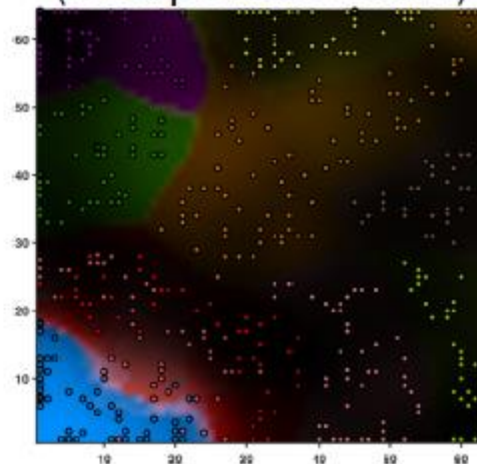
## Difference

(U-Matrix)

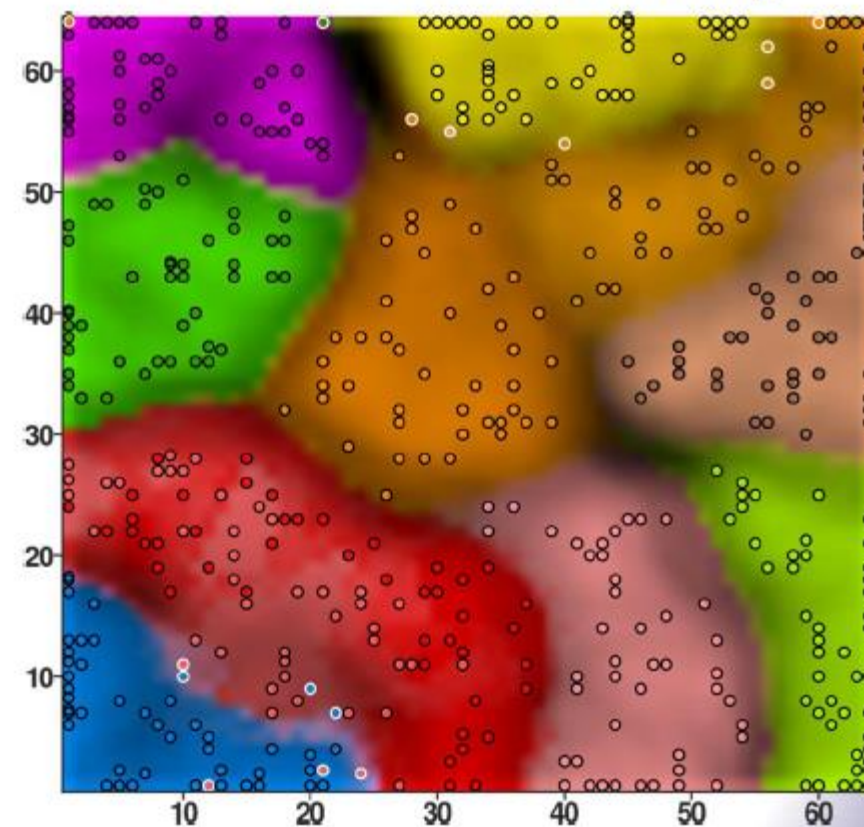


## Signals

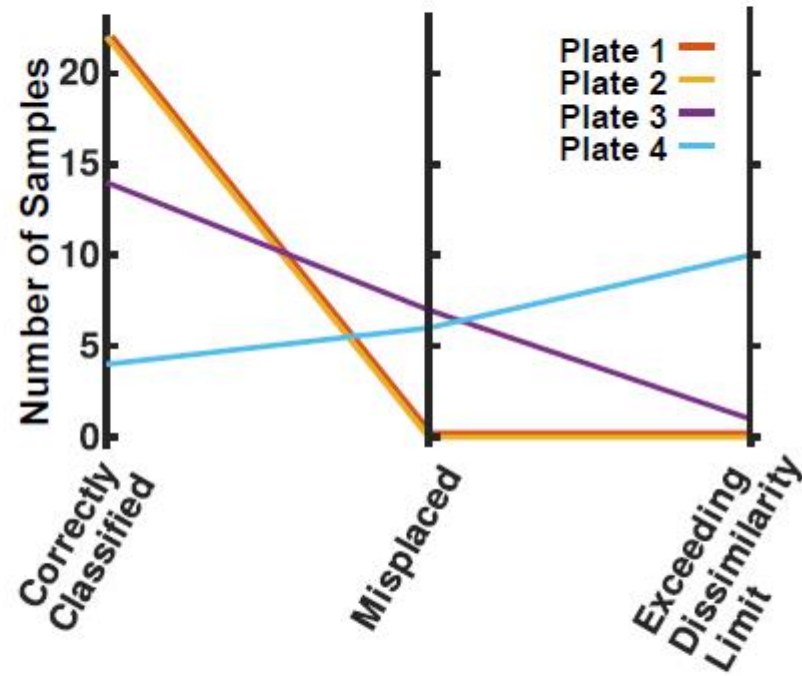
(Component Plane)



## Self-Organizing Map of HPTLC Videodensitometry

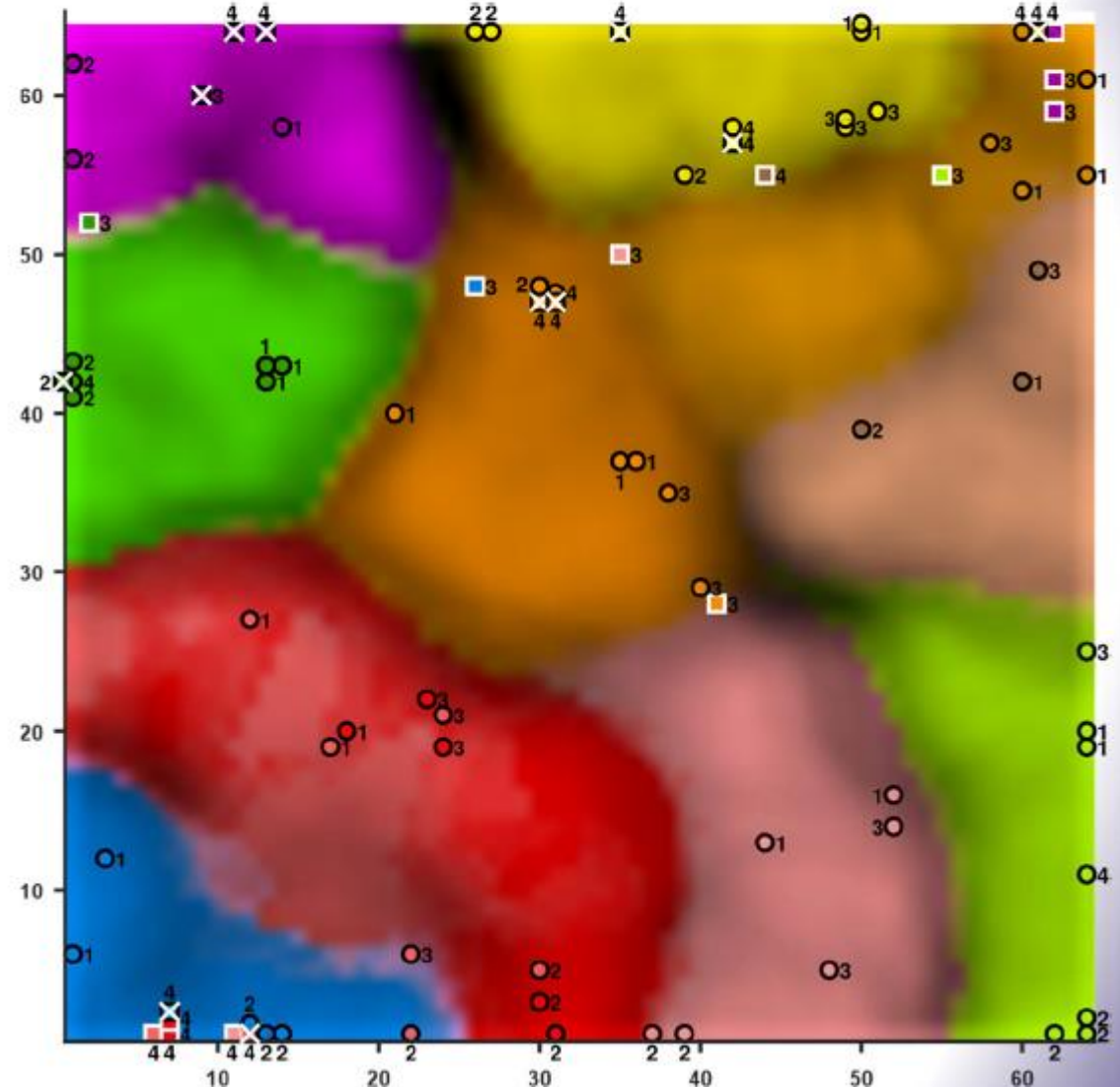


# Identification of Sample Class



**100% correct identification for correctly prepared plates.**

**Flaws cause misclassifications.**







## HPTLC IS A POWERFUL TOOL FOR IDENTIFICATION OF PROPOLIS BOTANICAL SOURCE

Etil Guzelmeric<sup>1</sup>, Nisa Beril Sen<sup>1</sup>, Ecesu Sezen<sup>2</sup>, Erdem Yesilada<sup>1</sup>

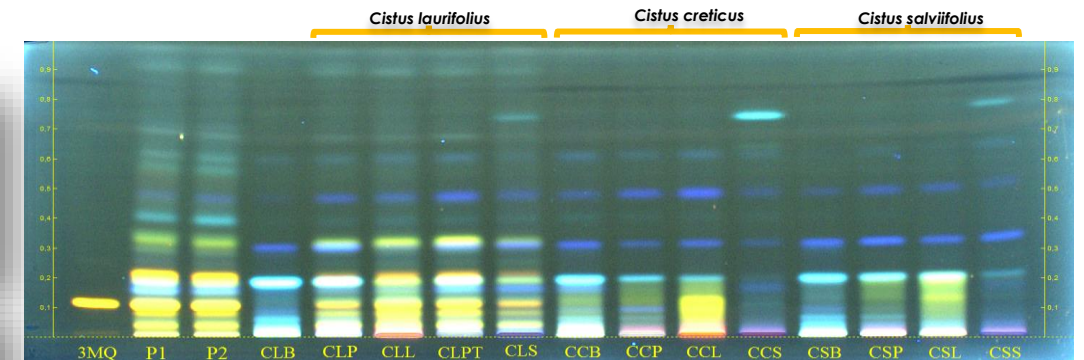
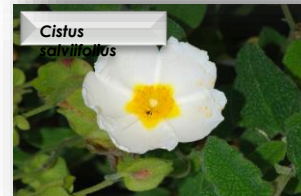
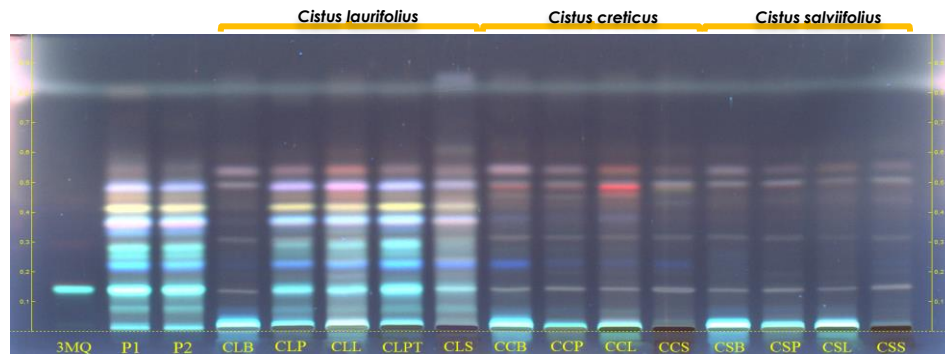
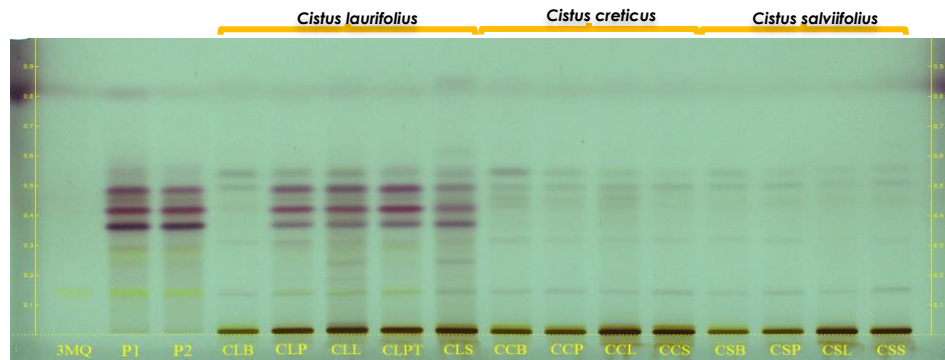
<sup>1</sup>Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy, Kayisdagi Cad., Atasehir, 34755, Istanbul, Turkey

<sup>2</sup>Yeditepe University, Faculty of Pharmacy, Kayisdagi Cad., Atasehir, 34755, Istanbul, Turkey

[ecesezen@gmail.com](mailto:ecesezen@gmail.com)

PRIX de POSTER

### Results



#### Result

Determination of the botanical source is highly important for the prediction of propolis' health benefits.

Chemical composition → Pharmacological activity

Consequently, HPTLC fingerprints of hydroalcoholic extracts of 3MQ-rich propolis were found to be similar to fingerprints of some plant parts of *C. laurifolius* extract.

**The botanical origin of propolis from *Cistus laurifolius* was confirmed for the first time by HPTLC analysis in this study.**





# CONCLUSIONS

an increasing number of papers in JCA  
shows that our user community is seriously growing  
(plants, lipids, sugars, API & EDA)

knowledge/training average situation is the issue

necessity to make good real science to be considered  
by the scientific analytical community

The power of the HPTLC technique is also our power !

# WHAT'S NEXT

Remember plans voted in BERLIN:  
COVER ALL WORLD

- 1 to enable all fans scientists to contribute at least every three years
- 2 to bring on board new industries when the topic comes into question
- 3 to help manufacturers to grow the users number and then their business

but our two main sponsors made another choice



"the question mark  
is to the wise what  
the exclamation  
point is to the fool"



...difficult to see: always in  
motion  
is the future



Many of the truth  
that we cling to  
depend on our  
point of view