HPTLC Asia 2018

International Symposium for High-Performance Thin-Layer Chromatography

Book of Abstracts

Bangkok, Thailand

November 27-30, 2018

HPTLC Asia 2018

1st Asia International Symposium for High-Performance Thin-Layer Chromatography

November 28-30, 2018, Bangkok, Thailand
www.hptlc.com

Welcome to Bangkok!

It is our pleasure to welcome you to the First International Symposium for High-Performance Thin-Layer Chromatography in Asia (HPTLC Asia 2018) which will take place in November 28-30, 2018, at Chulalongkorn University, Faculty of Pharmaceutical Sciences, Bangkok, Thailand.

HPTLC Asia 2018 represents a part of the traditional International HPTLC Symposium series that was first organized in 1980. The series has been held every three years since 2003 when Pierre Bernard-Savary organized the HPTLC 2003 in Lyon, followed by the ones in Berlin, Helsinki, Basel, Lyon and Berlin, respectively. The last one in Berlin was the 23rd HPTLC Symposium held in July 4-8, 2017. In that very successful event, however, some of our International Scientific Committee members expressed their concern about the small number of Asian participants involved in the meeting, despite a rapidly growing utilization of HPTLC-based techniques in Asia. The problems of long-distance travel and cost of participation were seen as an obstacle. Therefore, the HPTLC Asia symposium series was proposed in between the traditional European series, and the magnificent city of Bangkok was chosen as the first location of the ASIA series.

This First HPTLC Asia 2018 could attract more than 150 registrants with almost 90 abstract submissions. Among these, the problem-solving by HPTLC in the analytical field of "Botanicals and Traditional Medicines" was submitted about half of the total abstracts, and thus obviously the theme of this Symposium. The fields of "Effect-Directed Detection" and "HPTLC-MS for Compound Characterization" for have, interestingly, received considerable attentions from young researchers, based on the number of registrants (24 each). Certainly, we will enjoy seeing the views of academics, industries and student from various Asian countries in using HPTLC as powerful tools to solve their problems, from basic routine analytical work to advanced research problems.

We wish that all participants, especially those from Asia countries, will gain significant knowledge, techniques and applications of HPTLC from this Symposium, and will take them back to improve the quality of problem-solving of each group.

Finally, we also hope very much that this HPTLC-ASIA 2018 Symposium will cause expansion of the community of HPTLC users to a wider level, either through networking, mentoring or collaboration, which will speed up the innovation of HPTLC to wider fields with deep realization of its applications.

On behalf of the Scientific Committee and Organizing Committee of HPTLC Asia 2018,

Wanchai De-Eknamkul and Pierre Bernard-Savary

Content

The Series of International HPTLC Symposia

4-8 July 2017, **Berlin**

(23nd) International Symposium for High-Performance Thin-Layer Chromatography, HPTLC 2017

2-4 July 2014, **Lyon**

(22nd) International Symposium for High-Performance Thin-Layer Chromatography, HPTLC 2014

6-8 July 2011, **Basel**

(21st) International Symposium for High-Performance Thin-Layer Chromatography, HPTLC 2011

11-13 June 2008, **Helsinki**

(20th) International Symposium for Thin-Layer Chromatography, HPTLC 2008

9-11 October 2006, **Berlin**

(19th) International Symposium for Thin-Layer Chromatography, HPTLC 2006

29-31 May 2005, **Siofok**

(18th) Planar Chromatography 2005

23-25 May 2004, **Visegrad**

17th) Planar Chromatography 2004

15-18 October 2003, **Lyon**

(16th) International Symposium for TLC

21-23 June 2003, **Budapest**

(15th) Planar Chromatography 2003 (in honor of Professor Tyihak)

4-6 October 2002, **Novo mesto**

(14th) Planar Chromatography Today 2002

11-13 May 2002, **Keszthely**

(13th) Planar Chromatography 2002 (in honor of Doctor Geiss)

23-25 June 2001, **Lillafüred**

(12th) Planar Chromatography 2001

11-13 May 2000, **Lillafüred**

(11th) Planar Chromatography 2000 (in honor of Professor Kaiser)

16-19 May 1998, **Visegrad**

(10th) International Symposium on Instrumental Planar Chromatography (60 years TLC + 10 years JPC)

9-11 April 1997, **Interlaken**

(9th) International Symposium on Instrumental Planar Chromatography

5-7 April 1995, **Interlaken**

(8th) International Symposium on Instrumental Planar Chromatography

23-26 March 1993, **Brighton**

(7th) International Symposium on Instrumental Planar Chromatography

23-26 April 1991, **Interlaken**

(6th) International Symposium on Instrumental Planar Chromatography

21-24 February 1989, **Brighton**

(Fifth) International Symposium on Instrumental High Performance Thin-Layer Chromatography

22–25 September 1987, **Selvino**

(Fourth) International Symposium on Instrumental High Performance Thin-Layer Chromatography

17–19 April 1985, **Würzburg**

(Third) International Symposium on Instrumental High-Performance Thin-Layer Chromatography

2-6 May 1982, **Interlaken**

(Second) International Symposium on Instrumental High-Performance Thin-Layer Chromatography

18-21 May 1980, **Bad Dürkheim**

(First) International Symposium on Instrumentalized High-Performance Thin-Layer Chromatography

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International Scientific Committee
committee@hptlc.com

Co-Chair: Prof. Dr. Colin Poole, USA and Prof. Dr. Gertrud Morlock, Germany
Pierre Bernard-Savary, France
Dr. Vicente Cebolla, Spain
Prof. Dr. Wanchai De-Eknamkul, Thailand
Prof. Dr. Imre Klebovich, Hungary
Prof. Dr. Tadeusz Dzido, Poland
Prof. Dr. Lothar Kroh, Germany
Prof. Dr. Ilkka Ojanperä, Finland
Dr. Eike Reich, Switzerland
Prof. Dr. Joseph Sherma, USA
Prof. Dr. Mario Vega, Chile
Dr. Irena Vovk, Slovenia
Prof. Dr. Zheng-Tao Wang, China

Local Organizing Committee
info@hptlc.com

Chair: Prof. Dr. Wanchai De-Eknamkul, Thailand
Assist. Prof. Dr. Sornkanok Vimolmangkang, Thailand
Assist. Prof. Dr. Supakarn Chamni, Thailand
Dr. Chaisak Chansriniyom
Dr. Rossarin Tansawat, Thailand
Dr. Wanatchaporn Arunmanee, Thailand
Dr. Worathat Thitikornpong, Thailand
Jeerapat Doungchawee, Thailand

International Organizing Committee

Pierre Bernard-Savary, France
Louise Vicard, France

Poster Award Committee

Chair: Prof. Dr. Gertrud Morlock, Germany
Prof. Dr. Wanchai De-Eknamkul, Thailand
Pierre Bernard-Savary, France
Dr. Vicente Cebolla, Spain
Dr. Eike Reich, Switzerland
Prof. Dr. Jentaie Shiea, Taiwan
Prof. Dr. Zheng-Tao Wang, China
Dr. Irena Vovk, Slovenia

Program-at-a-Glance

Short Courses
November 27, 2018

Location: Chulalongkorn University, Faculty of Pharmaceutical Sciences,
7th Floor, Pharmaceutical Innovation Building, Pathumwan Road,
Bangkok 10330

Contact Person: A/Prof. Dr. Sornkanok (Aom) Vimonlmangkang
Email: Sornkanok.v@pharm.chula.ac.th
Mobile: +66(0)98-607-6446

8:45   Meet all together at Room 703 for Introduction
9:00   Start the Short Courses
              Room 702:   Herbal Standardization
              Room 703:   HPTLC for Research
              Room 710:   Quantitative Analysis

	Information	**Room 702** **(Herbal Standardization)**	**Room 703** **(HPTLC for Research**	**Room 710** **(Lipid Characterization** **and Quantification)**
9:00-12:00	**Course Code**	**SC-1**	**SC-5**	**SC-3**
	Topic	HPTLC method development	HPTLC-direct Bioautography	Quantitation and Validation in HPTLC
	No. of participants	17	24	5
	Lecturer (s)	Dr. Saikat Mallick	Prof. Dr. Gertrud Morlock	Pierre Bernard-Savary Dr. Jean-Marc Roussel
	Format	Lecture and Demonstration	Lecture and Demonstration	Lecture and Demonstration
	Lab Assistant	Ms. Khwanlada Kobtrakul	Dr.Siriluk Sintupachee	Ms. Sukanya Numsawad
12:00-13:00	Lunch (9th floor of the building)			
13:00-16:00	**Course Code**	**SC-2**	**SC-6**	**SC-4**
	Topic	Analysis of Botanicals in Compliance with USP and Pheur	HPTLC – MS for Characterization of Compounds	Lipid Characterization and Quantification
	No. of participants	17	22	6
	Instructor (s)	Dr. Eike Reich	Dr. Michaela Oberle Ivan Ng	Dr. Vicente L. Cebolla Pierre Bernard-Savary
	Format	Lecture and Demonstration	Lecture and Demonstration	Lecture and Demonstration
	Lab Assistant	Ms. Khwanlada Kobtrakul	Dr.Siriluk Sintupachee	Ms. Sukanya Numsawad
18:00-20:00	Cocktail Dinner at Pathumwan Princes Hotel (Vista Bar, 8th Floor)			

Symposium Program at a Glance

Location: Pharmacy Innovation Building, 10th Floor, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330

Wednesday, November 28, 2018

08:00	Registration – mounting of all posters
09:00	Opening
09:30-10:45	**Plenary Lecture (PL-1) of Session 1: Botanicals and Traditional Medicines**
10:45-12:15	6 Oral Presentations (OP-1 – OP-6)
12:15	Poster session with lunch (PP-1-PP-45)
13:30-14:15	**Plenary Lecture (PL-2) of Session 2: Effect-Directed Detection**
14:15-15:15	4 Oral Presentations (OP-7 – OP-10)
15:30-16:00	Manufacturer's Presentations (MP-1 – MP-2)
16:00-17:30	Poster Presentations (PP-1 – PP-45)

Thursday, November 29, 2018

09:00-09:45	**Plenary Lecture (PL-3) of Session 3: Pharm. and Toxicol. Analysis**
09:45-10:45	4 Oral Presentations (OP-11 – OP-14)
11:00-11:45	**Plenary Lecture (PL-4) of Session 4: Food, Dietary Supplements, Cosmetics and Biosamples**
11:45-12:30	3 Oral Presentations (OP-15 – OP-17)
12:30	Poster session with Lunch (PP-1-PP-45)
13:30-14:15	**Plenary Lecture (PL-5) of Session 5: HPTLC –MS for Compound Characterization**
14:15-15:15	4 Oral Presentations (OP-18 – OP-21)
15:30-16:00	Association/Manufacturer's Presentations (MP-3 and MP-4)
16:00-17:30	Poster Presentations (PP-1 – PP-45) and Poster Demounting

Friday, November 30, 2018

09:00-09:45	**Plenary Lecture (PL-6) Session 6: Strong Features of HPTLC**
09:45-10:15	2 Oral Presentations (OP-22 – OP-23)
10:30-10:45	Highlight Summary and Closing
11:15- 20:00	**Start Bangkok Tour** (with lunch box in the coach) to Grand Palace, a cruise to Icon-Siam, and Gala Dinner along Chaopraya River

Manufacturer / Association Presentations

Wed, Nov 28

Chair: Pierre Bernard-Savary

| 15:30 | MP-1 | Monika Bäumle | Merck, Germany | TLC/ HPTLC plates -Recent developments and applications |
| 15:45 | MP-2 | Pia Altenhofer | Biostep, Germany | Development of a universal set of system suitability testing (SST) standards for HPTLC |

Thu, Nov 29

Chair: Wanchai De-Eknamkul

| 15:30 | MP-3 | Eike Reich | HPTLC-Association Switzerland | Shaping the future of HPTLC: The international association for advancement of HPTLC |
| 15:45 | MP-4 | Ivan Ng | Waters, Singapore | WATERS' MS units for HPTLC-MS applications |

Social Program

Tue, Nov 27

| 18:00-20:30 | Welcome Cocktail Dinner | Pathumwan Princess Hotel, Vista Bar, 8th Floor | All Participants, including of Short Courses | Meet at the Place |

Wed, Nov 28

| 18:30-20:30 | Speakers' Dinner | SaSa International House | PL and PO Speakers and Accompany Persons | Meet at 18:00, on 2nd Floor of Hotel Lobby |

Thu, Nov 29

| 17.30-19.00 | Indian Get-Together Light Dinner | Room 902, 9th Floor, Pharmacy Innovation Building | All Indian Participants | Meet at the Room 902 |

Friday, Nov 30

| 11:15-20:00 | Bangkok Tour, River Cruise and Gala Dinner | From the Symposium Venue to the Grand Palace by Coach, to Icon Siam by Boat and River Cruise Dinner | All Participants | Take a Bus Right after the Symposium Closing |

HPTLC Asia 2018: Full Program

Short Course Program
November 27-30, 2018

Venue: Pharmacy Innovation Building, 7th Floor, Faculty of Pharmaceutical Sciences, Chulalongkorn University

Tuesday, November 27, 2018

ROOM 1	**HERBAL STANDARDIZATION (SC-1, SC-2)**
9:00-12:00	**SC-1:** HPTLC METHOD DEVELOPMENT
	Saikat Mallick
	Chief Chemist, ANCHROM Lab, Mumbai, India
13:00-16:00	**SC-2:** ANALYSIS OF BOTANICALS IN COMPLIANCE WITH USP AND PHEUR
	Eike Reich
	Director, CAMAG Laboratory, Muttenz Switzerland; President, HPTLC ASSOCIATION, Rheinfelden, Switzerland

ROOM 2	**QUANTITATIVE ANALYSIS OF HPTLC (SC-3, SC-4)**
9:00-12:00	**SC-3:** QUANTIFICATION AND VALIDATION IN HPTLC
	Pierre Bernard-Savary
	Chairman of Chromacim and French Club for Thin-Layer Chromatography, Moirans, France
	Jean-Marc Roussel
	Universities of Aix-Marseille and Bordeaux I, Analytical Methods Development and Validation Consulting, Co-Designer, NeoLiCy Software for Statistical Assessment of Analytical Method's Life Cycle, Mâcon, France
13:00-16:00	**SC-4:** LIPID CHARACTERIZATION AND QUANTIFICATION
	Vicente L. Cebolla
	Instituto de Carboquímica (ICB), Spanish National Research Council (CSIC), Zaragoza, Spain
	Pierre Bernard-Savary
	Chairman of Chromacim and French Club for Thin-Layer Chromatography, Moirans, France

ROOM 3	**HPTLC FOR RESEARCH (SC-5, SC-6)**
9:00-12:00	**SC-5:** HPTLC–DIRECT BIOAUTOGRAPHY
	Gertrud Morlock
	Chair of Food Science, Justus Liebig University Giessen, Giessen, Germany

13:00-16:00	**SC-6:** HPTLC – MS FOR CHARACTERIZATION OF COMPOUNDS
	Michaela Oberle
	TLC Specialist and Project Leader R&D, Merck KGaA, Darmstadt, Germany
	Ivan Ng
	Manager, System Solutions Business Development, Southeast Asia, Waters Pacific Pte. Ltd., Singapore
18:30-20:30	**Welcome Cocktail Dinner for All Symposium and Short-Course Participants (Pathumwan Princess Hotel, 8th Floor)**

International Symposium Program

Venue: Pharmacy Innovation Building, 10th Floor, Faculty of Pharmaceutical Sciences, Chulalongkorn University

Wednesday, November 28, 2018

| 8:00 | Registration |
| 9:00 | Opening |

SESSION 1:	**BOTANICALS AND TRADITIONAL MEDICINES**
	Chair: Prof. Dr. Wanchai De-Eknamkul
9:30-10:15	**Plenary Lecture (PL-1)**
	PL-1: APPLICATION OF HPTLC TECHNIQUE FOR HERBAL QUALITY CONTROL
	Prof. Dr. De-an Guo
	Chair Professor of Shanghai Institute of Materia Medica, Chinese Academy of Sciences; Director of National Engineering Laboratory for TCM Standardization Technology; Director of Shanghai Research Center for Modernization of Traditional Chinese Medicine, PR China
10:15-10:45	Coffee break
10:45-12:15	**6 Oral Presentations (OP-1 – OP-6)**
	OP-1: COMPREHENSIVE HPLTC FINGERPRINTING OFFERS ALTERNATIVES TO THE MARKER APPROACH FOR QUALITY CONTROL OF HERBAL MATERIALS
	Eike Reich
	Director, CAMAG Laboratory, Muttenz Switzerland; President, HPTLC Association, Rheinfelden, Switzerland
	OP-2: HPTLC AUTHENTICATION OF MULTI-ORIGINATED CHINESE HERBAL MEDICINES
	Zhengtao Wang
	Institute of Chinese Materia Medica, Key Laboratory of Standardization of Chinese Medicines of Ministry of Education; Shanghai University of Traditional Chinese Medicine, Shanghai, China

OP-3: HPTLC IMAGE ANALYSIS FOR ASSESSING THE FEASIBILITY OF THE EXTRACTIVE REFERENCE SUBSTANCE (ERS) OF CHINESE HERBAL MEDICINES IN QUALITY CONTROL

Longgang Guo

Guangdong Natural Medicine Engineering Technology Center and Guangzhou ImVin Pharmaceutical Co. Ltd., Guangzhou, China

OP-4: COMPARABILITY SPECTROSCOPIC AND CHROMATOGRAPHIC FINGERPRINT ANALYSIS OF GELSEMIUM ELEGANS

Mun Fei Yam

School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800, Pulau Pinang, Malaysia

OP-5: HPTLC FINGERPRINT-BASED RAPID CHEMOTAXONOMIC DISCRIMINATION OF DIFFERENT CLERODENDRUM SPECIES USING CHEMOMETRIC DATA ANALYSIS

Madhunita Srivastava

CSIR Central Institute of Medicinal and Aromatic Plants, Digras, India

OP-6: DEVELOPMENT OF SIMPLIFIED HPTLC SOLVENT SYSTEMS FOR THAI MEDICINAL PLANTS

Sornkanok Vimolmangkang

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

12:15-13:30	Lunch
SESSION 2:	**EFFECT-DIRECTED DETECTION**
	Chair: Prof. Dr. Gertrud Morlock
13:30-14:15	**Plenary Lecture (PL-2)**

PL-2: EFFECT-DIRECTED ANALYSIS OF FUNCTIONAL FOOD INGREDIENTS

Prof. Dr. Gertrud Morlock

Chair of Food Science, Justus Liebig University Giessen, Giessen, Germany

14:15-15:15   **4 Oral Presentations (OP-7 – OP-10)**

OP-7: TLC SCREENING AND EFFECT DIRECTED ANALYSIS OF CHOSEN PLANTS USED IN DEMENTIA TREATMENT

Irena M. Choma

Faculty of Chemistry, Maria Curie-Sklodowska University, Lublin, Poland

OP-8: A NOVEL HPTLC-HYPHENATED BIOAUTOGRAPHIC METHOD FOR IDENTIFICATION OF CARBOHYDRATE DIGESTING ENZYME INHIBITORY METABOLITES

Sayeed Ahmad

Bioactive Natural Product Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India

OP-9: EFFECT DIRECTED IDENTIFICATION OF ESTROGENIC SUBSTANCES IN FOOD CONTACT MATERIALS WITH HPTLC-BIOASSAYS AND HRMS

Alan Bergmann

Ecotox Center Eawag-EPFL, Dübendorf, Canton of Zürich, Switzerland

OP-10: HPTLC-EDA-UV/VIS/FLD-HESI-HRMS FOR TRACKING OF BIOACTIVE METABOLITES IN PRIMULA BOVEANA"

Ehab Mahran

Philipps University of Marburg, Marburg, and Justus Liebig University Giessen, Giessen, Germany

15:15-15:30	Coffee break
15:30-16:00	**MANUFACTURER'S PRESENTATIONS (MP-1 – MP-2)**

Chair: Pierre Bernard-Savary

MP-1: TLC/ HPTLC PLATES -RECENT DEVELOPMENTS AND APPLICATIONS

Monika Bäumle

Merck KGaA, Darmstadt, Sigma-Aldrich Chemie GmbH

MP-2: ARE THERE TRULY ESSENTIAL ROSE OILS IN COSMETICS AND FOOD?

Pia Altenhofer

Biostep GmbH, Glockenstr., Chemnitz, Germany

16:00-17:30	**Poster Presentations (PP-1 – PP-45)**

Thursday, November 29, 2018

SESSION 3:	**PHARMACEUTICAL AND TOXICOLOGICAL ANALYSIS**
9:00-9:45	**Plenary Lecture (PL-3)**

PL-3

9:45-10:45	**4 Oral Presentations (OP-11 – OP-14)**

OP-11: WHY IS IT NECESSARY TO USE HPTLC IN API MANUFACTURING PROCESS? - A REVIEW OF CASE STUDIES

Pierre Bernard-Savary

Chairman of Chromacim and French Club for Thin-Layer Chromatography, Moirans, France

OP-12: HPTLC ASSAY METHODS VALIDATION: WILL ICH MEET FDA/EMA/ USP?

Jean-Marc Roussel

Universities of Aix-Marseille and Bordeaux I, Analytical Methods Development and Validation Consulting, Co-Designer, NeoLiCy Software for Statistical Assessment of Analytical Method's Life Cycle, Mâcon, France

OP-13: USE OF BIOMARKERS AS QUALITY CONTROL INDICES FOR AYURVEDIC FORMULATIONS

Sunita Shailajan

Herbal Research Lab, Ramnarain Ruia College, Matunga (East), Mumbai, India

10:45-11:00	Coffee break

SESSION 4: **FOOD, DIETARY SUPPLEMENTS, COSMETICS AND BIOSAMPLES**

Chair: Dr. Vicente L. Cebolla

11:00-11:45	**Plenary Lecture (PL-4)**

PL-4: HPTLC OF LIPID-BASED MIXTURES IN DIFFERENT MATRICES: COMBINATION OF DENSITOMETRY AND MS FOR OBTAINING QUALITATIVE AND QUANTITATIVE SAMPLE INFORMATION

Dr. Vicente L. Cebolla

Instituto de Carboquímica (ICB), Spanish National Research Council (CSIC), Zaragoza, Spain

11:45-12:30	**3 Oral Presentations (OP-15 – OP-17)**

OP-15: HPTLC-FLD-SERS DETERMINATION OF FOLIC ACID WITH OZONE-INDUCED FLUORESCENCE

Yisheng Chen

The Key Laboratory of Food Science and Technology, School of Food Science and Technology, Collaborative Innovation Centre of Food Safety and Quality Control in Jiangsu Province, Jiangsu University, Wuxi, China

OP-16: HPTLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS EVALUATION OF PHENOLIC ACIDS AND FLAVONOIDS TO STUDY THE PROCESSING EFFECTS IN DIFFERENT MILLET GRAINS

Giridhar Goudar

TATA-NIN Center for Excellence in Nutrition, National Institute of Nutrition, Hyderabad, Telangana, India

OP-17: HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY: THE PERFECT ANALYTICAL TOOL FOR FOOD ANALYSIS

Mario Aranda

Department of Food Science, Nutrition and Dietetic, Faculty of Pharmacy, University of Concepcion, Concepcion, Chile

12:30-13:30	Lunch

SESSION 5: **HPTLC –MS FOR COMPOUND CHARACTERIZATION**

Chairs: Prof. Dr. Jentaie Shiea

13:30-14:15	**Plenary Lecture (PL-5)**

PL-5: HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY–MASS SPECTROMETRY (HPTLC-MS): A SIMPLE INTERFACE TO COMBINE HPTLC WITH ELECTROSPRAY IONIZATION MASS SPECTROMETRY

Prof. Dr. Jentaie Shiea

Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, Taiwan

14:15-15:15	**4 Oral Presentations (OP-18 – OP-21)**
	OP-18: HPTLC BASED METHOD DEVELOPMENT FOR CARDIOVASCULAR DISEASE CONTROLLING COMPOUNDS CONTAINING PLANT COLEUS FORSKOLII & COMPARISON OF SEPARATION EFFICIENCY OF TWO DIFFERENT HPTLC PLATES
	Ajai Prakash Gupta
	QC & QA, Indian Institute of Integrative Medicine, (CSIR-IIIM), Jammu, India
	OP-19: SIMPLE INTERFACE FOR SCANNING CHEMICAL COMPOUNDS ON DEVELOPED THIN LAYER CHROMATOGRAPHY PLATES USING ELECTROSPRAY IONIZATION MASS SPECTROMETRY
	Sychyi Cheng (will be presented by Suhail Muzaffar Bhat)
	Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, Taiwan.
	OP-20: TARGET BASED METHOD DEVELOPMENT USING EFFECT DIRECTED TLC - A STRAIGHT WAY FOR COMPOUND PURIFICATION
	Michaela Oberle
	TLC Specialist and Project Leader R&D, Merck KGaA, Darmstadt, Germany
	OP-21: HPTLC-MS IN API CLINICAL BATCHES MANUFACTURING
	Amélie Havard (will be presented by Pierre Bernard-Savary)
	Research Scientist, Oril Industrie, Servier, Bolbec, Le Havre Area, France
15:15-15:30	Coffee break
15:30-16:00	**Association/Manufacturer's Presentations (MP-3 and MP-4)**
	Chair: Prof. Dr. Wanchai De-Eknamkul
	MP-3: SHAPING THE FUTURE OF HPTLC: THE INTERNATIONAL ASSOCIATION FOR ADVANCEMENT OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY
	Eike Reich
	President, HPTLC Association, Rheinfelden, Switzerland
	MP-4: MASS DETECTION FOR ROUTINE ANALYSIS
	Ivan Ng
	Manager, System Solutions Business Development, Southeast Asia, Waters Pacific Pte. Ltd., Singapore
16:00-17:30	**Poster Presentations (PP-1 – PP-45)**

Friday, November 30, 2018

SESSION 6:	**STRONG FEATURES OF HPTLC**
	Chair: Dr. Irena Vovk
9:00-9:45	**Plenary Lecture (PL-6)**
	PL-6: HPTLC – DO WE REALLY KNOW IT AND HOW TO MAKE IT MORE POPULAR
	Dr. Irena Vovk
	Head, Department of Food Chemistry, National Institute of Chemistry, Ljubljana, Slovenia

9:45-10:15	**2 Oral Presentations (OP-22 – OP-23)**
	OP-22: DEVELOPMENT OF A UNIVERSAL SET OF SYSTEM SUITABILITY TESTING (SST) STANDARDS FOR HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY
	Manjusha Phanse
	Anchrom Enterprises (India) Pvt. Ltd., Mumbai, India
	OP-23: VALIDATED TLC DENSITOMETRIC METHOD FOR SIMULTANEOUS QUANTIFICATION OF SIX TERPENOIDS IN PUTRANJIVA ROXBURGHII-A PLANT USED IN INDIAN SYSTEM OF MEDICINE
	Sonali Mishra
	Analytical Chemistry Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow-226015, India
10:15-10:30	Coffee break
10:30-10:45	Highlights (Prof. Dr. Gertrud Morlock)
10:45-11:15	Poster Presentation Awards and Closure (Prof. Dr. Wanchai De-Eknamkul and Pierre Bernard-Savary)
11:15- 20:00	**Start Bangkok Tour (with lunch box in the coach) to Grand Palace, a cruise to Icon-Siam, and Gala Dinner along Chaopraya River**

List of Plenary and Oral Presentations

Pharmacy Innovation Building, 10th Floor, Faculty of Pharmaceutical Sciences, Chulalongkorn University
Phayathai Road, Pathumwan, Bangkok 10330

Wed, 28th

SESSION 1:   BOTANICALS AND TRADITIONAL MEDICINES
Chair: Prof. Dr. Wanchai De-Eknamkul

09:30	PL-1	De-an Guo	China	Comprehensive HPTLC fingerprinting offers alternatives to the marker approach for quality control of herbal materials
10:45	OP-1	Eike Reich	Switzerland	Comprehensive HPLTC fingerprinting offers alternatives to the marker approach for quality control of herbal materials
10:50	OP-2	Zhengtao Wang	China	HPTLC authentication of multi-originated Chinese herbal medicines
11:05	OP-3	Longgang Guo	China	HPTLC Image analysis for assessing the feasibility of the extractive reference substance (ERS) of Chinese herbal medicines in quality control
11:20	OP-4	Mun Fei Yam	Malaysia	Comparability spectroscopic and chromatographic fingerprint analysis of *Gelsemium elegans*
11:35	OP-5	Madhunita Srivastava	India	HPTLC Fingerprint-based rapid chemotaxonomic discrimination of different *Clerodendrum* species using chemometric data analysis
11:50	OP-6	Sornkanok Vimolmangkang	Thailand	Development of simplified HPTLC solvent systems for Thai medicinal plants

SESSION 2:   EFFECT-DIRECTED DETECTION
Chair: Prof. Dr. Gertrud Morlock

13:30	PL-2	De-an Guo	Germany	Effect-directed analysis of functional food ingredients
14:15	OP-7	Irena M. Choma	Poland	TLC Screening and effect directed analysis of chosen plants used in dementia treatment
14:30	OP-8	Sayeed Ahmad	India	A novel HPTLC-hyphenated bioautographic method for identification of carbohydrate digesting enzyme inhibitory metabolites
14:45	OP-9	Alan Bergmann	Switzerland	Effect directed identification of estrogenic substances in food contact materials with HPTLC-bioassays and HRMS
15:00	OP-10	Ehab Mahran	Germany	HPTLC-EDA-UV/VIS/FLD-HESI-HRMS for tracking of bioactive metabolites in *Primula boveana*

Thu, 29th

SESSION 3:   PHARMACEUTICAL AND TOXICOLOGICAL ANALYSIS

09:00	PL-3			
09:45	OP-11	Pierre Bernard-Savary	France	Why is it necessary to use HPTLC in API manufacturing process? - A review of case studies
10:00	OP-12	Jean-Marc Roussel	France	A Novel HPTLC-hyphenated bioautographic method for identification of carbohydrate digesting enzyme inhibitory metabolites
10:15	OP-13	Sunita Shailajan	India	Effect directed identification of estrogenic substances in food contact materials with HPTLC-bioassays and hrms
10:30	OP-14			

SESSION 4: **FOOD, DIETARY SUPPLEMENTS, COSMETICS AND BIOSAMPLES**
Chair: Dr. Vicente L. Cebolla

11:00	PL-4	Vicente L. Cebolla	Spain	HPTLC of lipid-based mixtures in different matrices: combination of densitometry and MS for obtaining qualitative and quantitative sample information
11:45	OP-15	Yisheng Chen	China	HPTLC-FLD-SERS determination of folic acid with ozone-induced fluorescence
12:00	OP-16	Giridhar Goudar	India	HPTLC method development and validation for simultaneous evaluation of phenolic acids and flavonoids to study the processing effects in different millet grains
12:15	OP-17	Mario Aranda	Chile	HPTLC: The perfect analytical tool for food analysis

SESSION 5: **HPTLC –MS FOR COMPOUND CHARACTERIZATION**
Chairs: Prof. Dr. Jentaie Shiea

13:30	PL-5	Jentaie Shiea	Taiwan	HPTLC-MS: A simple interface to combine HPTLC with electrospray ionization mass spectrometry
14:15	OP-18	Ajai Prakash Gupta	India	Hptlc based method development for cardiovascular disease controlling compounds containing plant coleus forskolii & comparison of separation efficiency of two different hptlc plates
14:30	OP-19	Sychyi Cheng	Taiwan	Simple interface for scanning chemical compounds on developed TLC plates using electrospray ionization mass spectrometry
14:45	OP-20	Michaela Oberle	Germany	Target based method development using effect directed TLC – A straight way for compound purification
15:00	OP-21	Amélie Havard	France	HPTLC-MS in API clinical batches manufacturing

Fri, 30th

SESSION 6: **STRONG FEATURES OF HPTLC**
Chair: Dr. Irena Vovk

09:00	PL-6	Irena Vovk	Slovenia	HPTLC – Do we really know it and how to make it more popular?
09:45	OP-22	Manjusha Phanse	India	Development of a universal set of system suitability testing (SST) standards for HPTLC
10:00	OP-23	Sonali Mishra	India	Validated TLC densitometric method for simultaneous quantification of six terpenoids in *Putranjiva roxburghii*-a plant used in Indian system of medicine

List of Poster Presentations

POSTER GROUP 1: BOTANICALS AND TRADITIONAL MEDICINES

PP-1	Li Pan	China	Establishment of a unified TLC identification method for *Sinapis semen, Raphani semen, Descurainiae semen*, and *Lepidii semen* and separation of its main specific components
PP-2	Moo-Seob Kim	China	A harmonized HPTLC analysis method of Ginseng, American ginseng, and Notoginseng
PP-3	Rui Rong Zheng	China	Improvement of TLC authentication methods of four Chinese medicinal materials
PP-4	Xia Li	China	Comparison of chemical constituents between green and brown *Forsythiae fructus*
PP-5	Lihua Gu	India	A specific HPTLC method for authentication of *Swertia mileensis* and its allied spp.
PP-6	Sharad Srivastava	India	Role of HPTLC in industrial chemotaxonomy
PP-7	Longgang Guo	China	HPTLC image analysis for assessing the feasibility of the extractive reference substance (ERS) of Chinese herbal medicines in quality control
PP-8	Poachanee Norfun	Thailand	Phytochemical Screening and HPTLC Fingerprinting profile of extracts of *Annona squamosa* L
PP-9	Nattaporn Chanthasakda	Thailand	HPTLC fingerprint profile of insecticidal alkaloids from *Stemona phyllantha* Gagnep
PP-10	Hayford Ofori	Ghana	HPTLC: A tool for characterizing sandalwood oils
PP-11	Karuna Shanker	India	Validated TLC densitometric method for simultaneous quantification of six terpenoids in *Putranjiva roxburghii* - A plant used in Indian system of medicine
PP-12	Misra Ankita	India	Chemotaxonomic studies on *Gloriosa superba* L. from different phyto-geographical zones of India through HPTLC
PP-13	Ramachandran Shakila	India	Quantification of active principle from Nilavembu Kudineer Chooranam - An anti-dengue Siddha formulation by HPTLC method
PP-14	Suresh Kumar	India	Estimation of bioactive marker compounds of *Baptisia tinctoria* using HPTLC
PP-15	Suhina Bhosale	India	Application of HPTLC in quality assessment of an Ayurvedic drug - *Myrica esculenta* Buch.-Ham ex D. Don
PP-16	Mayuresh Joshi	India	Standardization of ethyl acetate extracts of *Avicennia marina* and *Sonneratia apetala* using a validated HPTLC method
PP-17	Kesri N. Sharma	India	Standardization of herbal drugs by HPTLC methods
PP-18	Binit Dwivedi	India	Importance of HPTLC in analysis of homeopathic drug *Datura ferox*
PP-19	Archana P. Raina	India	Quantification of L-3,4-dihydroxyphenylalanine - An anti-Parkinson's compound from seeds of *Mucuna* species by HPTLC
PP-20	Ajai Prakash Gupta	India	HPTLC -dual wavelength spectro-densitometric method for simultaneous quantification of active compounds rosmarinic acid and caffeic acid in homeopathic plants *Rosmarinus officinalis* and *Ocimum gratissimum* as per USP
PP-21	Kartini Kartini	Indonesia	HPTLC fingerprints of some Indonesian medicinal plants used in "Scientific Jamu"

POSTER GROUP 2:   EFFECT-DIRECTED DETECTION

PP-22	X. L. Deng	China	TLC-bioautographic identification of new markers and modificationof HPTLC authentication of *Curculiginis rhizome*
PP-23	Tanushree Goswami	India	HPTLC hyphenated with bioassay for the screening of bioactive constituents of spice *Zanthoxylum rhetsa*
PP-24	Tanushree Goswami	India	HPTLC-DPPH method for the detection of antioxidant compounds from *Zanthoxylum rhetsa*
PP-25	Alan Bergmann	Switzerland	Performance of measuring estrogenicity with planar-yeast estrogen screen compared to 96-well plate yeast estrogen screen
PP-26	Supakarn Chamni	Thailand	Determination of steroid 5α-reductase activity of furano-naphthoquinones by non-radioactive and direct dihydrotestosterone detection using HPTLC

POSTER GROUP 3: PHARMACEUTICAL AND TOXICOLOGICAL ANALYSIS

PP-27	Tom Sostaric	Australia	Quantification of tacrolimus ointment using HPTLC
PP-28	Samir Patel	India	Validated TLC densitometric method for stress degradation and accelerated stability study of asiaticoside from *Centella asiatica* and its marketed formulation
PP-29	Surender Sehrawat	India	Development of new solvent systems for the analysis of atorvastatin extracted from blood using HPTLC plate
PP-30	Manoj Shrawan Charde	India	Simultaneous estimation of drotaverine HCl and nimesulide in pharmaceuticals by HPTLC method
PP-31	Reena Mascarenhas	India	A validated HPTLC method for quantification of kaempferol from rat plasma and its application to pharmacokinetic study using sparse sampling method
PP-32	Archita Patel	India	Development and validation of stability-indicating HPTLC method for simultaneous estimation of ilaprazole and levosulpiride in capsules using design of experiment approach
PP-33	Pallavi Choudharyi	India	Simultaneous determination and method validation for opioids, cannabinol, and nicotine in postmortem whole blood using HPTLC-mass spectrometry
PP-34	Nishit Patel	India	Comparative evaluation of glass containers and laminated plastic pouches in maintaining chemical integrity of *Ashwagandhadi lehya*
PP-35	Sunita Shailajan	India	Pharmacokinetic evaluation of a polyherbal formulation using high-performance TLC technique
PP-36	Yogeshkumar Gavhane	India	Development of validated HPTLC method for the estimation of eugenol in marketed herbal formulation of muscle and joint hrx pain relieving oil

POSTER GROUP 4:   FOOD, DIETARY SUPPLEMENTS, COSMETICS AND BIOSAMPLES

PP-37	Laksana Charoenchai	Thailand	HPTLC densitometry simultaneously determined sesamin and sesamolin in cold pressed sesame oil
PP-38	Vesna Glavnik	Slovenia	Determination of anthraquinones in Japanese knotweed extract by HPTLC – mass spectrometry methods
PP-39	Vicente Luis Cebolla	Spain	A high-throughput combinatorial platform of fluorophores for obtaining fingerprints for analyte discrimination

POSTER GROUP 5:   HPTLC - MS FOR COMPOUND CHARACTERIZATION

PP-40	Urška Jug	Slovenia	Isolation of bioactive compounds from Japanese knotweed rhizomes using HPTLC methods followed by NMR characterization
PP-41	Mario Aranda	Chile	Identification of bioactive compounds in *Aristotelia chilensis* by HPTLC-bioassay-mass spectrometry
PP-42	Mario Aranda	Chile	HPTLC-ESI-MS/MS determination of fining-related allergen proteins in Chilean wines
PP-43	Mario Aranda	Chile	Identification of acetylcholinesterase inhibitors compounds in in Annona cherimola Mill. by HPTLC-bioassay-mass spectrometry
PP-44	Karem Henríquez-Aedo	Chile	Analysis of polyphenols in Chilean wine by planar chromatography-mass spectrometry-bioassay
PP-45	Darlene Peterssen Fonseca	Chile	Chemical and functional evaluation of fresh and black Chiloe's giant garlic (*Allium ampeloprasum* L.) by HPTLC -autography coupled to mass spectrometry

PL-01

Application of High-performance Thin-layer Chromatography Technique for Herbal Quality Control

De-an Guo

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It is well known that high-performance thin-layer chromatography (TLC) (HPTLC) was called as "planar chromatography" at its preliminary stage, and only in 2003, the name of HPTLC was formally used in the symposium entitled "International symposium on HPTLC." When "planar chromatography" was firstly "invented," China introduced it into quality control (QC) of Traditional Chinese Medicine (TCM) and Chinese Pharmacopoeia (CHP) had widely adopted this technique in the quality monographs of Chinese herbal drugs and their finished products. The first HPTLC paper entitled "differentiation and evaluations of ginsengs and their preparation by means of HPTLC fingerprint analysis" by Prof. Xie Peishan was published in the first issue of Joint Parliamentary Committee in 1988. Since then, HPTLC was widely considered as the most convenient and feasible tool for the identification of crude drugs and finished products as it can be witnessed by the different editions of CHP (CHP 1990, 1995, 2000, 2005, 2010, and 2015). In the 1990s, 16 HPTLC-based assays for markers of TCM drugs were introduced to CHP. In 1993, the first CHP TLC Atlas for crude drugs was published followed by another Atlas for granules. Following this trend, CHP HPTLC Atlas with two volumes in Chinese and one volume in English were published in 2005. Moreover, currently, the most recent issue of the CHP HPTLC Atlas is under preparation. Now, China Food and Drug Administration has equipped 150 mobile laboratories for herbal QC by HPTLC, and all provincial drug control institutes perform HPTLC for QC as well. CHP is also conducting trainings throughout the country to educate drug control institutes and industry for the best use of HPTLC. Although China also develops multimarker approaches (high-performance liquid chromatography/mass spectrometry/biological tests) for an introduction to CHP monographs, HPTLC still plays a significant and pivotal role for herbal identification and QC. Dr. Eike Reich from CAMAG is now collaborating with my group, the United States Pharmacopoeia (USP) and Ph. Eur for which a standardized HPTLC methodology USP<203>/PhEur 2.8.25 has been adopted.

In this lecture, a number of Chinese herbal drugs will be exemplified for their HPTLC identification and differentiation of similar and easily adulterated species, including Panax species suites, Angelica sinensis, Schisandra chinensis, and many more. It is hard to overstate how important HPTLC is for the herbal identification and QC. It is expected that HPTLC will become more and more popular in the application of herbal identification and other field with the technical advances.

Prof. De-an Guo is currently as the Chair Professor at Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Director of National Engineering Laboratory for TCM Standardization Technology and Director of Shanghai Research Center for Modernization of TCM. He has focused his research on modernization of TCM for >30 years and established a research model for analysis of active components of TCM and constructed a holistic QC system for TCM, which have been successfully applied in CHP, the USP, and Europe Pharmacopoeia for the herbal standards. These remarkable achievements were considered to have a great global impact and widely recognized through numerous awards and prizes received over the years, including State National Science Award in 2012 and National Science and Technology Progress Award in 2016. Professor Guo is the first Chinese scientist to elaborate TCM standards for USP and European Pharmacopoeia. He has published over 430 papers in science citation index (SCI) cited journals with over 7000 SCI citations.

PL-02

Effect-directed analysis of functional food ingredients

Gertrud Morlock

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Gertrud Morlock

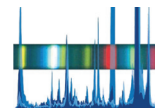*Justus Liebig University Giessen, Chair of Food Science, Giessen, Germany.*

On the one hand, the separation of thousands of the compounds in a complex extract is thrilling but maybe still unsatisfactorily due to coelutions. Hence, the question arises when to stop in high-sophisticated comprehensive separation science? On the other hand, the separation itself does not imply an effect-directed answer to questions such as "Which compounds out of the thousands are active?" Hence, a straightforward methodology is presented that is able to answer these effect-directed questions without the need for a comprehensive separation.[1] The first part of the streamlined methodology is an effect-directed screening (direct bioautography) of up to 22 raw extracts in parallel. It is a non-targeted chromatography-based bioprofiling. Depending on the selected assay, for example, antibiotics, estrogens, and androgens as well as inhibitors of acetylcholinesterase, α-/ß-glucosidase, α-amylase, xanthine oxidase, or tyrosinase are detected in complex samples. Examples of the methodology are given for functional food ingredients and plant extracts, taking 3–20 min per sample for the discovery of bioactive components. The second part is a highly targeted characterization of the effective compounds discovered through the hyphenation to high-resolution mass spectrometry and further structure elucidating techniques. Information on effective compounds in a complex sample and their molecular formulae can be obtained from the same chromatographic separation. The pictorial side-by-side sample comparison, the matrix tolerance for raw extracts, the avoidance of carry over and discrimination (the full sample is in the focus), the always fresh adsorbent for a separation, the comparatively low-tech operation, and the multievaluation of the separated sample are treasured features of the straightforward methodology.

REFERENCE

1. Morlock GE. Bioassays and further effect-directed detections in chromatography. In: Worsfold PJ, Poole C, Townshend A, Miro M editors. Reference Module in Encyclopedia of Analytical Science. 3ʳᵈ ed. Amsterdam: Elsevier Science; 2018.

Gertrud Morlock is full professor at the Justus Liebig University Giessen in Germany. She holds the Chair of Food Science and, currently, also chairs the Institute of Nutritional Science. She is a Director of the Trans MIT Center for Effect-Directed Analysis. She has made about 130 peer-reviewed original research paper since 2006, 74 further scientific papers and 14 book chapters, is editor of the free-of-charge CAMAG CBS journal and its online database CCBS containing ca. 11000 abstracts on TLC/HPTLC, conducted about 70 workshops, presented about 240 posters and 260 lectures at symposia, and is active in several scientific committees.

PL-04

HPTLC of lipid-based mixtures in different matrices: Combination of densitometry and mass spectrometry for obtaining qualitative and quantitative sample information

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In complex samples, such as derived from food or lipidomics (LC), hundreds of analytes can be present. A substantial part of these compounds has not yet been identified. As the quantification is still based on the availability of target compounds as calibration standards, it becomes too costly or directly impossible to obtain standards for all possible unknown compounds. In this context, while performance of modern analytical instruments progressively increases, semi-quantitative approaches make sense. As it has been recognized in LC, there is no self-sufficient technique for lipid analysis. Despite the high separation efficiency of column LC using reversed phase, quantification by LC–mass spectrometry (MS) is complicated due to the high dependence of response factors on ionization conditions, even for structurally similar lipids eluted at different retention times. High-performance thin-layer chromatography (HPTLC) has been very popular in lipid analysis. Unlike LC, most of the separation of lipid samples are performed on silica gel plates, which allow to group lipids in classes according to their polar head, each class including different lipid fatty acid chain lengths. A strong point of HPTLC is multiple detections. Coupling of ultraviolet/focal length scanning densitometry with MS opens the door to obtain representative profiles and deep identifications of lipid molecular species in complex samples, with the possibility of selectively detecting a desired zone of plate. All these aspects, as well as some strategies for obtaining reliable semi-quantitative information, will be discussed in the light of recent literature concerning glycerophospholipids, (glyco)sphingolipids, fatty acids, glycerolipids, and other lipids found in different matrices of interest.

Vicente L. Cebolla, Ph.D in 1987 at CNRS (Institut Recherches Sur la Catalyse, France) and University of Zaragoza (Spain). Visiting Prof. in Metz University (2001). Richard H. Glenn Award 2002 from the American Chemical Society (Division of Fuel Chemistry). At present, CSIC Staff Senior Researcher at the CSIC, Instituto de Carboquímica (Zaragoza, Spain). Research interests: HPTLC of complex samples (fuels and lipids); development of fluorescence-based detection techniques for the characterization of organic materials.

PL-05

High-Performance Thin-Layer Chromatography–Mass Spectrometry (HPTLC–MS): A Simple Interface to Combine High-Performance Thin-Layer Chromatography with Electrospray Ionization Mass Spectrometry

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Mass spectrometry (MS) is one of the most sensitive analytical techniques for the detection of chemical compounds. Previously, several interfaces for coupling high-performance thin-layer chromatography (HPTLC) with MS have been developed to characterize the analytes on developed HPTLC plates. However, the detachment of HPTLC gel particles during HPTLC–MS analysis may affect the detection efficiency of analyte ions and damage turbomolecular vacuum pumps if gel particles enter the mass analyzer. This study developed an extremely simple interface to combine HPTLC with MS for the detection of analytes on an HPTLC plate with minimum detachment of silica gel particles. The technique was applied to characterize the dye mixtures and drugs adulterated in herbal products as well as alkaloids in plant extracts, which were all separated on the HPTLC plate, respectively.

The track on an alumina-based HPTLC plate was centrally bifurcated with scissors to form two sawtooth HPTLC pieces, with each piece containing 17 triangle tips. The sawtooth HPTLC pieces were then positioned on an XYZ stage, with the first triangular tip on one HPTLC piece pointed toward the inlet of the mass analyzer. A drop of methanol and high direct current voltage (5 kV) was applied at the tip to induce electrospray ionization (ESI) for the following MS detection of analytes at the tip. The HPTLC piece was then moved so the second triangular tip was pointed toward the MS inlet for the next analysis. The process was repeated until all 34 triangular tips on two sawtooth HPTLC pieces were analyzed. Sawtooth HPTLC-ESI/MS allows the detection of analytes on both visible and non-visible bands, and the spatial resolution of the approach was determined to be approximately 1.5 mm/band (50 mm/34). Sawtooth HPTLC-ESI/MS was utilized to characterize a mixture of dye standards and food dyes extracted from candy. The limits of the detection of dye standards such as rhodamine B and patent blue V were 0.25 ng/band. The technique was also applied to determine active ingredients in herbs such as ginseng and traditional Chinese medicines (TCM). In addition to the active and toxic alkaloids in herbal plants, drugs such as sildenafil, acetaminophen, chlorpheniramine, and dextromethorphan laced in TCM were also detected. The experimental results obtained in this study have demonstrated that sawtooth HPTLC-ESI/MS is a useful technology in the areas of food safety, herbal medicine, and natural products.
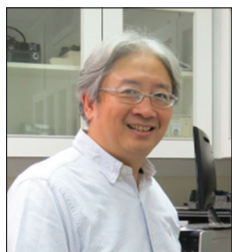
Jentaie Shiea, Professor of Chemistry, (NSYSU); Fellow of Royal Society of Chemistry; Director of Rapid Screening Research Center for Toxicology and Biomedicine at NSYSU (Education: BS, 1981, Nat'l Chung-Hsing University; MS, 1988, Montana State University; PhD, 1991, Montana State University; Postdoctoral, Penn. State University, 1991). Advisory or editorial board member of Journal of the American Society Mass Spectrometry, Clinical Mass Spectrometry, International Journal of Mass Spectrometry, Mass Spectrometry, Mass Spectrometry Let., Cur. chromatography, and Technology. Special Clinica Chimica Acta, Mass Spectrometry, Journal Food Drugs Analysis, and Rapid Communications Mass Spectrometry Research interests: (1) Developing novel ambient ionization techniques and applying them to on-site detection of chemical compounds regarding food safety, antiterrorism, anti-doping, forensic science, and environmental chemistry; (2) interfacing mass spectrometryMS with HPTLC, high-, and gas chromatography; and (3) applying modern mass spectrometryMS for clinical diagnoses.

PL-06

High-performance thin-layer chromatography - Do we really know it and how to make it more popular
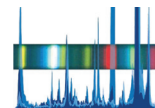
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High-performance thin-layer chromatography (HPTLC) is not a new technique but is a well-established powerful chromatographic technique, which is unfortunately often overlooked. However, in the "era of fast and faster," some HPTLC users are not aware of the details that influence the results. Therefore, the results can be false or even irreproducible. HPTLC is also unique - not only because the separation results in an image but also because it enables a variety of *in situ* bioactivity testing under the umbrella of the so-called effect-directed analyses. The fact is that the potential of HPTLC has not been fully explored. Detection techniques (e.g., image analysis, densitometry, and mass spectrometry) that can be used before or after post-chromatographic derivatization provide a lot of data about the analytes and other compounds present in the chromatograms (in the sample). However, currently available instrumental and software setup - even when upgraded with chemometrics - cannot combine all collected data for efficient evaluation. HPTLC can also easily provide complementary data to other chromatographic techniques and is for those who know it an indispensable tool. We will present the potential of HPTLC (from one dimensional to multidimensional) combined with different detections (ultraviolet, vis, fluorescence, and mass spectrometry [MS]/[MS]) before and after derivatization in targeted and non-targeted analyses of the different group of compounds using different stationary phases. The influence of the sorbent, pre-developing, and developing solvents on stability of the analytes (before and after development) and ion suppression in HPTLC-MS and HPTLC-MS/MS analyses will also be discussed. An innovative simultaneous hyphenation of HPTLC with a triple quadrupole and an ion trap mass analyzer that enabled a reliable and straightforward non-targeted characterization of the analytes will also be presented.

Irena Vovk: B.Sc. (1989), M.Sc. (1993), and D.Sc., 1998, in chemistry at the University of Ljubljana (Slovenia). Since 2008 head of the Department of Food Chemistry at the National Institute of Chemistry in Ljubljana. Research interests: Determination, isolation, and identification of biologically active natural compounds (polyphenols, carotenoids, triterpenoids, etc.) from plant materials and food supplements by chromatographic and hyphenated techniques, as well as the development of new chromatographic methods for the determination of compounds of the interest for food and pharmaceutical industry.

OP-01

Comprehensive high-performance thin-layer chromatography fingerprinting offers alternatives to the marker approach for quality control of herbal materials

Eike Reich

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In monographs, pharmacopoeias prescribe a suite of tests for identity, purity, potency, and content of one or more constituents (and many other characters) to ensure the quality of herbal materials. In reality, and to cut costs, the focus often lies on high-performance liquid chromatography assays of marker compounds to establish potency or minimum content. Such model fits single compound pharmaceuticals and botanicals with constituents of known therapeutic activity. However, for most herbal materials active constituents are not known. As alternative, "analytical" markers, typically selected for quantitative description of manufacturing processes, are assayed instead and thus become associated with quality of a material even though their percentage in the material is small. The concept "comprehensive high-performance thin-layer chromatography (HPTLC) fingerprinting" combines image data generated during the identification of herbal materials and quantitative information available after converting such data into peak profiles. This way qualitative and quantitative analysis can be performed on one set of data, thus by eliminating a separate experiment for the assay, reducing the overall analytical cost. Through several case studies, the paper investigates the possibilities offered by "comprehensive HPTLC fingerprinting" for linking the identity of material with its amount at hand (minimum content). Those include:

- Absolute quantification or minimum content test of known constituents as in conventional assays.
- Expressing content of one or more markers, individually or as a sum parameter, against a common reference substance.
- Quantitative assessment of peak profiles relative to calibration standards.

A suitable validation of the individual approaches assures that samples passing acceptance criteria of comprehensive HPTLC fingerprinting will also meet the criteria of the typical assay.

Eike Reich received his doctorate degree in Natural Products Chemistry in 1989 from Humboldt University Berlin. From 1990 to 1998, he was Associate Professor of Chemistry at Longwood College, Virginia, USA. In 1998, he moved to Switzerland to join CAMAG as Head of Laboratory. His research focuses on applications of high-performance thin-layer chromatography, particularly in the field of botanicals. Dr. Reich has published numerous papers on the subject and contributed chapters to textbooks and encyclopedias. He is author of the book "HPTLC for the analysis of medicinal plants." He is founding member and since 2018 President of the International Association for the Advancement of HPTLC.

OP-02

High-performance thin-layer chromatography authentication of multioriginated Chinese herbal medicines

Zhengtao Wang

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[1]*Shanghai R&D Center for Standardization of Chinese Medicines, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai, China,* [2]*The MOE Key Laboratory of Standardization of Chinese Medicines, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai, China.*

Traditional Chinese medicine (TCM) herbs are derived from medicinal plants, as well as animals and minerals. As a source of natural medicines, the ecological environments, the collecting and agricultural practices, even the drying, storage, and shipping processes may greatly affect their quality. Furthermore, many TCM herbs are genetically multioriginated or confused by substitutes/adulterants due to the morphological similarity resources shortage. All in all, quality evaluation and standards development of TCM herbs are the key point to ensure the safety and effectiveness in clinical application. With the development of advanced analytical instruments and technologies, chromatographic such as thin-layer chromatography (TLC), high-performance liquid chromatography, and gas chromatography, spectroscopic including ultraviolet, infrared, mass spectrometry, and nuclear magnetic resonance, and the hyphenated techniques are all extensively used in the quality evaluation of TCM herbs. Among them, TLC, as one of the most conventional chromatographic techniques, has been used for the identification of TCMs since Chinese Pharmacopoeia 1977 edition. Not only chemical reference substance but also "the reference crude drugs" have been introduced to some selected Chinese crude drugs and patent medicines (formulated preparations) in TLC identification for enhancing its specificity since Chinese Pharmacopoeia 1990 edition.In our present study, a combination of high-performance thin-layer chromatography (HPTLC) and biological activity screening and natural products chemistry was applied for the identification and isolation of the marker substances, then the HPTLC methods established and validated for the authentication of TCM crude drugs of agarwood of *Aquilaria,* herb of *Swertia*, and seeds of *Cruciferae* plants such as *Descurainia, Lepidium,* Sinapis, and Brassica species.

Zhengtao Wang, Liaoning College of Traditional Chinese Medicine (B.Sc., 1982), Shenyang Pharmaceutical University (M.Sc., 1984), China Pharmaceutical University (Ph.D., 1989). Professor, China Pharmaceutical University, Department of Pharmacognosy (1989–2013). At present, Chair Professor, Shanghai University of Traditional Chinese Medicine, Head, Institute of Chinese Materia Medica; Director, MOE Key Laboratory of Standardization of Chinese Medicines, and Shanghai R&D Center for Standardization of Chinese Medicines. Research fields: Quality evaluation and standardization of traditional Chinese medicines and natural products; resources of medicinal plants; and natural products chemistry.

OP-03

Using extractive reference substance for the quality control of Chinese herbal medicines by High-performance thin-layer chromatography image analysis

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The high-performance thin-layer chromatography (HPTLC) technology needs promotion and innovation in China. In colleges and universities, there are no professional courses for HPTLC technology. The levels of different users are seriously polarized. The promotion of HPTLC technology is very necessary. Nowadays, the HPTLC image can be easily scanned, compared with the HPLC analysis, the most unique feature of HPTLC is the dimension of color, which means that HPTLC has the advantages that cannot be replaced by HPLC in image recognition. Combined with the computer intelligent technology, the efficiency of TLC identification for drug analysis will be greatly improved, especially in traditional Chinese medicine (TCM) and natural medicine fields. This might be the new innovation of HPTLC technology. Extractive reference substances (ERS) from traditional Chinese medicinal herbs are one of the research hotspots in recent years. It is a standard reference substance for the comprehensive analysis of TCM. It can be used for both identification and quantification of TCM. There are four basic criterions for ERS (ASCS): Authenticity (identify the source of the crude drug as the staring material); specificity (highly match the chromatographic pattern with the source herbal drug); consistency (keep the well consistence between different batches of ERS products); and stability (accepted stability of all elements in the ERS within the validated period. HPTLC analysis can play the important role for QC of the ERS during the total process. The dedicated picture-like HPTLC image reawakens to pay close attention to the once-ignored unique feature of the image itself in which the detected components are illustrated in detail. Moreover, a simple but professional investigation can easily distinguish the true or false, assess the bad from the good of the testing samples more effective than awkward literal description. It is showing that monitoring the quality of herbal's ERS within the overall process based on the benchmark of ASCS, HPTLC image analysis works well. ERS can play the important role during the promotion and innovation process of HPTLC technology. The ERS is the material foundation of the TCM quality standard. The HPTLC image is the database of the TCM quality standard. The combination of these two technologies will have a far-reaching impact on the comprehensive quality control of TCM.
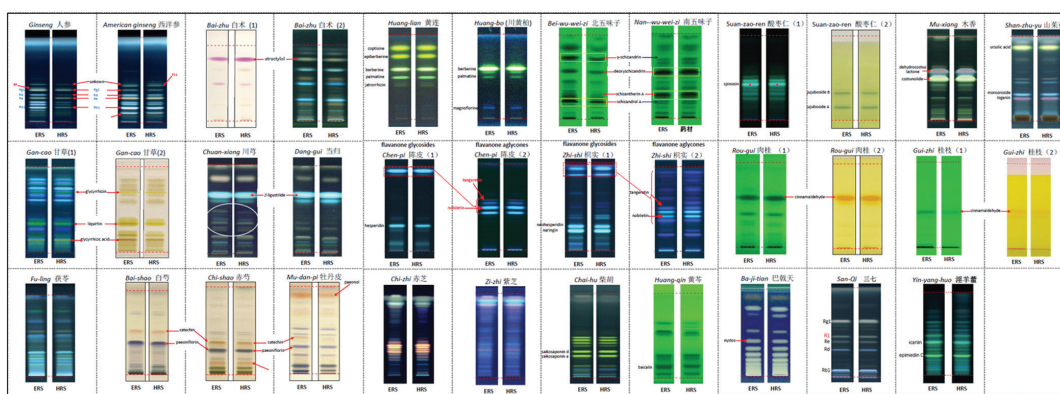
Figure 1: The atlas collection of high-performance thin-layer chromatography images of the herbal extractive reference substance (ERS) (lab-made ERS) with the corresponding herbal drugs

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OP-04

Comparability spectroscopic and chromatographic fingerprint analysis of *Gelsemium elegans*

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Fourier transform infrared spectroscopic (FT-IR) tri-step identification, nuclear magnetic resonance (NMR), high-performance thin-layer chromatography (HPTLC), and ultra performance liquid chromatography–tandem mass spectrometer (UPLC-MS) are methods that are widely used for the quality control of herbal medicines. Recent advances show that a combination of these methods can create an overall chemical profile for each herb. The purpose of this study was to compare different analytical methods with *Gelsemium elegans* from Fujian and Guangxi province as the object of study to distinguish between the stem, leaf, and root as they contained different amount of indole alkaloid that contributed to its toxicity. The spectroscopic and chromatographic fingerprint of the plant was studied by all four instruments, and different parts of *G. elegans* can be successfully distinguished by the four instruments. Besides that, the most abundant active compound present in *G. elegans*, koumine was qualitatively studied in HPTLC and UPLC-MS. Besides that, HPTLC and UPLC-MS were developed for quantitative determination of koumine in *G. elegans*. Both methods showed good precision, recovery, stability, and repeatability. The content of koumine in *G. elegans* measured by HPTLC and UPLC-MS showed positive correlation. To have a well-resolute comparison, multivariate analysis and principal component analysis (PCA) were performed based on the data obtained by FT-IR and NMR to discriminate the three parts. Through this study, all three parts of *G. elegans* were successfully identified and discriminated through the spectroscopic and chromatographic fingerprint, and PCA of all the instruments studied. It can be concluded that the discrimination of different parts of *G. elegans* should be performed from various angle including, fingerprints, quantification, and multivariate analysis, to provide a more conclusive outcome.
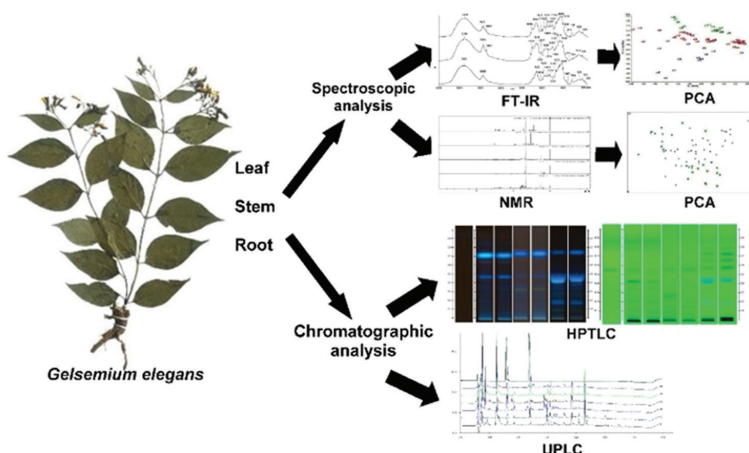
Mun Fei Yam, Universiti Putra Malaysia (B.Sc. (Hons), 2001), Universiti Sains Malaysia (M.Sc., 2006; Ph.D., 2012), Fujian University of Traditional Chinese Medicine, China (B.Med., 2012; Doctor of Medicine, 2016). Currently appointed as senior lecturer in School of Pharmaceutical Sciences, Universiti Sains Malaysia. Research filed: Pharmacology, cardiovascular research, pain research, TCM fingerprint, and acupuncture.

OP-05

High-performance thin-layer chromatography fingerprints based rapid chemotaxonomic discrimination of different *Clerodendrum* species using chemometric data analysis

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Clerodendrum viscosum is mentioned in classical *Ayurveda* like "*Samhitas* and *Nighantus*" for the treatment of fever, pain, dysentery, colic, and piles. *Clerodendrum* species differentiation has always been debatable and challenging due to their morphological and phytochemical similarity. Chemometric analyses along with chromatographic techniques are widely used in the chemotaxonomic classification and chromatographic profiling. Chemometric analysis coupled to validated high-performance thin-layer chromatography (HPTLC) profile of four key biogenic chemicals is used to distinguish three closely related *Clerodendrum* species, namely *Clerodendrum inerme*, *Clerodendrum multiflorum*, and *Clerodendrum viscosum*. PRISMA approach has resulted in effective HPTLC fingerprint with optimum separation and selectivity of key secondary metabolites in all three species. This study not only provides simultaneous quantitation of biogenic markers but also gives scope to established taxonomic differentiation by fingerprint-based similarity analysis, a chemotaxonomic study using hierarchical clustering analysis (HCA), and principal component analysis (PCA). The link of HPTLC and chemometrics analysis provides a rapid approach for defining the similarities and dissimilarities in different Clerodendrum spp. HPTLC method provides rapid and sensitive quantitation of key biogenic markers (one diterpene and three triterpenoids) of *Clerodendrum* species produced by diverse biosynthetic processes. Distribution of these metabolites has assisted in defining *Clerodendrum* species using with PCA loadings. To the best of our knowledge, it is the first report on the application of PCA and HCA with HPTLC for the distinction of *Clerodendrum* species for therapeutic uses. Chemometric analysis is successfully applied for distinguishing and identification of *Clerodendrum* spp. based on HPTLC fingerprints of four key metabolites.

Madumita Srivastava, CCS University (B.Sc., 2006), Banasthali Vidyapeeth University (M.Sc., 2008), CSIR-CIMAP, India (Ph.D., 2016-present) as an ICMR-Senior Research Fellow in Dr. Karuna Shanker's laboratory, Analytical Chemistry Department, CSIR-CIMAP, Lucknow, India. Research fields: Applications of chromatographic techniques in quality evaluation, fingerprinting and marker-based standardization of medicinal plants, isolation of natural products. Email: madhumitasrivastava05@gmail.com

OP-06

Development of simplified high-performance thin-layer chromatography solvent systems for Thai medicinal plants

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Thailand has a long history of using medicinal plant that has considerable pharmaceutical potential. At present, a lot of medicinal plants are in demand as important raw materials in pharmaceuticals and cosmetics. Identification of herbs is the main requisite to ensure the accuracy and quality of raw materials before production. Chromatographic and fingerprint analysis plays an important role in the quality control of medicinal plants. High-performance thin-layer chromatography (HPTLC) is a technique of choice to identify the raw materials in the production of herbal products because this method is efficient, fast, and reliable, thus reducing the time and quantity of sample. However, there is a myriad of plants using various developing systems making it difficult to perform the experiments. Herein, we aim to develop simplified HPTLC developing systems for various medicinal plants, ultimately reducing the performance time. In the present study, 24 plants frequently presented in Thai herbal products were analyzed. The plants were grouped on the basis of plant family and compound structures present in them. The groups included *Zingiber, Curcuma, Senna, Phyllantus, Centella, Acanthaceae,* and *Piperaceae* family and the plant containing terpenes/flavonoids compounds. The sample preparations were tested by a protocol suggested by the HPTLC association and Thai herbal pharmacopoeia (THP). The HPTLC Association method is preferred because it is simpler and faster than THP method. The development systems were also tested using both methods. At the present, nine separation conditions were successfully developed to separate each group. Plants in the same group can be identified and differentiated from each other using a single system. This development could help in the reduction of time and cost to perform the analysis for quality control and standardization of herbs.

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OP-07

Thin-liquid chromatography screening and effect-directed analysis of chosen plants used in dementia treatment

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Plants are valuable sources of biologically active compounds used in pharmaceutical preparations and drugs. Still, there are thousand plant components of biological properties to be revealed, for example, using effect-directed detection (EDD) such as thin layer chromatography combined with direct bioautography (TLC-DB). TLC-DB is used to search for biologically active compounds even in very complex matrices. It can be based on any biological effect, for example, antibacterial, antifungal, antioxidant, antiestrogenic, or enzyme inhibition. The compounds detected by EDD should be further identified using spectroscopic methods, predominantly mass spectrometry, which can be carried out directly on a TLC plate. Supplementation with herbal preparations may reduce the risk of many diseases including those associated with the senility. *Schisandra chinensis*, *Rhodiola rosea*, *Ginkgo biloba*, *Yerba mate,* and *Ginseng* are known for their adaptogenic and anti-dementia properties. They are rich in antioxidants and acetylcholinesterase (AChE) inhibitors. AChE inhibitors are the largest group of medicines used to treat the early stages of Alzheimer's disease. The search for new compounds from this group is currently particularly important. Thin-layer chromatography (TLC) with biodetection based on an enzymatic test is a method of choice in searching new anti-Alzheimer drugs. It was proved that among the aforementioned plants, *S. chinensis* is the most abounded in AChE inhibitors while *R. rosea* and *Y. mate* extracts have very strong antioxidant properties.

Short CV*:* Dr. Hab. Irena Choma, Prof. UMCS. Chromatographic Method Department, Faculty of Chemistry, Maria Curie-Sklodowska University. Researcher and Academic Teacher. Fields of interest: HPLC, TLC, EDA, and food and plant analysis.

OP-08

A novel high-performance thin-layer chromatography-hyphenated bioautography method for identification of carbohydrate-digesting enzyme inhibitory metabolites

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In Indian traditional system of medicine, the flower of *Butea monosperma* Lam. has been various pharmacotherapeutic effects. The aim of our study was to identify the antidiabetic metabolites. Aqueous extract of *B. monosperma* flower was prepared and subjected to polarity- and metabolite-based fractionations. The prepared fractions were screened for antidiabetic and antioxidant activity through *in vitro* approach. Hypoglycemic properties of the best bioactive fractions were tested in streptozotocin (STZ)-induced diabetes in rats and high-fat diet-fed-induced (HFD) Type 2 diabetes in mice. A new method has been developed for the screening of metabolites responsible for the inhibition of α-amylase activity. New high-performance thin-layer chromatography (HPTLC)-based bioautography method has been developed to identify antioxidant and antidiabetic metabolites in glycoside enriched fraction. Polarity-based four fractions (hexane, ethyl acetate, dichloromethane, and water) and three metabolite-based fractions (alkaloid, glycoside, and phenols) were prepared. Glycoside enriched fraction was found to be the best antidiabetic fraction as compared to the other fractions. Glycoside enriched fraction was separated in HPTLC silica gel 60 plate using toluene and ethyl acetate (7:2, v/v). A new, simple bioautography method has been developed and validated for the identification of α-amylase and β-glucosidase inhibitory metabolites. Aurone (R_F 0.43), lupinine (R_F 0.26), and otosenine (R_F 0.14) showed antioxidant and α-amylase inhibitory potential while butrin (R_F 0.46) showed antioxidant and β-glucosidase inhibitory activity. Metabolites separated at R_F 0.57 and identified as butrin showed antioxidant, α-amylase, and β-glucosidase inhibitory activities. In STZ-induced diabetic rats, a dose of 25 mg/kg produces a maximum fall of 47.86% in acute effect, whereas, in chronic effect, it was 44.5% as compared to toxic control. Whereas, a complete reversal of diabetes was observed in HFD-induced diabetic mice at a dose of 45 mg/kg. The level of fasting blood glucose, lipid profiles, and antioxidant marker enzymes was significantly ($P < 0.05$) ameliorated in both HFD and STZ experimental model. The newly developed bioautography method can be used for the identification of antidiabetic metabolites and glycoside enriched fraction in low dose was effective in both types of diabetes.

Dr. Sayeed Ahmad (B.Pharm, 2000, M.Pharm, 2002, and Ph.D, in 2005, from Jamia Hamdard, New Delhi, in Pharmacognosy and Phytochemistry) honored with the University Gold Medal 2002, DST Fast Track Young Scientist Award (2007), CST-UP Young Scientist Award (2008-2009), DST BOYSCAST, AICTE Career Award (2009-2010), as well as UGC Research Award 2016 and SFE Young Ethnopharmacologist Award, 2017, and various best paper awards for his contribution in Natural Product Research. He did his postdoc from Albert Einstein College of Medicine, New York, USA (2011), also worked as Visiting Scientist in Sultan Qaboos University, Muscat, Oman (2012), as well as invited as expert pharmacognosist at University of Khartoum Sudan and National Medicinal and Poison Board, Sudan (2017 and 2018). He is currently working as Assistant Professor and in charge Bioactive Natural Product Laboratory at the Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi. His field of interest in research is metabolomics, chromatographic analysis including HPTLC, HPLC, and GCMS for targeted and untargeted metabolites in biological systems and quality control.

OP-09

Effect-directed identification of estrogenic substances in food contact materials with high-performance thin-layer chromatography-bioassays and high-resolution mass spectrometry

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Alan Bergmann, Daniel Olbrich, Teresa Mairinger, Eszter Simon, Juliane Hollender, Andreas Schönborn

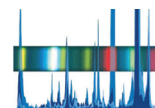*Etienne Vermeirssen Ecotox Center Eawag- École Polytechnique Fédérale de Lausanne, Dübendorf, Canton of Zürich, Switzerland.*

Materials used in food packaging (food contact materials [FCMs]) may impart chemicals to food that are hazardous to human health. Non-intentionally added substances, for example, manufacturing byproducts, are of particular interest because they are largely unidentified and can occur in complex mixtures. To protect public health, manufacturers and regulators need sensitive methods for detecting hazards in FCM and identifying the responsible toxicants. To this end, we are developing effect-directed analysis (EDA) approaches with high-performance thin-layer chromatography (HPTLC) for the identification of estrogenic substances in FCM. We used a planar-yeast estrogen screen [1] to detect native estrogen activity in food simulant migrates of food cans with plastic resin lining. We performed EDA by extracting active zones from parallel HPTLC plates with a TLC–mass spectrometry (MS) elution head and measuring with liquid chromatography (LC)-high-resolution mass spectrometry (HRMS)/MS. With 30 chemicals representative of (xeno)estrogens from FCM, we developed an LC-HRMS/MS acquisition method and a non-target screening workflow to identify suspect toxicants in FCM migrates. We evaluated the procedure with migrates spiked with the known (xeno)estrogens bisphenol A (BPA), estrone, and benzophenone-3 (BP-3). Recovery of spiked chemicals extracted from active zones in parallel plates was about 50%. The spiked chemicals BPA and BP-3 were identified in active fractions, while detecting estrone was limited by the difference in sensitivity between the bioassay and chemical analysis. Potentially responsible toxicants identified in natively bioactive FCM will also be discussed. This work demonstrates the capabilities of HPTLC-EDA by successfully identifying spiked chemicals in a complex mixture and prioritizing unknown chemicals as potentially responsible toxicants in FCM.

REFERENCE

1. Schoenborn A, Schmid P, Bram S, Reifferscheid G, Ohlig M, Buchinger S. Unprecedented sensitivity of the planar yeast estrogen screen by using a spray-on technology. J Chromatogr A 2017;1530:185-91.

Alan James Bergmann, Western Washington University (B.Sc., 2011), Oregon State University (Ph.D., 2017), presently, postdoctoral researcher in the Swiss Centre for Applied Ecotoxicology. Research fields: Bio- and chemical- analytical evaluation of environmental samples and consumer products; Identification of toxicants in complex mixtures.

OP-10

High-performance thin-layer chromatography-effect-directed analysis-UV/vis/FLD-heated electrospray ionization-high resolution mass spectrometer for tracking of bioactive metabolites in *Primula boveana*

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Primula boveana (Primulaceae) is an endangered endemic plant to Sinai peninsula in Egypt. Mostly, endemic plants grow indefinite area(s) with a little number of populations. Hence, relying on traditional methods for their chemical and/or biological investigation is practically inefficient since it requires relatively large quantities of plant material. Hence, for such threatened plants, effect-directed analysis (EAD) linked to high-performance thin-layer chromatography (HPTLC) offers an ideal solution for a material saving (as less as few milligrams) biological/biochemical screening.In the present study, antimicrobial and inhibitory activities of the less polar extract of *Primula boveana* leaves on *Aliivibrio fischeri* and *Bacillus subtilis* bacteria, as well as acetyl/butyrylcholinesterase, were studied directly on the developed HPTLC plate [Figure 1]. Detected bioactive compounds were structurally characterized by HESI-HRMS and UV/vis/FLD analysis. The most intense acetylcholinesterase inhibition zone was compared with rivastigmine as a standard inhibitor. The results showed that the apparent multi-inhibitory activity traced to the presence of flavone. Three further active metabolites were tentatively assigned as hydroxy, methoxy, and tetramethoxyflavones.
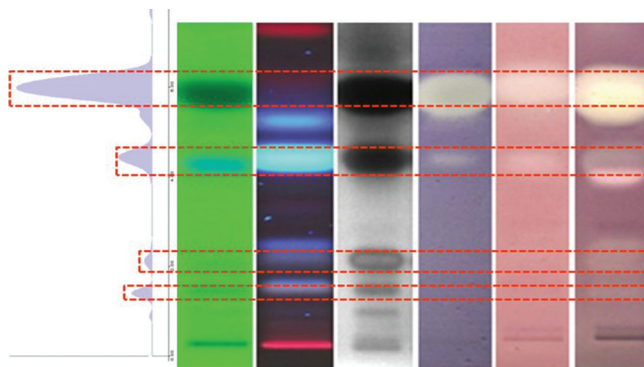
Figure 1: High-performance thin-layer chromatography densitogram at 254 nm, chromatograms at UV 254/366 nm, as well as *Aliivibrio fischeri*, *Bacillus subtilis*, butyryl, and acetylcholinesterase autograms of Sinai primrose leaf extract.

Ehab Mahran, Philipps-Universität Marburg, and Justus Liebig Univ., Germany (Ph.D. researcher, 2016 - till present), Claflin University, USA (Research Associate, 2015–2016), Al-Azhar Univ., Egypt (M.Sc., 2013), Al-Azhar Univ., Egypt (B.Sc., 2006). Research interests: Isolation and identification of bioactive metabolites from plants; utilization of mass spectrometry and NMR as powerful tools for structure elucidation of compounds.

OP-11

Why is it necessary to use high-performance thin-layer chromatography in active pharmaceutical ingredients manufacturing process? A review of case studies

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High-performance thin-layer chromatography (HPTLC) is for sure the fastest method to start and get a quick result, and any sample can be applied automatically on the plate with nearly no limitations in terms of sample type. The capabilities of detection through staining which may complete the direct UV analysis give sometimes information which other methods do not. These advantages of HPTLC versus other chromatographic techniques are well known. Through three real examples where HPTLC has been favored by the analysts over the other techniques, details will be given why this choice was made. The first example is an esterification follow-up where the goal was to set up and determine the best process. The speed and the simplicity of the method were a key element. Stability of the compounds orientated the choice on a CN plate. The second example is an environmental problem. An antibiotic manufacturing process was requested to validate that less than a limit of 2.5 ppm, but HPTLC was sensitive until 1 ppm (mg/L). The matrix effect was also acceptable and do not need sample preparation. The third example is a fermentation process follow-up. The HPTLC method was retained as the first choice because impurities were hardly detected without a staining by phosphomolybdic reagent. This method was also chosen because also of the easy migration of polar compounds, which are too much retained in RP phase. Moreover, finally, no sample preparation was needed. HPTLC has in the environment of active pharmaceutical ingredients manufacturing plants a good role to play. Main arguments for this method are speed and easiness in the laboratory as in the workshop of the plant, low risk of breaks and low cost, adaptability of the method to the final analytical goal, and no matrix effect which enables analysis of raw samples.

Pierre Bernard-Savary is currently president and founder of the Club de CCM and Chromacim SAS. He started to study chromatography at Merck in the 80s. From 1992, he went in charge of TLC plates and instruments for this group in France as product manager until his resignation in 2001. In 1998, he founded the HPTLC French Club and a training center on HPTLC. In 2001, he was elected by the committee of the Interlaken Symposia series as chairman and succeeded to Alfred Studer, Nestlé. He founded Chromacim SAS in 2003 for the exclusive sales of CAMAG equipment. He organized from the 90s then over 40 French meetings and seven international meetings dedicated to HPTLC. He promotes in France, and worldwide, this HPTLC method for over 20 years.

OP-12

High-performance thin-layer chromatography assay methods validation: Will ICH meet FDA/EMA/USP?

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Since the 90s and the publication of ICH Q2 guideline on analytical methods validation, the classical assay methods validation designs usually include the assessment of specificity, precision, linearity, and accuracy. Unfortunately, modern high-performance thin-layer chromatography (HPTLC) assay methods may require other calibration functions. Moreover, separating precision and accuracy studies make it impossible to simultaneously assess trueness and precision, which is, by definition, accuracy. To avoid these pitfalls, the use of a balanced validation design has already been described for pharmaceutical industry[1-3] and is included in some standards for foodstuff[4] and environmental[5] analysis. This validation design is perfectly suited for the assessment of the calibration function by means of back-calculated concentrations statistical analysis, whatever is the chosen calibration function, and of accuracy with the estimation of the so-called prediction intervals and/or content tolerance intervals. Furthermore, it has been demonstrated that measurement uncertainty can be directly obtained from prediction intervals information.[4,6] Using several HPTLC methods validation data and results, we will point out the limits of the ICH Q2 requirements and show how the more recently published recommendations and standards can provide the analyst with complete accuracy assessment and uncertainty of measurement estimation. Does this mean that the most recent recommendations are in opposition to the "old" ICH Q2? Certainly not and we demonstrate that, for instance, the new USP <1210> chapter[7] proposal can take the best from the information given by a classical method validation design. Moreover, we may be confident in the next future since the announcement in June 2018 of the revision of ICH Q2 guideline. Finally, we show that HPTLC assay methods validation can be done in an easy and convenient way, with a very reasonable number of analysis, as far as steady intermediate precision conditions are met during this process.

REFERENCES

1. Hubert P, Nguyen-Huu JJ, Boulanger B, Chapuzet E, Chiap P, Cohen N, et al. Harmonization of strategies for the validation of quantitative analytical procedures: A SFSTP proposal-part I. J Pharm Biomed Anal 2004;36:579-86.
2. USP PF. <1210> Statistical Tools for Procedure Validation; 2014.
3. EMA. Guideline on Bioanalytical Method Validation; 2012.
4. NF V03-110. Analysis of Agri-Foodstuffs - Protocol of Characterization for the Validation of a Quantitative Method of Analysis by Construction of an Accuracy Profile; 2010.
5. NF T90-210. Water Quality - Protocol for the Initial Method Performance Assessment in a Laboratory; 2009.
6. Roussel JM, Botalla S, Beltramo JL, Belveze A, Berthier M, Legras Y, et al. Measurement uncertainty of the analytical methods in drug monitoring. Commission SFSTP. STP Pharm Prat 2017;27:171-248.
7. USP 41. <1210> Statistical Tools for Procedure Validation; 2018.

Jean-Marc Roussel, Aix-Marseille Univ. (M.Sc., 1984 and Ph.D., 1988). At present, Independent Consultant on Assay Methods Development and Validation. President of the French Society for Pharmaceutical Sciences (SFSTP) commission on Uncertainty of Measurement. Co-Designer of NeoLiCy®, software for Analytical Methods' Life Cycle Statistical Assessment.

OP-13

Use of biomarkers as quality control indices for ayurvedic formulations

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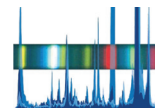sunitashailajan@gmail.com

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Herbal drugs, singularly or in the form of formulations, contain a myriad of compounds. These compounds synergistically contribute to the overall efficacy of the formulation. This creates a challenge in establishing quality control standards for raw materials and standardization of finished herbal drugs. Moreover, as these formulations are easily available over the counter and marketed under the claims of being a cosmaceutical and nutraceutical, standardization of this formulation, and assurance of authenticity of the ingredients used holds prime importance. The variation, thus, caused by the use of improper ingredients or adulteration can bring about serious ill effects. *Pushyanuga churna* is one of such non-hormonal herbomineral formulations which consist of 25 medicinal plants and one mineral drug. Ayurvedic Formulary of India prescribes it for various female reproductive disorders and is being prepared and marketed by various manufacturers such as Dabur, Baidyanath, Arkashala, and by local Vaidyas. The presence of potent biomarkers such as ursolic acid, beta-sitosterol, lupeol, berberine, and quercetin has been reported from PC; hence, in the current research work, the method has been validated as per the ICH guidelines and applied to estimate the content of these markers from various marketed formulations of PC in comparison to the in-house formulation. Further, high-performance thin-layer chromatography (HPTLC) fingerprints were developed for all the formulations using a simple toluene-methanol mobile phase separation was achieved on silica gel 60 F_{254} HPTLC plates, and a fingerprint of the individual ingredients was also developed. Densitometric quantitation was done at 254 and 366 nm using CAMAG TLC Scanner 4 and photodocumentation was done using CAMAG Reprostar 3 at 366 nm. These HPTLC-based fingerprint methods form a simple, quick, and a reliable tool to detect and eliminate adulteration and the use of substandard ingredients in the preparation of PC. The data generated from the chromatographic analysis not only supports the first report of these markers from PC but also can be used as a simple and rapid method to authenticate the ingredients on the basis of the marker content.

Prof. Dr. Irena Shailajan (Dean, Research, Ramnarain Ruia Autonomous College, Mumbai, India). Area of specialization: Quality evaluation of traditional formulations using HPTLC and HPLC techniques, pharmacological (toxicity and efficacy), and pharmacokinetics. Patents: 02 (generated from DST project).

OP-15

High-performance thin-layer chromatography-fluorescent densitometry-surface-enhanced raman spectroscopy determination of folic acid with ozone-induced fluorescence

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Folic acid (FA) played irreplaceable roles in many physiological reactions of normal metabolism. Moreover, it had been evidenced that remarkably higher risks of many diseases such as cancer were tightly related to chronic insufficiency of dietary FA. Concerning the malnutrition of FA represented a worldwide problem, the ability to quickly screen FA in food/feed was urgently desired. Herein, a high-performance thin-layer chromatography-fluorescent densitometry-surface-enhanced Raman spectroscopy (HPTLC-FLD-SERS) method tailored for the sensitive and confirmative screening of FA was established. For the first time, ozone gas was used for the visualization of separation results on HPTLC plates, which gifted strong fluorescence to the parent molecules of FA. After that, FLD measurements were carried out for quantitative purpose, offering linearity $R^2 > 0.999$, LOD within 0.07-0.35 mg/kg. In addition, the applicability and reliability of the established method were validated with real food and feed samples, which agreed well with conventional HPLC-UV benchmark. Apart from that, the obtained SERS fingerprint of bands under optimized conditions gave unambiguous identification of the targeted compound, serving as a efficient tool to prevent potential mistake by false positive results [Figure 1].
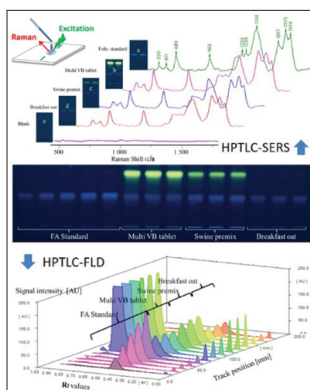
Figure 1: Schematic illustration of high-performance thin-layer chromatography-fluorescent densitometry- surface-enhanced Raman spectroscopy hyphenation

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OP-16

High-performance thin-layer chromatography method development and validation for simultaneous evaluation of phenolic acids and flavonoids to study the processing effects in different millet grains

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Millet grains are one of the important minor cereals grown extensively in the tropics which have been serving as the staple food for the low-income groups in some countries of the world. Several scientific evidence have proved that they help in reducing the risk of chronic diseases including cancer and heart disease. Hence, there is a need to optimize selective and sensitive methodologies using sophisticated analytical equipment to accurately quantify the levels of bioactive compounds such as phenolic acids and flavonoids in millet grains. In the present study, the different processing effects on the bioactive compounds were evaluated in different millet grains, namely proso millet, Kodo millet, and barnyard millet. The phenolic acids and flavonoids were identified and quantified using high-performance thin-layer chromatography (HPTLC), wherein the effect of thermal processing (roasting) on these bioactive compounds was studied, which was compared between the raw dehulled grains and roasted grains. Extraction process of phenolic compounds in the grain samples was done using ethyl alcohol (70% v/v). Method validation was done for the parameters: Linearity, precision, and accuracy. The four-point linear calibration curves were linear in the range of 250–1000 ng for linearity. The precision and accuracy of the method were assessed by calculating the relative standard deviation percentage of the assay results of six different standard concentrations each in three replicates. HPTLC was performed on silica gel 60 F_{254} (0.25 mm) aluminum backed TLC plates as stationary phase with automatic sample applicator using the 10 μl microsyringe. The chromatograms were developed using the mobile phase of the solvents; ethyl acetate, toluene, formic acid, and methanol in the ratio of 6:6:1.6:0.4 v/v. The spectral detection was performed using TLC scanner with scanning from 200 to 700 nm, the λmax for individual phenolic compounds were further evaluated based on their absorption maxima. Post-chromatographic derivatization was done with natural product reagent by spraying, and the R_F values obtained for reference standards were compared with the R_F values of the bands for the sample extracts. The peak height and area were utilized for evaluation and quantification of phenolic acids and flavonoids. Hence, this HPTLC method developed for evaluating phenolics in millet grains to study the processing effects is a very simple, precise, and accurate comparatively.

OP-17

High-performance thin-layer chromatography: The perfect analytical tool for food analysis
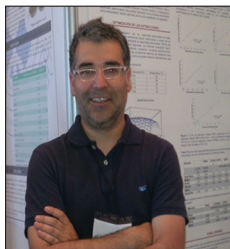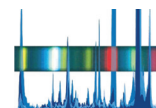
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Nowadays, bioactive molecules detection is an important task in food analysis. Several analytical techniques have been applied to identify this kind of molecules, but each one has critical drawbacks. In this scenario, high-performance thin-layer chromatography (HPTLC) emerges as the perfect analytical tool for bioactive molecules detection. This presentation describes the detection and identification of two types of bioactive molecules, that is, functional ingredients and food contaminants. For the first case, we will show the application of effect-directed analysis HPTLC/bioassay/mass spectrometry to detect and identify food biologically active components that exert healthy effects beyond of intrinsic nutritional effect, which may reduce the risk of suffer chronic non-communicable diseases (CNCD). These pathologies are characterized for showing long duration and slow progression. The most relevant ones are cardiovascular, cancer, diabetes, and chronic respiratory diseases. It is estimated that CNCD is responsible for 38 million of deaths per year, which represents 68% of all deaths worldwide. For the second case, we will show a new fast and selective method for biogenic amines determination in wines and beers. Biogenic amines are bioactive compounds with relevant physiological activities, but its consumption at high concentrations may produce several problems for human health, for example, headaches, hypo- or hyper-tension, kidney failure, anaphylactic shock, and even death. It will show how improvements of sample preparation and chromatographic conditions allow relevant reductions in analysis time (ca. 88%). With this new method, only one development is required to selectively separate eight dansylated amines, that is, tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine, in wines, and beers.

Professor Dr. Mario Aranda, University of Concepcion, Chile (Pharmacist, 2000), University of Concepcion (M.Sc., 2005), University Hohenheim, Germany (Ph.D., 2008). Presently, Professor, University of Concepcion, Faculty of Pharmacy. Research fields: Functional foods and food safety. His research is focused in detection and evaluation of bioactive molecules with potential use against chronic non-communicable diseases as well as those which can represent a risk for human health.

OP-18

HPTLC-based method development for cardiovascular disease controlling compounds containing plant Coleus forskohlii and comparison of separation efficiency of two different HPTLC plates

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Coleus forskohlii is an important indigenous medicinal plant of India. The plant extract has been used in traditional Ayurvedic medicine for curing various disorders. The plant is reported to have several bioactive compounds including the diterpenoid forskolin. Forskolin is useful in the treatment of congestive heart failure, glaucoma, asthma, and certain type of cancers. Ultrasonication technique was used for the extraction of the samples at 40°C for 30 min in three different solvents. In comparison to ethyl acetate and chloroform, acetonitrile was found to be the better solvent for the extraction of forskolin, 9-deoxy-forskolin, 1,9-dideoxy-forskolin, and deacetyl forskolin. The benzene-ethyl acetate (17:3 v/v) solvent system was used for the separation of forskolin from other compounds. High-performance thin-layer chromatography (HPTLC) plate was derivatized with anisaldehyde-sulphuric acid derivatizing agent followed by heating at 105°C for 3–5 min. Two different types of plates were investigated, whereby HPTLC LiChrospher Si $60F_{254s}$ was preferred to HPTLC Si $60F_{254s}$ plates due to better separation efficiency. The developed method will be used for quality control of raw materials and finished products.
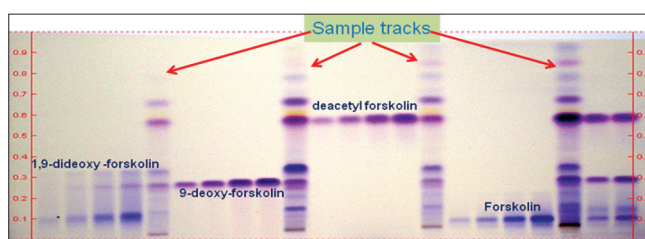
Figure 1: High-performance thin-layer chromatography LiChrospher Si 60F254s

Dr. Ajai Prakash Gupta CIMAP, INDIA (Ph.D., 1998), Presently, I am working as Senior Technical Officer and Assistant Professor AcSIR (Chemical Science) in Quality control and Quality Assurance at Indian Institute of Integrative Medicine, (IIIM-CSIR), Jammu. His expertise includes method development, validation, and analysis of tissue culture samples, bioactive constitutions of microbes and medicinal plants using LC–MS/MS. The area of expertise also includes pharmokinetics and pharmodynamics (PKPD) studies, drug metabolites, and mechanistic study through HPLC, HPTLC, and LC–MS/MS. He is also involved in quality control and quality assurance of cGMP grade herbal formulations and monograph preparations for various pharmacopoeias. I am also recognized Jammu and Kashmir FDA approved Quality Control Person.

OP-19

Simple interface for scanning chemical compounds on developed thin-layer chromatography plates using electrospray ionization mass spectrometry

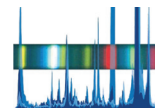**Sychyi Cheng**

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Suhail Muzaffar Bhat, Sychyi Cheng

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Thin-layer chromatography (TLC) has combined with electrospray ionization mass spectrometry (ESI/MS) to characterize a mixture. A V-shaped piece that held analyte spots was cut out of an aluminum-based TLC plate. A solvent and high DC voltage was applied on the V-shaped piece to elute analytes toward the sharpened tip at which an ESI plume was induced. Although the technique was useful to generate analyte ions for the following MS detection, it was laborious to cut the entire TLC track to V-shaped pieces for analysis. Therefore, the technique was commonly used to analyze selected sample spots, and the chemical information of unselected spots was lost. This study developed a simple TLC-ESI/MS interface to scan the developed TLC plates. A zigzag cut scissors were used to rapidly cut the developed TLC track into two sawtooth pieces that were positioned on an XYZ stage so that one of the triangular tips was pointed toward the MS inlet. A drop of methanol and high DC voltage was applied at the tip to induce ESI. After the analytes in the first tip were analyzed, the TLC piece was moved so that the second triangular tip was pointed toward the MS inlet for analysis. The process was repeated until all the triangular tips on the piece were analyzed. In this manner, the analytes, no matter visible or non-visible bands, were scanned and characterized. Since 4.8 cm long TLC track was cut to 32 triangles on two sawtooth pieces for analysis, the spatial resolution of using the sawtooth TLC-ESI/MS for analysis is 1.5 mm/band. A mixture of dye standards and plant extracts were analyzed to demonstrate the capability of sawtooth TLC-ESI/MS on scanning and characterizing chemical compounds on the TLC plates. The limits of detection of the dye standards were between 0.25 and 2.5 ng/band.

Suhail Muzaffar Bhat received his master's degree at Barkatullah University in 2012. He is now a Ph.D. student in the Department of Chemistry at National Sun Yat-Sen University (NSYSU). His research focuses on (i) the development of flame-induced atmospheric pressure chemical ionization (FAPCI) for ambient MS analysis and (ii) coupling TLC to MS for analyses of food dyes and drugs.

OP-20

Target-based method development using effect directed thin-layer chromatography - A straightforward process design for the identification and isolation of bioactive natural products

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Thin-layer chromatography (TLC) is still by far the most important technique to isolate and identify novel bioactive compounds from natural sources. It is easy to use, offers the possibility to detect specific groups of compounds through post-chromatographic derivatization or staining and can be combined with identification tools such as mass spectrometry and bioautography. In addition, the separation conditions, which have been optimized on the TLC plate, can be transferred directly to the preparative liquid chromatography process to obtain the compound of interest in larger amounts. We will show examples for the straightforward process design starting with the identification of bioactive compounds using TLC, optimizing the separation by appropriate choices of the solvent system and the transfer of the separation to preparative liquid chromatography. After the transfer from the TLC plate to the column, we will also show the possibilities of scaling up to preparative liquid chromatography. We will show the advantages of bioautographic tests on TLC plates to identify unknown compounds with specific physiological activities during the method development and optimize the separation conditions for a further scaling up to preparative liquid chromatography for isolation.

Michaela Oberle R&D Project leader and specialist for Thin-layer Chromatography within Instrumental analytics at Merck KGaA, Darmstadt, Germany. Bachelor of Science in Business Chemistry (Fresenius Hochschule Idstein, 2014). Joint Merck in 1992 and took over responsibilities in various departments as a specialist for analytical, preparative and flash chromatography; extraction and analysis of natural compounds; cosmetic efficacy screenings including skin and hair applications; product development, customer requests, TLC applications, and international trainings for TLC issues.

OP- 21

High-performance thin-layer chromatography-mass spectrometry and mass spectrometry prepartive in API clinical batches manufacturing

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[1]Oril Industrie, Bolbec, France, [2]Chromacim Sas and Club de CCM, Pommiers la Placette, France.

Clinical batches manufacturing is a very challenging domain where competition is always very hard to reduce as much as possible the time of the production of a batch of a pure compound which is expected to have pharmacologic interesting properties without negative effects on health. The time to bring a molecule on the market is always very long and reduce of the time at any steps is very valuable. This is the reason why Oril chose a very straight and robust method to achieve both the best throughput and purity of compound possible. Two aspects will be discussed: The production yield of the whole process to get the necessary amount of pure product for early-stage testing and the purity and reliability of the obtained compound. With these needs, thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) are coupled with preparative chromatography and TLC/HPTLC–mass spectrometry (MS) is very complementary to the setup of this manufacturing process. The method will be described and especially how to use the TLC separation method development to transfer directly to columns up to 450 mm diameter. Once the purification is obtained, all the fractions are controlled through an automated HPTLC analysis for saving time, doing the analysis right in the workshop and having an absolute safety on the obtained quality. On TLC, one sees always everything. Any problem with process which is only controlled by U/HPLC is, therefore, checked on TLC–MS. Not only the problem is then solved for future processes but the workshop may also isolate any unexpected compound. The benefit of this approach is at several steps of the whole process which is a key in the development of a new drug. Due to the cost of the separated compound, this approach is also very valuable for Oril company which is now ready to share is long-lasting experience with the third parties as CRO-CDMO for 1 year.

Pierre Bernard-Savary is currently president and founder of the Club de CCM and Chromacim SAS. He started to study chromatography at Merck in the 80s. From 1992, he went in charge of TLC plates and instruments for this group in France as product manager until his resignation in 2001. In 1998, he founded the HPTLC French Club and a training center on HPTLC. In 2001, he was elected by the committee of the Interlaken Symposia series as chairman and succeeded to Alfred Studer, Nestlé. He founded Chromacim SAS in 2003 for the exclusive sales of CAMAG equipment. He organized from the 90s then over 40 French meetings and seven international meetings dedicated to HPTLC. He promotes in France, and worldwide, this HPTLC method for over 20 years.

OP-22

Development of a universal set of system suitability test standards for high-performance thin-layer chromatography

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High-performance thin-layer chromatography (HPTLC), a powerful, yet simple, and rapid analytical technique is constrained due to the large variation in results, due to its thermodynamically open nature. Standardization of the methodology, e.g., by accepting parameters in accordance with the United States Pharmacopoeia (USP) chapter/Pharmacopoeia Europaea (Ph. Eur.) chapter 2.8.25 makes HPTLC significantly more reproducible. A system suitability test (SST), which is an integral part of gas and liquid chromatographic methods, verifies that the resolution and reproducibility of the chromatographic system are adequate for the analysis at hand. Therefore, the SST is now also incorporated in all HPTLC methods of the USP and Ph. Eur. It is typically based on two reference substances which have close Rf values but are still separated. From a practical point of view, the SST must qualify the chromatographic system and does not necessarily have to utilize compounds that are expected in the samples analyzed with the corresponding method. A study was designed to find a set of substances for an universal SST, from which during method development a subset can be selected to qualify the chromatographic system. The standards had to be inexpensive, easily available, stable, well detectable under ultraviolet (UV) 254 nm and UV 366 nm, and finally not restricted. 117 substances were evaluated in three mobile phase with different polarity and selectivity. Of those 27 standards of mixed origin, i.e., synthetic and herbal were selected. Examples were elaborated to demonstrate the usefulness of the concept [Figure 1].
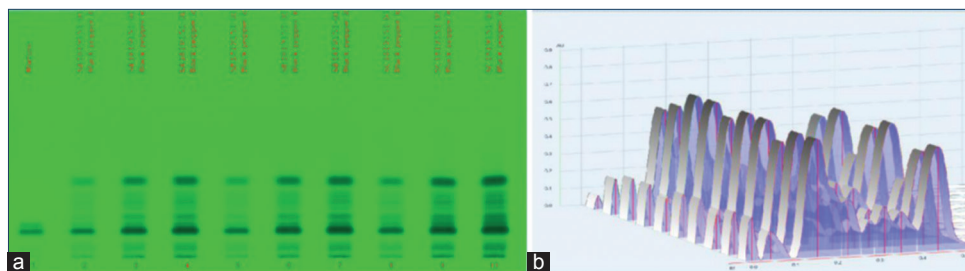
Figure 1: (a) Black pepper fingerprint track I-2 system suitability test standards track 3–10 black pepper sample 2, 57 μl. (b) Three-dimensional of chromatogram - black pepper fingerprint

Manjusha Phanse - B.sc., 2003 (Pune University), M.Sc., 2005 (Pune University), India. University of Mumbai (Ph.D., 2018), Worked as research fellow at Agharkar Research Institute, Pune, and S.P. College, Pune, India (2005–2010). Research field: Standardization of herbal products and nutraceuticals.

OP-23

Validated thin-layer chromatography densitometric method for simultaneous quantification of six terpenoids in *Putranjiva roxburghii* - A plant used in Indian system of medicine

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Sonali Mishra, Karuna Shanker

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Putranjiva (*Putranjiva roxburghii* Wall., syn. *Drypetes roxburghii* family- Euphorbiaceae) is an Indian medicinal plant and widely grown all over Asia. In traditional system of medicine, it is used for azoospermia, diuretic, ophthalmopathy, catarrh, and constipation. The objective of the present study is to develop and validate a method for the standardization of *Putranjiva* bark and its dried products for quality assurance. Six characteristics terpenes 3-oxo-25-norfriedel-9(11)-ene, methyl putranjivate, putric acid, Friedlin, Friedlin-3,7-dione, and 3β-acetoxycycloart-24-en-23-one were isolated from Putranjiva bark. The optimum separation of the six triterpenes was achieved over thin-layer chromatography (TLC) plate using hexane:ethyl-acetate (90:10, v/v). The densitometric analysis at 425 nm after derivatization with vanillin-sulphuric acid was sensitive (the limit of detection: 0.71–2.68 ng/spot; limit of quantification: 2.47–8.94 ng/spot). Linear regression analysis data for the calibration plot of the compounds showed linear relationship (r^2 = 0.9981–0.9981) in the concentration range of 4 ng–20 ng per spot. The established method showed good precision and accuracy with overall intra- and inter-day variations of 1.61–2.97% and 1.74–2.42%, respectively, and overall recoveries of 93.35–97.43% for the six compounds analyzed. The method was applied to evaluate the quality of *Putranjiva* samples to select elite chemotype. A simple, rapid, selective, and quantitative high-performance TLC (HPTLC) method was validated for sensitivity, linearity, accuracy, precision, specificity, and recovery as per the International Conference on Harmonization guidelines for the quantification of six characteristic analytical markers in the *Putranjiva* bark.

Sonali Mishra, Lucknow University (B.Sc., 2012), Integral University (M.Sc., 2014), CSIR-CIMAP, India (Ph.D., 2015-present) as a DST-INSPIRE Senior Research Fellow in Dr. Karuna Shanker's laboratory, Analytical Chemistry Department, CSIR-CIMAP, Lucknow, India. Research fields: Applications of chromatographic techniques in quality evaluation, fingerprinting, and marker-based standardization of medicinal plants, isolation of natural products. Email: snlmishra631@gmail.com

PP-01

Establishment of a unified thin-layer chromatography identification method for *Sinapis semen*, *Raphani semen*, *Descurainiae semen*, and *Lepidii semen* and separation of its main specific components

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The establishment of existing quality standards for traditional Chinese medicines is basically based on the establishment of qualitative or quantitative analysis of one (or some) component of traditional Chinese medicines. The significance of establishing the quality standard of traditional Chinese medicine lies in the identification of "true or false" and "good or bad," while the premise of "good or bad" is "true or false," "variety is wrong, it is all wrong." The search for "special components" cannot be limited to the medicinal materials themselves. The common components of the same genus and even the same family plants are the majority, and only in comparison can the "exclusive" be embodied. This study attempted to establish a uniform thin-layer chromatography (TLC) identification method, which is suitable for the qualitative identification of the same family plants. Taking the cruciferous seed medicinal materials collected in the Chinese Pharmacopoeia 2015 as an example, including Sinapis semen, which include the seeds of *Sinapis alba* L. (SS) and the seeds of *Brassica juncea* (L.) Czern. et Coss. (BS), *Raphani semen* (RS), *Descurainiae semen* (DS), and *Lepidii semen* (LS). The TLC method in the pharmacopoeia could not be used to identify these herbs effectively, and the reference substances of SS and RS were both Sinapine thiocyanate, which was also found in BS, DS, and LS. Sinapine thiocyanate is not specific to them. In this study, the TLC method includes adding 20 ml of methanol to 2 g of the powder, ultrasonicate for 30 min, cool, filter, evaporate to dryness, and then dissolve the residue in 1 ml of methanol as the test solution, a mixture of ethyl acetate, formic acid, and water (10:1.8:1.5) as the developing solvent on HPTLC silica gel F_{254} precoated plate and a 10% solution of sulfuric acid in ethanol as the chromogenic agent, then heating at 105°C for 3 min and examining under ultraviolet light at 254 nm before derivatization, at 365 nm after derivatization. Under the condition of the TLC method, their specific identification points were separated by Sephadex LH-20 and preparative liquid chromatography and identified by nuclear magnetic resonance and MS, which provided an effective reference value for the study of the quality standards of these medicinal materials [Figure 1].
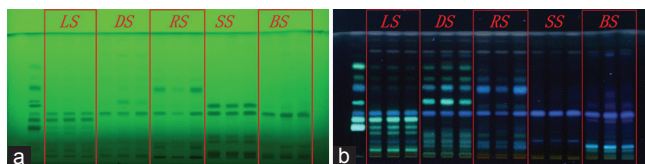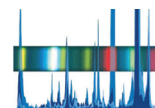

Figure 1: HPTLC chemical profiles of various samples viewed under 254nm (a) and 366nm (b).

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PP-02

A harmonized high-performance thin-layer chromatography analysis method of Ginseng, American ginseng, and Notoginseng

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Moo-Seob Kim[1,2], Li-Hua Gu[1,2], Zheng-Tao Wang[1,2]

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For thousands of years, ginseng, American ginseng, and notoginseng have been widely used in the world. Especially, they are important traditional herbal materials in Asia. They are similar in morphology and many common components, but their pharmacological effects are different and the main efficacy of them comes from ginsenoside. In terms of marker components, sample extraction method, or test condition, the criteria of them for identification are different from various pharmacopoeias or enterprises. It is difficult and plagued by the market circulation and quality evaluation. So far, there is no quick and accurate unified method to identify them. In addition, the present thin-layer chromatography (TLC) methods cannot identify these herbs effectively. Hence, it is very important to identify them precisely for ensuring the safety of medication.Hence, the goal of this study is to establish a common high-performance thin-layer chromatography (HPTLC) method for simultaneous identification of them. First, three methods of extracting medicinal materials were unified. Then, it is run one new HPTLC development system. After all, the improved HPTLC identification method is so simple and quick to operate and efficient for the verification compared to that of before [Figure 1].
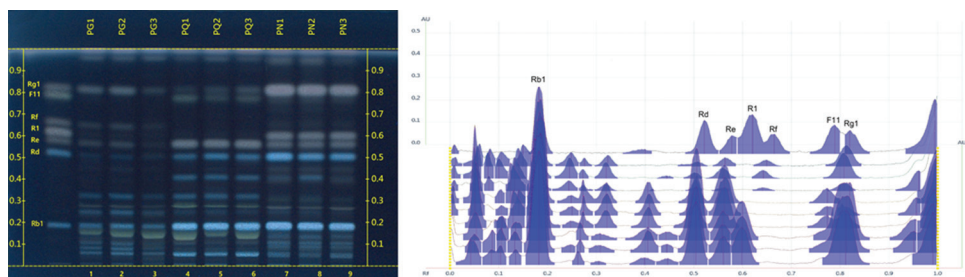
Figure 1: High-performance thin-layer chromatography analysis of ginseng (1-3), American ginseng (4-6), and notoginseng (7-9)

Moo-Seob Kim, Beijing University of Chinese Medicine (B.Sc., 2012), Beijing University of Chinese Medicine (M.Sc., 2015). Presently, Ph.D., Shanghai University of Traditional Chinese Medicine, China. Research fields: Chemical composition and activity of botanicals and traditional herbal medicines; research on international standardization of Asian ginseng and American ginseng and notoginseng.

PP-03

Improvement of thin-layer chromatography authentication methods of four Chinese medicinal materials

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Thin-layer chromatography (TLC) methods have become routine methods for quality evaluation of herbal medicines and applied in national monographs worldwide. However, some of TLC conditions for herbs were non-specific or provided poor chemical information. In this study, the HPTLC methods of four herbs recorded in Chinese Pharmacopoeia (*ChP*), namely *Tinosporae radix* [Figure 1a], *Dioscoreae rhizoma* [Figure 1b], *Trachelospermi caulis et Folium* [Figure 1c], and Phragmitis rhizoma [Figure 1d], were revised and validated. *As the marker substance of T. radix, the band column was covered with other constituents in the TLC identification as described in ChP (2015 Ed), the mobile phase has been revised as the upper layer of the mixture of n-hexane, ethyl acetate, methanol, and strong ammonia TS (8:9:2:1), the Rf (=0.52) was more moderate and the resolution was optimized. D. rhizoma is a commonly used herb, but no specific marker was identified until our study. A systematic chemical investigation led to the discovery of a specific bibenzyl derivative batatasin, which is used as the marker to establish TLC authentication. As for Trachelospermi Caulis et Folium and P. rhizoma, the derivatization reagents have been changed to a 10% solution of sulfuric acid in ethanol to provide more characteristic bands on the TLC images. The revised TLC authentication methods for these four herbs have been validated and hopefully accepted by the new version of ChP.*
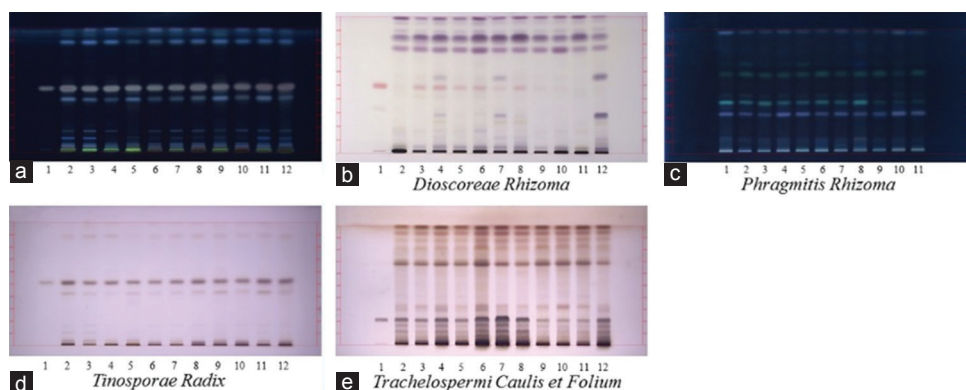
Figure 1: (a) *Tinosporae radix,* (b) *Dioscoreae rhizoma,* (c) *Trachelospermi caulis et folium,* (d) *Phragmitis rhizoma*

Rui Rong Zheng, Shanghai Univ. of Traditional Chinese Medicine. (B.Sc., 2015). At present, Ph. D student, Shanghai Univ. of Traditional Chinese Medicine, China. Research fields: Chemical composition and activity of botanicals and traditional herbal medicines; Research on searching natural compounds with anti-benign prostatic hyperplasia activities.

PP-04

Comparison of chemical constituents between green and brown *Forsythiae fructus*
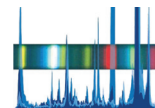
Xia Li

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Forsythiae fructus is the dried fruits of *Forsythia suspensa* (Thunb.) Vahl., and it mainly contains benzo sides, lignans, and terpenoids. *F. fructus* was divided to green *F. fructus* (known as "Qingqiao") and brown *F. fructus* (known as "Laoqiao") according to harvest times, both of them were considered different in clinic application due to difference of chemical constituents. Here, based on studies of chemical constituents of fruits of *F. suspense*, we focused on analyzing the chemical differentiation of Qingqiao and Laoqiao by high-performance thin-layer chromatography (HPTLC). Two HPTLC methods were established according to polarity of chemical constituents. The test solution was prepared with 0.5 g of the drug powder in 5 ml of methanol, ultrasonicate for 30 min and filter. The markers were dissolved in methanol, respectively, to produce 1 mg/ml of reference solutions. 5 μl of each of the test solutions and 4 μl of each of the reference solutions were applied in bands. After developing and removal of the solvent, the plates were sprayed with a 10% solution of sulfuric acid heated at 105°C until the bands clear and examined in daylight. For detecting 4 benzo sides forsythoside A, (+)-pinoresinol-β-D-glucoside, cornoside, and forsythin, the mixture of cyclohexane, ethyl acetate, and formic acid (7:2:0.2) was used as the mobile phase; detecting 6 lignans and terpenoids, pinoresinol, phillygenin, ursolic acid, betulinic acid, β-sitosterol, and dammar-24-ene-3β-acetate-20S-ol, the mixture of chloroform, ethyl acetate, methanol, and formic acid (25:5:10:1) was used as the mobile phase. The comprehensive results showed that the quality of Qingqiao was much higher than Laoqiao.

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PP-05

A specific high-performance thin-layer chromatography method for authentication of *Swertia mileensis* and its allied species
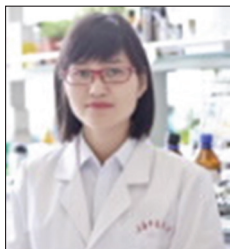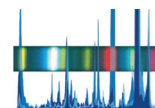
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The whole herb of *Swertia mileensis* Ho and Shih is a common Chinese herb widely used in both prescribed and herbal compound preparation for its hepatoprotective activities. However, in recent years, the scarcity of the resources of *S. mileensis* leads to confusing use of various allied species. Based on systematic pharmacognostical and chemical studies, the high-performance thin-layer chromatography (HPTLC) analysis method was established for authenticating various Swertia species in this study. To look for characteristic constituents, *S. mileensis* and its allied species plants were treated in parallel. Their methanol extracts were dried and dissolved in water then transferred into a manual MCI solid phase extraction column (GEL CHP 20P, 75~150 mm, 2 g, and column inner diameter 1.5 mm), eluted with 20 ml of water and discarded, collected successively 80% methanol and methanol eluates, dried and dissolved in 1 ml of methanol as fractions I and II. Two mobile phases chloroform, methanol, water, and formic acid (8:2:0.2:0.2) and chloroform, acetone, and formic acid (30:1:0.2) were selected for fractions I and II, respectively, totally 28 specific bands were presented on HPTLC chromatograms by detecting both under UV_{254} directly and then under Ultraviolet $(UV)_{365}$ after derivatization with 1% $AlCl_3$ reagent. Chemical isolation was quickly carried out by preparative TLC combined with preparative high-performance layer chromatography method based on above specific bands, and ten xanthones compounds were isolated and identified by hydrogen nuclear magnetic resonance and 13C NMR Spectra. Based on above results, a specific HPTLC method was established for authentication of eleven medicinal species derived from the same genus *Swertia* using varied xanthones as markers.

Lihua Gu, Anhui University of TCM. (B.Sc., 2002), Shanghai University of TCM. (M.Sc., 2007; Ph.D., 2017), Visiting scholar in Professor Annie Bligh's laboratory, University of Westminster (2015–2016), At present, Associate Proffesor, Shanghai University. of TCM., Institute of Chinese Materia Medica, and Shanghai R&D Center for Standardization of Chinese Medicines. Research fields: Chemical analysis and quality control of Chinese Herbal Medicines; HPTLC research of Chinese Herbal Medicines.

PP-06

# Role of high-performance thin-layer chromatography in industrial chemotaxonomy

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Accurate plant identification and right quality raw material from the best location of different phytogeographical zones are the foundation of effective usage of plant-based natural health products in herbal industries. Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge are important. The use of chromatographic techniques and marker compounds to standardize botanical preparations has proven industrial usage for commercial exploitation of medicinal diversity, their variable sources and chemical complexity. This has huge opportunity in the area of drug development and discovery, where variation in metabolite content plays an important role. A chemotypic evaluation provides an optimal characterization of botanical materials. This present contribution provides an overview such studies conducted using high-performance thin-layer chromatography as a powerful tool in identifying the best location of quality raw material from different phytogeographical zones of India.
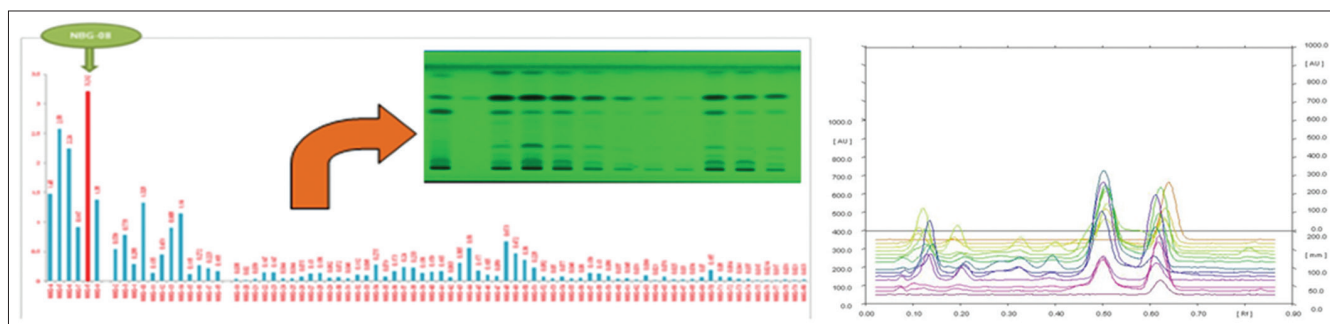
Dr. Sharad Srivastava is Principal Scientist in Pharmacognosy Division at CSIR-National Botanical Research Institute; Lucknow, India. He made significant contributions to quality control of crude drugs/products, chemotaxonomy, bioprospection, and natural product development and have developed quality parameters of single crude drugs (>70 medicinal plants) and also identified biomarkers for their quality control. He contributed 30 monographs of single herbal drugs in *Ayurvedic Pharmacopoeia of India* and identified some elite chemotypes of high-value medicinal plants. He has 137 publications in peer-reviewed journals, 16 patents and developed some technologies/formulations, few has already been transferred to industry.
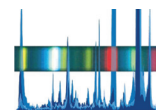
PP-07

High-performance thin-layer chromatography image analysis for assessing the feasibility of the extractive reference substance of Chinese herbal medicines in quality control

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Longgang Guo[1,2], Peishan Xie[1,2]

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Extractive reference substances (ERS) from traditional Chinese medicinal herbs is one of the research hotspots in recent years. It is a standard reference substance for comprehensive analysis of traditional Chinese medicine (TCM). It can be used for both identification and quantification of TCM. There are four basic criterions for ERS (ASCS): Authenticity (identify the source of the crude drug as the staring material); specificity (highly match the chromatographic pattern with the source herbal drug); consistency (Keep the well consistence between different batches of ERS products); and stability (accepted stability of all elements in the ERS within the validated period. High-performance thin-layer chromatography (HPTLC) analysis can play the important role for quality control (QC) of the ERS during the total process. In addition to all of the other benefits, the current HPTLC image elaborated by means of the up-to-date material and sophisticated instruments with the careful standardized operation. Hence, the dedicated picture-like HPTLC image reawakens to pay close attention to the once-ignored unique feature of the image itself in which the detected components are illustrated in detail. Morever, a simple but professional investigation can easily distinguish the true or false, assess the bad from the good of the testing samples more effective than awkward literal description. It is showing that monitoring the quality of herbal's ERS within the overall process based on the benchmark of ASCS, HPTLC image analysis works well. HPTLC analysis is a key technology for the application of ERS. On the other way, the increasing application of ERS will play an important role in promoting HPTLC technology. The combination of these two technologies will have a far-reaching impact on the comprehensive QC of TCM [Figure 1].
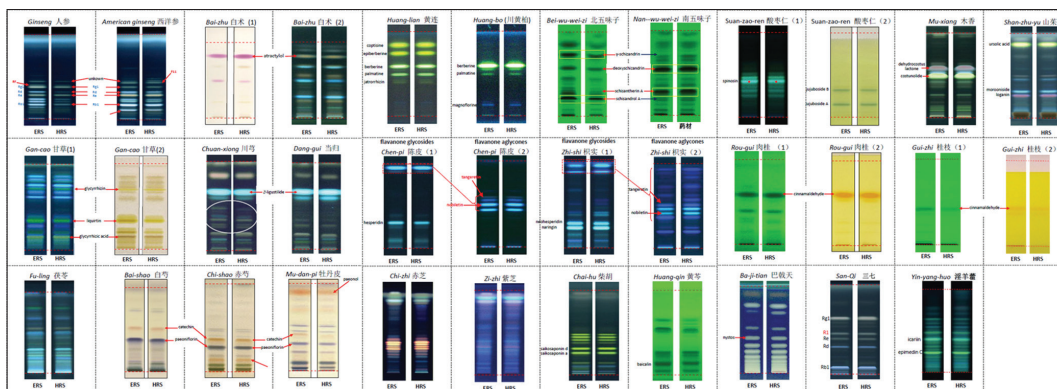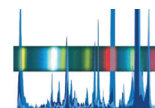

Figure 1: The atlas collection of high-performance thin-layer chromatography images of the herbal extractive reference substance (lab-made extractive reference substances) with the corresponding herbal drugs (Herbal Reference Substance; HRS)

Longgang Guo, Master degree of Pharmaceutical analysis, Sun Yet-Sen University; Director of research and development center of Guangzhou ImVin Pharmaceutical Co. Ltd., Director of Guangdong natural drug Engineering Research Center. Research field: Quality control of traditional Chinese medicine; New drug research of traditional Chinese medicine.

PP-08

Phytochemical screening and high-performance thin-layer chromatography fingerprinting profile of extracts of *Annona squamosa* L.
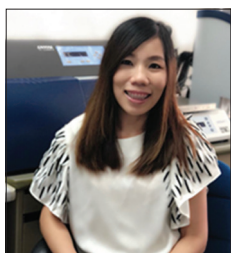
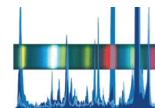**Poachanee Norfun**

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Poachanee Norfun, Panneeka Attanon, Pukwarin Santiteerarod

Agricultural Production Sciences Research and Development Division, Department of Agriculture, Bangkok, Thailand.

The present communication attempts to evaluate the efficacy and the fingerprinting profile of sugar apple (*Annona squamosa* L.) seed extract against *Plutella xylostella* L. In this study, seeds of sugar apple were extracted with methanol, and the crude extract was then separated and identified by high-performance thin-layer chromatography (HPTLC). CAMAG-HPTLC system comprising of a TLC Visualizer and Linomat 5 sample applicator was used in this study. The separation was performed using TLC aluminum plates pre-coated with silica gel 60 F254. Ethyl acetate:methyl tertiary butyl ether:methanol (93:5:2 v/v) was used as mobile phase. The developed plates were visualized under visible, ultraviolet (UV) 254 and 366 nm light. As post-derivatization, the plates were sprayed with Dragendorff's reagent. HPTLC fingerprinting of the methanolic sugar apple seed extract under UV 366 light reveals the presence of five components and marked as F1, F2, F3, F4, and F5 and gives the total of eight different alkaloids. The insecticidal activities of five fractions against *P. xylostella* L. were studied; the results showed that fraction F2 caused the highest mortality. The results indicate that alkaloid with RF 0.57 is an active compound. Therefore, the study revealed that specific qualitative HPTLC data can be used as a diagnostic tool for the correct identification of the plant, and it is useful as a phytochemical marker and also a good estimator for the plant for future standardization work.

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PP-09

High-performance thin-layer chromatography fingerprint profile of insecticidal alkaloids from *Stemona phyllantha* Gagnep.

Nattaporn Chanthasakda
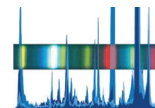
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Stemona phyllantha Gagnep., belonging to the family *Stemonaceae*, is locally known as "Non Tai Yak" and commonly used in Thailand as botanical pesticides. A high-performance thin-layer chromatography (HPTLC) method was developed for a fast analysis of a fingerprint of active substances in *S. phyllantha* Gagnep. *S. phyllantha* Gagnep. roots were sequentially extracted with hexane, dichloromethane, ethyl acetate, and 50% ethanol. These extracts were tested against the 2nd instar diamondback moth (*Plutella xylostella* L.) by leaf dipping method. The highest insecticidal activity was observed from hexane and dichloromethane extract of *S. phyllantha* Gagnep. Preliminary phytochemical screening of crude extracts affecting on diamondback moth (*P. xylostella* L.) death showed the presence of alkaloids group. HPTLC fingerprint of alkaloids was performed by dichloromethane:ethyl acetate:methanol:ammonium hydroxide (50:45:4:0.1 v/v) as mobile phase. Post-chromatographic derivatization was employed by spraying with Dragendorff's reagent to visualize the spots. HPTLC fingerprint of *S. phyllantha* Gagnep. revealed two peaks of alkaloids with RF values of 0.46 and 0.67. This method can be used to identify alkaloids group as a marker of *S. phyllantha* Gagnep. from plant raw material and also other commercial products. It can be developed as a standard method in the future.

Nattaporn Chanthasakda, Chula University (B.Sc., 2011), Chula University (M.Sc., 2014), At present, Researcher, Department of Agriculture, Agricultural Production Sciences Research and Development Division, Agriculture toxic substance research group, Botanical pesticide research subgroup. Research fields: Botanical pesticides.

PP-10

High-performance thin-layer chromatography: A tool for characterizing sandalwood oils
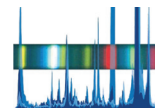
Hayford Ofori

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Hayford Ofori[1,4], Dhanushka Hettiarachchi[2], Tomislav Sostaric[3], Frankie Busetti[1], Mary C. Boyce[1]

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Sandalwood is a hemi-parasitic tree of genus *Santalum* (Family Santalaceae) comprised approximately 17 species from Asia Pacific region. Sandalwood essential oil distilled from heartwood of several *Santalum* species is in high demand as in the perfumery and cosmetic industry. The unique aroma of sandalwood is produced by two sesquiterpene alcohols, namely α-santalol and β-santalol; however, the oil is composed of many other sesquiterpenes and sesquiterpene alcohols, which contribute to the olfactory characteristics for each species. At present, gas chromatography (GC) is the only method used for qualitative and quantitative assessment of sandalwood oil. High-performance thin-layer chromatography (HPTLC) was explored as an alternative to GC and its ability to characterize oils from different sandalwood species. HPTLC solvent system and wavelength were used as the detection method, and chromatographic images were processed and analyzed using HPTLC software (visionCATS, CAMAG). There were variations in the profile of bands (R_F values and color) and peak profiles displayed by the essential oils across and within five sandalwood species with some bands being unique to the individual species. Despite the samples being unique, all the samples had a prominent band at R_F 0.39 (orange) under 366 nm after derivatization and under white light after derivatization. The band at R_F 0.39 was extracted and identified as α-santalol using CAMAG TLC- mass spectrometry (MS) Interface 2 and GC-MS, respectively. A HPLTC fingerprint for sandalwood oil has been established. The ability of HPTLC in identifying unique bands in sandalwood oils from different species was demonstrated in the present study. HPTLC can serve as a quality control tool in authenticating sandalwood oils from adulterated oils.

Hayford Ofori is Univ. of Cape Coast, Ghana (BSc, 2002), Umea Univ., Sweden (MSc, 2006). At present, Ph.D. student, Edith Cowan Univ., Australia, Research fields: Trace metal and aflatoxin contaminants in food, characterization of sandalwood essential oil using chromatographic techniques.

PP-11

Validated thin-layer chromatographic densitometric method for simultaneous quantification of six terpenoids in *Putranjiva roxburghii* - A plant used in Indian system of medicine

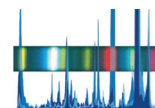**Karuna Shanker**
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Putranjiva (Putranjiva roxburghii Wall, Syn. *Drypetes roxburghii* family - Euphorbiaceae) is an Indian medicinal plant and widely grown all over Asia. In the traditional system of medicine, it is used for azoospermia, diuretic, ophthalmopathy, catarrh, and constipation. The objective of the present study is to develop and validate a method for the standardization of *Putranjiva* bark and its dried products for quality assurance. Six characteristics terpenes 3-oxo-25-norfriedel-9(11)-ene, Methyl putranjivate, Putric acid, Friedlin, Friedlin-3,7-dione, and 3β-acetoxycycloart-24-en-23-one were isolated from Putranjiva bark. The optimum separation of the six triterpenes was achieved over thin-layer chromatographic (TLC) plate using hexane:ethyl-acetate (90:10, v/v). The densitometric analysis at 425 nm after derivatization with vanillin-sulfuric acid was sensitive (the limit of detection: 0.71–2.68 ng/spot), limit of quantification: 2.47–8.94 ng/spot). Linear regression analysis data for the calibration plot of the compounds showed a linear relationship (r^2 = 0.9981–0.9981) in the concentration range of 4–20 ng per spot. The established method showed good precision and accuracy with overall intraday and interday variations of 1.61–2.97% and 1.74–2.42%, respectively, and overall recoveries of 93.35–97.43% for the six compounds analyzed. The method was applied to evaluate the quality of *Putranjiva* samples to select elite chemotype. A simple, rapid, selective, and quantitative high-performance TLC (HPTLC) method was validated for sensitivity, linearity, accuracy, precision, specificity, and recovery as per the International Conference on Harmonization guidelines for the quantification of six characteristic analytical markers in the *Putranjiva* bark.

Sonali Mishra, Lucknow University (B.Sc., 2012), Integral University (M.Sc., 2014), CSIR-CIMAP India (Ph.D., 2015-present) as a DST-INSPIRE Senior Research Fellow in Dr. Karuna Shanker's laboratory, Analytical Chemistry Department, CSIR-CIMAP, Lucknow, India. Research fields: Applications of chromatographic techniques in quality evaluation, fingerprinting, and marker-based standardization of medicinal plants, isolation of natural products. Email: snlmishra631@gmail.com

PP-12

Chemotaxonomic studies on *Gloriosa superba* L. from different phytogeographical zones of India through high-performance thin-layer chromatography

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The study aimed for chemotaxonomic studies on *Gloriosa superba* collected across the country for the identification of high colchicine containing elite chemotype(s). A total of 128 samples (tuber) were collected from seven different phytogeographic regions of India (31 from Gangetic plain(s), 26 from Western and Eastern Himalayas, 9 from Central India, 25 from Western Ghats and Malabar Coast, and 37 from Eastern Ghats). The method was developed and validated on HPTLC precoated silica gel 60 F_{254} plates in tertiary solvent system, consisting of chloroform:acetone:diethylamine (5:4:1). Densitometric quantification at 350 nm reflects the significant variation in colchicine content, varying from 0.0052% to 0.860% (dry wt. basis); maximum content was recorded in NBG-06 from Western Ghats, which is the highest content reported till date in Indian population of *G. superba*. The identified elite chemotype(s) are useful to industry as quality raw material for manufacturing efficacious product as well as quality planting material to promote the commercial cultivation in site-specific locations of elite germplasm. This will provide a source of income generation to farmers and constant supply of quality raw material to industry [Figure 1].
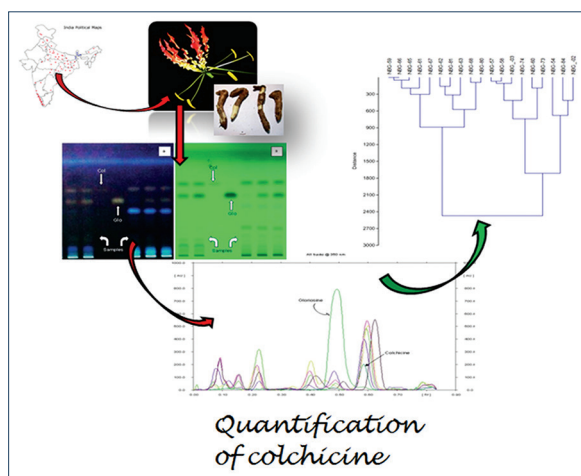
Figure 1: Chemotying of colchicine in *Gloriosa superba*

I am working in the area of Pharmacognosy, specifically on the modeling for new drug development, taking plant and phytochemical compounds as the lead moieties. The current area of research includes collection of herbs from the wild sources, identification, activity guided fractionation, and development of scientifically validated and standardized herbal products for common ailments. These extraction, isolation, and characterization of active markers from plants are done using sophisticated instrumentation techniques such as OPLC, HPLC, HPTLC, and other spectroscopic techniques. I had published 30 original research papers with total impact factor 31.982 in peer-reviewed journals (h index 4 and 64 citations, since 2013), authored two books, two bulletins, and have two patents. One patented technology is ready for commercialization and under negotiation with industry.

PP-13

Quantification of active principle from Nilavembu Kudineer Chooranam - An anti-dengue Siddha formulation by high-performance thin-layer chromatography method

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Nilavembu Kudineer Chooranam is a classical Siddha formulation referred in Siddha Vaidya Thirattu, p. 291 and also enlisted in "The Siddha Formulary of India," Part-I, Government of India, p. 161. Nilavembu Kudineer Chooranam is a polyherbal formulation containing nine plants, namely Andrographis paniculata Nees. (whole plant), Vetiveria zizanioides (Linn.) Nash. (root), Plectranthus vettiveroides Jacob. (root), Santalum album Linn. (heartwood), Trichosanthes cucumerina Linn. (whole plant), Cyperus rotundus Linn. (rhizome), Zingiber officinale Roxb. (rhizome), Piper nigrum Linn. (fruit), and Mollugo cerviana (L.) Ser. (whole plant). This Nilavembu Kudineer Chooranam is a remedy for all types of fever. Furthermore, its efficacy over dengue was well experienced by the affected patients in India, especially in Tamil Nadu in the recent past years. The active principle responsible for the anti-dengue property of Nilavembu Kudineer Chooranam is andrographolide, and hence, authors aimed to estimate the andrographolide present in Nilavembu Kudineer Chooranam. The drug is readily available in the market in the form of coarse powder; however, Kudineer (water extract) is prepared at the time administration and consumption within 3 h as per the advice of the physician. The drug (6–12 g) is added with water (120–240 ml) and boiled to reduce to one-fourth. This is the therapeutic dose recommended based on the age and weight of the patient. Hence, the content of the active principle, andrographolide was estimated in the water extract prepared as per the decoction procedure from Nilavembu Kudineer prepared in the laboratory and four market samples. The extract was applied on the thin-layer chromatography (TLC) plate precoated with silica gel $60F_{254}$. The solvent system used was chloroform:methanol (9:1, v/v). The presence of andrographolide was first ascertained by qualitative TLC, then quantitatively estimated in the Kudineer at 231 nm. The calibration curve for the standard andrographolide in ethanol was obtained for the concentration levels of 2 μg–6 μg. The linear regression equation for the calibration curve obtained was Y=19.5021X+4974.4347 and the correlation coefficient was 0.99852 [Figure 1]. The standard deviation was 1.66% (relative standard deviation 3.439%). The R_F of andrographolide was observed at 0.35.
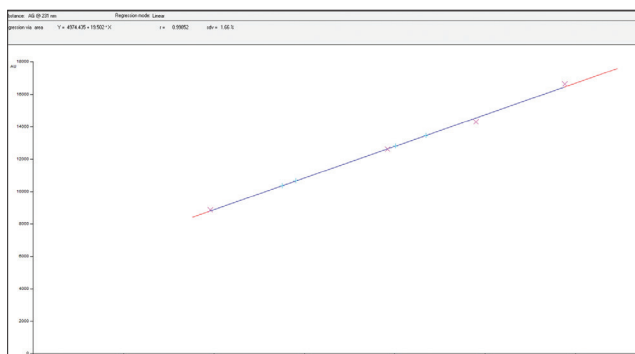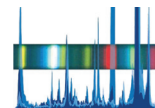

Figure 1: Calibration curve of the standard andrographolide

Shakila R, Madras University (B.Sc., 1994), Anna University (M.Sc., 1996), Alagappa University (M.Phil, 2009), Madras University, India (Ph.D, 2017). At present, Research Officer (Chemistry), Siddha Central Research Institute (Central Council for Research in Siddha, Ministry of AYUSH, Government of India). Research field: Standardization of Siddha formulation, isolation of secondary metabolites, method development, and validation for quantification of chemical markers by different chromatographic techniques.

PP-14

Estimation of bioactive marker compounds of *Baptisia tinctoria* using high-performance thin-layer chromatography
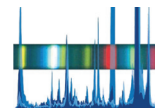
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Standardization of the plant on the basis of bioactive marker is required to ensure its efficacy and safety. High-performance thin-layer chromatography (HPTLC) plays an important role in the estimation of marker compounds in natural products. The method development and validation are quite easy in standardizing plants on the basis of bioactive marker compounds using HPTLC system. Due to wide applicability, efficiency, and reproducibility of results with HPTLC system, it has gained great recognition in the field of natural product analysis. *Baptisia tinctoria* (L.) R. Vent. is one of such traditional plants, which has potential to be developed as anticancer drug. Thus, it was considered worthwhile to standardize plant material on the basis of bioactive marker compounds. Maackiain and trifolirhizin are major bioactive constituents of *B. tinctoria*. An attempt was made to develop an HPTLC method for the estimation of maackiain and trifolirhizin in *B. tinctoria* roots. Linearity of calibration plots of maackiain and trifolirhizin was achieved between 300–800 and 250–1500 ng, respectively. The contents of maackiain and trifolirhizin in plant were found to be 7.95% and 2.74% w/w, respectively. The quantitative HPTLC method was validated for sensitivity, precision, and accuracy for each marker. Limit of detection for maackiain and trifolirhizin was found to be 9 ng and 20 ng, whereas limit of quantification was 35 ng and 68 ng for maackiain and trifolirhizin, respectively. Inter- and intra-day precision was complied with the limits prescribed in ICH guidelines. Average percent recovery was found to be more than 98.70% w/w for maackiain and 99.35% w/w for trifolirhizin.

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PP-15

Application of high-performance thin-layer chromatography in quality assessment of an Ayurvedic drug - *Myrica esculenta* Buch.-Ham ex D. Don
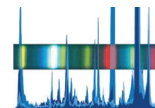
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Myrica esculenta Buch.-Ham ex D. Don (Myricaceae) is commonly known as Katphal. *M. esculenta* is utilized for its bark, flower, fruit, and leaves in Ayurvedic and Unani system of medicine against various ailments such as menorrhagia, asthma, and other menstrual disorders. *M. esculenta* is reported for its innumerable pharmacological activities such as hepatoprotection, antifungal, and anti-inflammatory. Due to the varied application, it is a part of various Ayurvedic formulations such as *Katphaladi churna* and *Pushyanuga churna*. The use of appropriate raw material is necessary to maintain its therapeutic potency and prevent its adulteration. Thus, the aim of the study was to check the quality of *M. esculenta* in terms of bioactive marker using high-performance thin-layer chromatography (HPTLC) technique. In the present research work, the powder sample (1.0 g) was extracted using ethanol (10.0 mL), vortex mixed for a minute and kept on shaker for overnight extraction for 6 h followed by filtration through Whatman filter paper no. 1. Using HPTLC, the chromatographic separation of the phytochemical constituents was achieved on HPTLC plates precoated with silica gel 60 F_{254} (0.2 mm thickness) on aluminum sheet support. Mobile phase, toluene:ethyl acetate:chloroform:formic acid (8:1:0.5:0.3 v/v/v/v) was used to develop the fingerprint. Further, content of gallic acid from *M. esculenta* was estimated using ICH guidelines, toluene:ethyl acetate:formic acid (4:4:1 v/v/v) was used as mobile phase. After development, the plate was dried in a current of air at room temperature. Further, TLC Camag scanner 4 was used for densitometric scanning and the source of radiation was a deuterium lamp (278 nm). All measurements were performed at 22 ± 1°C. Plate was photodocumented using Camag Reprostar 3 under ultraviolet radiation (254 nm). The effect of extraction of plant sample in solvents of varying polarity, various extraction techniques, and different morphological parts in terms of marker content was analyzed. The limit of detection and limit of quantification levels were found to be 8 μg/mL and 10 μg/mL, respectively, with a linear response range of 10–500 μg/mL, linear equation y=127.9x+60.87, and a correlation coefficient $r^2 > 0.99$. Intra- and inter-day precision were within the acceptance limit of 85–115%. The percent recovery was found to be 90%. The concentration of phytochemical marker varied in samples collected from different regions of Uttarakhand. Variation was also observed in the samples extracted using solvents of varying polarity, from different morphological parts, marketed formulation, and using different extraction techniques. The HPTLC fingerprint can be used as quality control tool for the use of authentic sample of *M. esculenta* which is the key ingredients of many herbal formulations. The data can be recommended for the detection of gallic acid from other biological matrices. The variation in the content of marker may be due to the climatic condition.

Ms. Suhina Bhosale is a Ph.D. student in Bioanalytical Sciences at Ramnarain Ruia Autonomous College, affiliated to University of Mumbai, Maharashtra, India. She has completed M.Sc. in the subject of Bioanalytical Sciences at Birla College, University of Mumbai. She is pursuing her Ph.D. and also working as a Senior Research Fellow on the project funded by University Grants Commission, Government of India, in the area of standardization of medicinal plants and evaluation of their therapeutic efficacies. She has two publications and presented her work in this field at various national and international conferences.

PP-16

Standardization of ethyl acetate extracts of *Avicennia marina* and *Sonneratia apetala* using a validated high-performance thin-layer chromatography method
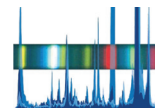
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Mangroves play various important roles in human lives. Recent studies have shown that mangroves possess priceless phytoconstituents which might play a significant role in their traditionally reported therapeutic potential due to constant environmental stress. *Avicennia marina* Forssk. and *Sonneratia apetala* Buch.-Ham. are two common mangroves found around estuarine coasts of India whose therapeutic potential has not been studied extensively, but investigations so far have led to the discovery of several novel compounds with prospective medicinal value for the discovery of new chemotherapeutic agents. Hence, in the present research work, high-performance thin-layer chromatography (HPTLC) method has been developed for the simultaneous estimation of five biomarkers, namely ursolic acid, betulinic acid, betulin, beta-sitosterol, and lupeol, from the standardized ethyl acetate extract of the leaves of these mangroves. Chromatographic separation was achieved on 60F254 HPTLC plate with toluene:acetone (9:1.2, v/v) as mobile phase after double development and derivatization with 10% methanolic sulfuric acid reagent. The method was validated in terms of linearity, precision, accuracy, and sensitivity as per the ICH guidelines. Accuracy was checked by conducting recovery studies and average recovery was found within acceptable limits. Solvent of extraction was optimized using this method and ethyl acetate was chosen as solvent of preference based on the marker content. The total content of these five markers was found to be highest in the sample collected from Airoli (8.648 ± 0.4350 mg/g) and BKC area (8.138 ± 0.1487 mg/g) in the case of *A. marina* and *S. apetala*, respectively. The band pattern established can also be used as fingerprint and may assist in quality evaluation and standardization in the absence of markers. This data can be used to establish a cross talk between chromatographically established phytochemistry and bioassays to provide an assurance of efficacy and consistency of plant extracts.

Dr. Mayuresh Joshi has received his Ph.D degree in Botany under the guidance of Prof. Sunita Shailajan, HOD, Department of Botany, Ramnarain Ruia Autonomous College. At present, he is working as an assistant professor in botany and his topic of research has been the biomarker-based standardization and evidence-based evaluation of hepatoprotective potential of the leaf extracts of two mangroves, namely *A. marina* and *Sonneratia apetala*. He has attended/presented his research works at more than 40 national and international conferences, seminars, and workshops in the past 5 years and has published six papers in peer-reviewed journals.

PP-17

Standardization of herbal drugs by high-performance thin-layer chromatography methods

Kesri N. Sharma

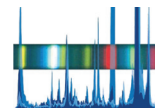sharma_kesri@rediffmail.com

Kesri N. Sharma

Central Drugs Testing Laboratory, Medical and Health Department, Government of Rajasthan.

The therapeutic history of plants is well known over many decades, and plant-derived products are continuously being used in the traditional health-care system. Herbal medicines widely used in health care are complex chemical mixtures extracted and prepared from plants. According to the WHO, about 80% of the world's population is depended on herbs and other traditional medicines for their primary health-care needs. Herbal formulations have reached widespread acceptability as therapeutic agents for curing of diabetics, arthritics, liver diseases, cough remedies, cancer, etc. The efficacy and safety of natural products have turned the major population to shift toward them for pharmaceutical and nutraceuticals demands. Characterization and standardization of herbal drugs comprise sophisticated analytical techniques for the identification and bioassay of active principles. As on date, there is no legal control model over medicinal plants. Different countries claim medicinal plants or natural products derived from different ways and have adopted different approaches for licensing and dispensing to ensure their safety, quality, and efficacy. Fingerprinting of herbal medicines is used for the authentication and quality control of herbal medicines and also for herbal preparations. Chemical fingerprints obtained by chromatographic techniques have become the most efficient tool for the quality control of bioactive compounds. The advancement of analytical techniques serves as an important tool in the herbal research, and it allows the pharma companies/manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory bodies for efficacy, safety, and high shelf life of herbal drugs. High-performance thin-layer chromatography (HPTLC) is a very powerful and versatile chromatographic technique for the separation and analysis of natural products as compared to other techniques such as HPLC, spectrophotometry, and titrimetric. It has ability to analyze crude samples containing multicomponents. The separation process is easy to follow, especially with colored compounds. Several samples can be separated parallel to each other on the same plate, resulting in a high output, time-saving, and a rapid low-cost analysis. Choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step. HPTLC can combine and consequently be used for the different modes of evaluation, allowing identification of compounds having different absorption spectra. HPTLC method can help to minimize the risk of exposure to toxic organic effluents and significantly reduces its disposal problems, consequently reducing environmental pollution.

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PP-18

Importance of high-performance thin-layer chromatography in analysis of homeopathic drug *Datura ferox*
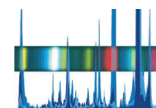
Binit Dwivedi

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High-performance thin-layer chromatography (HPTLC) and UV spectroscopy are an important tool for the qualitative and quantitative phytochemical, chemo profiling, and marker compound analysis of homeopathic drugs/tinctures (HT). According to the phytoequivalence concept, the full HT can be seen as the active compound because several constituents act together being responsible for its therapeutic effect. *Datura* is a genus of 13 species, i.e., *Datura candida*, *Datura ceratocaula*, *Datura discolor*, *Datura dolichocarpa*, *Datura ferox*, *Datura innoxia*, *Datura kymatocarpa*, *Datura leichhardtii*, *Datura metel*, *Datura reburra*, *Datura stramonium*, and *Datura wrightii* of poisonous vespertine flowering plants belonging to the family Solanaceae. They are commonly known as *Daturas*, *D. ferox* in homeopathy frequently used in the treatment of bronchitis in asthma, mental illness, respiratory disorders, attention deficit hyperactivity, and drug detoxification. The present study reveals the limit of determination (4.09%), total ash (1.99%), alcohol extractive values (5.24%), water extractive values (2.94%), total solids (0.52%), wt/ml (0.84 g), and alcohol content (72%). HPTLC analyses of in house HT were performed on silica gel 60 F_{254} precoated plate using chloroform-methanol (9:1, v/v) as mobile phase. Under UV light (254 nm), two spots appear at R_f 0.44, 0.53 (brown). Under U.V (366 nm), five spots appear at R_f 0.36, 0.43, 0.51, 0.58, and 0.81 (blue). After spray with anisaldehyde-sulfuric acid, eight spots appear at R_f 0.36 (light orange), 0.43 (orange), 0.57 (blue), 0.62 (purple), 0.66 (blue), 0.69 (brown), 0.78 (purple), and 0.83 (blue). The present physicochemical and phytochemical data are to be considered as pharmacopoeial standards for aforesaid drug.

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PP-19

Quantification of L-3,4-dihydroxyphenylalanine - An anti-Parkinson's compound from seeds of Mucuna species by high-performance thin-layer chromatography

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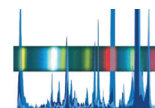[2]ICAR - National Bureau of Plant Genetic Resources, Regional Station Cuttack, Odisha, India.

Mucuna species are an important medicinal plant of India which are the best known natural source of bioactive compound L-3,4-dihydroxyphenylalanine (L-DOPA) used as potential drug for the treatment of Parkinson's disease. The plant is widely distributed throughout India and in other parts of the tropics including Central and South America. In India, 14 species of Mucuna are found in the foothills of the Himalayas, the plains of West Bengal, Madhya Pradesh, Karnataka, Kerala, Andhra Pradesh, Uttar Pradesh, and the Andaman and Nicobar islands. Of these, *Mucuna pruriens*, *Mucuna Monosperma*, and *Mucuna gigantea* are widely distributed all over; while *Mucuna nigricans* is distributed mainly in the North-Eastern parts of India. *M. pruriens* (L.) DC. is widely used in traditional Ayurvedic system of Indian medicine for the management of male fertility, nervous disorders, and as an aphrodisiac. Keeping in view the increasing demand for herbal drugs, importance of this genus, and vast diversity of Mucuna germplasm available in Indian continent, efforts were made to collect diverse germplasm of Mucuna species from wild habitats of the state of Odisha, India. Five species of *Mucuna*, namely *M. monosperma*, *M. nigricans*, *M. gigantea*, *M. pruriens*, and *Mucuna utilis*, collected from wild habitats were analyzed for the presence of L-DOPA by high-performance thin-layer chromatography (HPTLC) method standardized in our laboratory.[1,2] *Mucuna* seed powder (1 g) was refluxed twice with 50 ml 0.1 N HCl on boiling water bath for 30 min and filtered using Whatman filter paper. Total volume of the pooled extract was made 100 ml with 0.1 N HCl for further estimation of L-DOPA. Separation of L-DOPA in seed extracts of *Mucuna* species was done on HPTLC silica gel plates 60F254 (20 cm × 10 cm) purchased from *E. Merck* (Darmstadt, Germany) using mobile phase of n-butanol:acetic acid glacial:water (4:1:1, v/v) saturated for 30 min. Stock solution of standard L-DOPA (1 mg/ml) procured from Sigma chemicals was used in the concentration range of 100–1000 ng/spot. A TLC spot corresponding to pure L-DOPA standard was observed at Rf value of 0.35 ± 0.02. Maximum absorption of compound was observed at 280 nm wavelength under UV and L- DOPA peak was confirmed in seed extract by overlaying spectra of sample with the pure compound spectra. Results of the present studies indicated a wide range of variability in seed size, color, and L-DOPA content among the diverse germplasm collections. Among the five Mucuna species, highest L-DOPA content was found in *M. pruriens* germplasm varying between 4.91% and 7.09%. Other Mucuna species having high L-DOPA were *M. gigantea* (6.76%), *M. nigricans* (6.16%), and *M. monosperma* (4.61%), while *M. utilis* showed least L-DOPA content of 1.22%. Promising accessions with high L-DOPA content (>6%) were identified IC599290, IC599342, IC599336, IC599361, and IC599350. Highest L-DOPA content of 7.09 ± 0.43% was found in black seeds of IC599290. Superior accessions along with other rarely occurring *Mucuna* species can be exploited for large-scale cultivation of this drug plant for pharmaceutical utilization and can be used in future breeding programs for developing high L-DOPA yielding varieties.

REFERENCES

1. Archana RP, Khatri R. Quantitative determination of L-DOPA in seeds of *Mucuna pruriens* germplasm by high performance thin layer chromatography. Indian J Pharm Sci 2011;73:459-62.
2. Archana RP, Tomar JB, Dutta M. Variability in *Mucuna pruriens* germplasm for L-DOPA, an anti parkinsonian agent. Genet Resour Crop Evol 2012;59:1207-12.

Archana P. Raina, H.P University (B.Sc., 1989), Dr. Y.S. Parmar University of Horticulture and Forestry, Solan, H.P (M.Sc., 1991), IARI, Delhi (Ph.D.,1995). At present, Principal Scientist in Biochemistry at ICAR-National Bureau of Plant Genetic Resources, New Delhi-110012. Research Fields: Characterization and evaluation of medicinal and aromatic plants germplasm for quality traits and diversity analysis, development of analytical methods for bioactive compounds in medicinal plants, chemical profiling of medicinal and aromatic plants, and identification of chemotypes and value-rich superior germplasm.

PP-20

HPTLC dual wavelength spectrodensitometric method for the simultaneous quantification of active compounds rosmarinic acid and caffeic acid in homeopathic plants *Rosmarinus officinalis* and *Ocimum gratissimum* as per USP
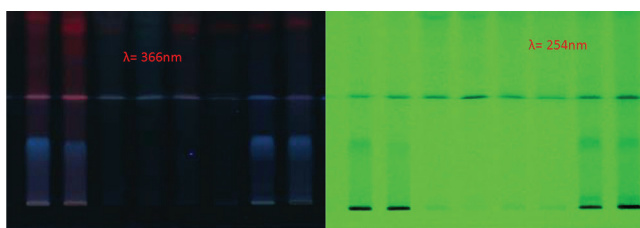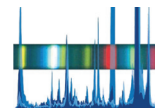
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Homeopathy developed about 200 years ago by the German Physician Samuel Hahnemann has over time become one of the most common and safe complementary and alternative medicine procedures. The quantification of rosmarinic acid and caffeic acid in mother tincture and different plant extract by performed by high-performance thin-layer chromatographic (HPTLC) using following chromatographic conditions Stationary phase: Precoated HPTLC Silica gel 60 F_{254} plate (20 cm × 10 cm); developing system:ethyl acetate:toluene:formic acid (5:5:0.7; v/v/v). The analysis was carried out on CAMAG HPTLC equipped with ATS4, TLC III scanner, TLC visualizer, and WINCATS software which revealed that both rosmarinic acid and caffeic acid are present in mother tincture of both the plants in comparison to other plant extracts. The overall relative standard deviation for retention time and area rosmarinic acid and caffeic acid was 0.01–0.11% and 0.14–1.23% for intraday and 0.02–0.22% and 0.31–1.41% for interday, respectively. The recovery of compounds was ranged between 97.35 and 102.50%. The method was validated according to the ICH guidelines. Consequently, this profile examination would prepare for the future homeopathic preparation use in our lives.

Dr. Ajai Prakash Gupta Cimap, India (Ph.D., 1998), Presently, I am working as Senior Technical Officer & & Assistant Professor AcSIR (Chemical Science)in Quality control & Quality Assurance at Indian Institute of Integrative Medicine, (IIIM-CSIR), Jammu. His expertise includes method development, validation & analysis of tissue culture samples, bio-active constitutions of microbes and medicinal plants using LC-MS/MS. The area of expertise also includes pharmo-kinetics & pharmo-dynamics (PKPD) studies, drug metabolites, mechanistic study, through HPLC, HPTLC and LC-MS/MS. He is also involved in quality control & Quality assurance of cGMP grade herbal formulations and monograph preparations for various Pharmacopoeias. I am also recognised Jammu & Kashmir FDA approved Quality Control person. Email: ajai747@yahoo.co.in/apgupta@iiim.ac.in

PP-21

High-performance thin-layer chromatography fingerprints of some Indonesian medicinal plants used in "Scientific Jamu"

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The use of herbal medicine in Indonesia is still very popular. However, it has not been recognized by medical doctors as an alternative method of health care. Therefore, the government created a "Scientific Jamu" program, which is scientific proof of herbal medicine through health care-based research. To succeed this program supports from various aspects is needed, including ensuring the authenticity and quality of the raw materials. In this research, the high-performance thin-layer chromatography (HPTLC) fingerprint of seven medicinal plants (i.e.: *Orthosiphon stamineus*, *Centella asiatica*, *Phyllanthus niruri*, *Apium graveolens*, *Caesalpinia sappan*, *Stelechocarpus burahol*, and *Curcuma domestica*) used in "Scientific Jamu" was established. Optimization of the mobile phase was designed with the *mixture design* method to generate separation with the highest number of bands and the best separation. Validation methods included stability, precision (intraday and interday), and specificity. Each plant was sonicated using methanol and then proceeded for HPTLC analysis using the following condition. A Camag TLC system comprising Linomat 5 sample applicator, twin-through chamber, TLC visualizer 2, and visioncats software were used. Chromatography was performed on HPTLC plates (silica gel 60 F_{254}) with a 100-µl Camag syringe. Samples were spotted under a flow of N_2 as 6 mm bands, 15 mm from the left edge, 10 mm from the bottom edge, and 10 mm of track distance. Development (80 mm) was carried out in a chamber previously equilibrated with a mobile phase which was optimized for each plant extract. The plates were dried under warm air and derivatized using a specific reagent. Fingerprints evaluation was carried out under TLC visualizer. The fingerprints of the seven plants are presented at Figure 1. Method validation exhibited that the method used meets the requirements of stability, precision, and specificity. It can be concluded that developed HPTLC fingerprint can be used to ensure the authenticity of plants used in "Scientific Jamu."
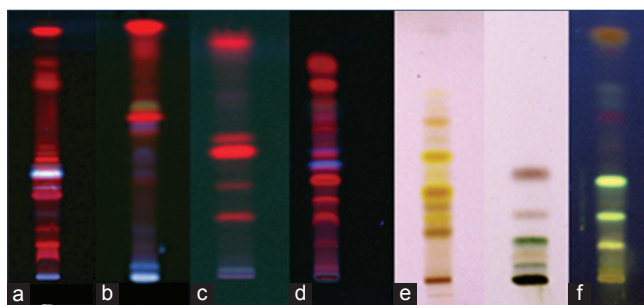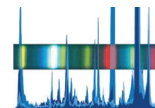

Figure 1: High-performance thin-layer chromatography fingerprint of seven plants used in Scientific Jamu: *Orthosiphon stamineus* (a), *Centella asiatica* (b), *Phyllanthus niruri* (c), *Apium graveolens* (d), *Caesalpinia sappan* (e), *Stelechocarpus burahol* (f), and *Curcuma domestica* (g)

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PP-22

Thin-layer chromatography-bioautographic identification of new markers and modification of high-performance thin-layer chromatography authentication of *Curculiginis rhizoma*

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[1]*Shanghai R and D Center for Standardization of Chinese Medicines, Shanghai, China, [2]The MOE Key Laboratory of Standardization of Chinese Medicines, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai, China*

Curculiginis rhizoma is the rhizome of *Curculigo orchioides* Gaertn. (family Amaryllidaceae) recorded in the Chinese Pharmacopoeia, which is used for the treatment of impotence, limb limpness, arthritis of the lumbar and knee joints, and watery diarrhea in China. To establish a reasonable quality standard of *C. rhizoma* for clinic application, the thin-layer chromatography (TLC)-guided isolation method was applied for screening and isolating characteristic chemical compounds of C. rhizoma. First, several characteristic bands were determined from TLC and TLC-bioautographic images; then, seven compounds were isolated using TLC coupled with other isolation techniques and identified as nyasicol, nyasicoside, 1-*O*-butylnyasicosides, curculigoside, orcinol, orcinol glucoside, and 2,6-dimethoxyphenol by ^{1}H and ^{13}C-NMR spectra. At last, a high-performance TLC (HPTLC) identification method was established using curculigoside and orcinol glucoside as reference substances. Test solution: To 2 g of the powder, add 20 ml of ethanol, hot reflux for 30 min. Filter and evaporate the filtrate to dryness. Dissolve the residue in 1 ml of ethanol. Reference solution: Dissolve a quantity of curculigoside and orcinol glucoside in ethanol to produce a solution containing 0.2 mg/ml. Stationary phase: HPTLC silica gel precoated plate (Yantai Institute of Chemical Industry), mobile phase: Dichloromethane-acetone-formic acid (5:2:1), and derivatization: Spray with a solution of 2% vanillin in 10% ethanol sulfate, heat at 105 until the color of the bands appears distinctly. Detection: Examine in daylight [Figure 1].
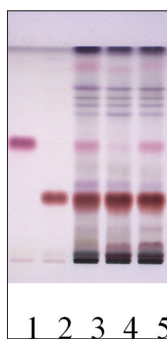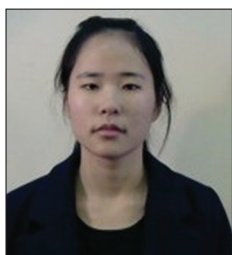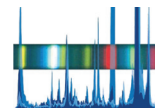

Figure 1: High-performance thin-layer chromatography silica gel precoated plate (Yantai Institute of Chemical Industry. (1) Curculigoside, (2) orcinol glucoside, (3-5) sample

Xueli Deng, Hubei Univ. of Chinese Medicine (B.Sc., 2016). At present, Master, Shanghai Univ. of Traditional Chinese Medicine, China. Research fields: Chemical composition and activity of botanical and traditional herbal medicines; research on standardization of *Curculiginis rhizoma*.

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PP-23

High-performance thin-layer chromatography hyphenated with bioassay for the screening of bioactive constituents of spice *Zanthoxylum rhetsa*

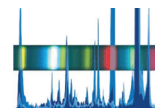**Tanushree Goswami**

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Medicinal plants have played an important role in discovery and development of new pharmaceuticals as clinically useful drugs over the years due to the enormous structural and chemical diversity. The isolation and characterization of antibacterial compounds are relevant because of increasing occurrence of opportunistic infections and multidrug resistance. Plants possess plethora of phytochemicals which mainly include secondary metabolites but only few of them exhibit bioactivity. The screening and analysis of these bioactive components become difficult due to the presence of large number of other compounds. *Zanthoxylum rhetsa* is a medicinally important plant of the family Rutaceae. In the present study, a fingerprint profile was developed followed by the identification of bioactive compounds through high-performance thin-layer chromatography (HPTLC)-bioautography. HPTLC analysis was performed on aluminum plates precoated with silica gel 60 F_{254}. The ethyl acetate extract was applied as bands of 10 mm width with the help of CAMAG Linomat V applicator. The plates were developed in a presaturated twin trough chamber and scanned at wavelength of 254 nm and 366 nm using CAMAG TLC Scanner. Then, the plate was derivatized with anisaldehyde-sulfuric acid reagent and heated at 105°C on hot plate. The fingerprint can be used as a quality control tool for authentication and comparison of subtle differences among samples of identical plant resources. The antibacterial activity of separated compounds was tested by agar-overlay bioautography against the food pathogen *Staphylococcus aureus* ATCC 25923. Antibacterial compounds on the TLC-plate were detected as white bands against purple-pink background. Thus, TLC-bioautography is an effective and inexpensive assay for the rapid and precise detection of biologically active substances in complex mixture of plant extracts. It can be concluded that bioassay-coupled chromatography is a simple and pragmatic approach for target-directed analysis of bioactive compounds.

Tanushree Goswami, VES College, Mumbai University (B.Sc., 2010), Khalsa College, Mumbai University (M.Sc., 2012). Presently working as a research scholar at Guru Nanak Khalsa College, Mumbai University. Research interests: Isolation and characterization of bioactive compounds from natural sources, essential oils studies, and standardization of medicinal plants.

PP-24

High-performance thin-layer chromatography 2,2-diphenyl-1-picrylhydrazyl method for the detection of antioxidant compounds from *Zanthoxylum rhetsa*

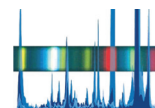**Tanushree Goswami**

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Zanthoxylum rhetsa is an important, indigenous medicinal plant of India. The different parts of the plant have been used in traditional Ayurvedic medicine for curing various disorders. Characteristic secondary metabolites include lignans, alkaloids, flavonoids, terpenes, steroids, amides, and coumarins. The secondary metabolites produced by plants should be isolated and identified to use them as active principles in medicinal preparations. One of the simplest, economic, rapid, and sensitive techniques is high-performance thin-layer chromatography (HPTLC)-bioautography. Hence, the aim of the present study was to develop a suitable solvent system followed by detection of antioxidant compounds with the help of the detecting reagent 2,2-diphenyl-1-picrylhydrazyl (DPPH) in *Z. rhetsa*. For chromatographic separation, different combinations of mobile phases were used, and the one who gave the best separation was selected for further study. After migration of the sample, the HPTLC plate was sprayed with DPPH reagent. The active antioxidant constituent was detected as creamish spot produced through reduction of DPPH by resolved bands against the purple background on the TLC plate. HPTLC-DPPH technique was very useful as the direct link to effective compounds is achieved in the same chromatographic separation bed. Sophisticated technical equipment for the bioassay coupling is not necessary. Thus, HPTLC-DPPH assay which is precise, inexpensive, and reproducible aided the target-directed detection of the antioxidant compound from *Z. rhetsa*.

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PP-25

Performance of measuring estrogenicity with planar-yeast estrogen screen compared to 96-well plate yeast estrogen screen

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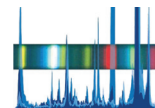alanjames.bergmann@oekotoxzentrum.ch

Alan Bergmann, Andrea Schifferli, Eszter Simon, Etienne Vermeirssen, Andreas Schönborn

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Food contact materials contain many non-intentionally added substances (NIAS) that could migrate into food. To identify potentially hazardous NIAS in food contact materials, effect-directed analysis (EDA) is useful to identify potential toxicants such as endocrine-disrupting chemicals. Development of fast and robust methods for EDA is still necessary before it is practical to use in routine analyses. The yeast estrogen screen (YES) performed directly on high-performance thin-layer chromatography plates (planar-YES) is a promising tool for screening and EDA of (xeno)estrogens, and it has recently experienced several improvements. The planar-YES eliminates the need for carrier solvents and can be performed immediately after chromatographic separation. However, there has been no direct comparison of the planar-YES to a standard 96-well plate version of the YES. To fill this gap, we tested several chemicals in the planar-YES and a 96-well plate YES (lyticase-YES, L-YES). We found the planar-YES to be more sensitive to the reference substance (17β-estradiol) than in the L-YES by 5-fold. For both assays, we present the bioactivity of 20 chemicals chosen for their relationship to food packaging materials. 14 of the 20 chemicals were bioactive. The bioassays may be susceptible to losing test substances when the extract is transferred into the assay, for example, through evaporation in the planar-YES or sorption to the test chambers in the L-YES. Therefore, we explore the bioactivity of the 20 chemicals with respect to their physicochemical properties. This work helps to understand the advantages and limitations of both assays for estrogen screening and EDA toward the goal of identifying toxic NIAS in food contact materials.

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PP-26

Determination of steroid 5α-reductase activity of furanonaphthoquinones by non-radioactive and direct dihydrotestosterone detection using high-performance thin-layer chromatography

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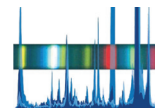
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Steroid 5-alpha-reductase or 3-oxo-5α-steroid 4-dehydrogenases is an enzyme controlled the reduction of testosterone to dihydrotestosterone, which both are the important androgen hormones involved in several human diseases such as prostate cancer, benign prostatic hyperplasia, acne, hirsutism, and androgenic alopecia. An evaluation of steroid 5-alpha-reductase activity has been developed by employing human keratinocytes (HaCaT) cell-based bioassay in conjugation with direct detection of the enzymatic product dihydrotestosterone using a non-radioactive high-performance thin-layer chromatography (HPTLC). In this study, a series of furanonaphthoquinones was screened as the potential steroid 5-alpha-reductase inhibitors. After treating HaCaT cells with a series of interesting compounds at subtoxic concentration and addition of testosterone as enzyme substrate, a supernatant was collected and extracted with ethyl acetate followed by evaporating to dryness. Then, the resulting extract was reconstituted in methanol and spot on an aluminium silica gel 60-F254-HTLC plate. A mixture of cyclohexane-ethyl acetate-triethylamine (1.5:1:0.1, v/v/v) was used as the mobile phase. After separation, the HPTLC plate was stained with phosphoric acid and heat at 120°C for 20 min before visualizing under 366 nm ultraviolet light. The produced dihydrotestosterone that directly derived from steroid 5α-reductase was clearly identified in both qualitative and quantitative applications. The results showed that avicequinone B, avicequinone C, and their analogues exhibited activity of steroid 5-alpha-reductase at IC$_{50}$ 0.2–4.5 μm concentrations.

Supakarn Chamni, Thammasat University, Thailand (B.Sc., 2005), Texas A&M University (Ph.D., 2011); Assistant Professor at Faculty of Pharmaceutical Sciences, Chulalongkorn University (2012-present); Research fields: Medicinal chemistry and structure-activity relationship of natural products and their synthetic derivatives, Member of Professor. Wanchai De-Eknamkul's laboratory.

PP-27

Quantification of tacrolimus ointment using high-performance thin-layer chromatography
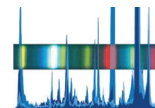
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Tacrolimus is macrolide lactone obtained from *Streptomyces tsukubaensis* fermentation broth. Due to its immunosuppressant properties, it is administered in ointment formulations as a steroid-free treatment for controlling the symptoms of atopic dermatitis and psoriasis in adults and children. Quantification of tacrolimus ointments using high-performance liquid chromatography analysis can be challenging as there tends to be an insufficient recovery of the drug from the ointment base before analysis. This study, therefore, aimed to explore high-performance thin-layer chromatography (HPTLC) as a potential alternative analysis tool for the determination of drug content in topical tacrolimus formulations.The investigated tacrolimus ointment was prepared using isopropyl myristate (1%), propylene glycol (1%), liquid paraffin (30%), and white soft paraffin (68%) as an ointment base. The ointment was dissolved in a mixture of cyclohexane and chloroform (2:1 V/V) and the resulting solution (3–6 μl) developed in a mobile phase consisting of toluene, ethyl acetate, and glacial acetic acid on silica gel F_{254} HPTLC plates followed by derivatization with anisaldehyde reagent. The resulting tacrolimus bands were analyzed at white light and quantified against a tacrolimus standard curve obtained by the same method.The developed analysis protocol allowed for reliable and accurate quantification of tacrolimus in the ointment base with drug content as low as 0.05% without prior drug extraction from the vehicle and thus presents a novel avenue for the quantification of tacrolimus in this formulation. The findings also highlight that HPTLC analysis should be considered more widely for the quality control of topical pharmaceutical formulations.

Tom Sostaric. (B.Sc., 1998), Edith Cowan Univ. (M.Sc., 2001), Edith Cowan University, Adjunct research fellow, presently at University of Western Australia, Faculty of Health and Medical Sciences. Research fields: Honey and Honey product fingerprinting, quantification methods using HPTLC for problematic pharmaceutical preparations.

PP-28

Validated thin-layer chromatographic densitometric method for stress degradation and accelerated stability study of asiaticoside from *Centella asiatica* and its marketed formulation

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The present work is to develop and validate the stability-indicating high-performance thin-layer chromatographic (TLC) method for *Centella asiatica* and its marketed formulation using quality by design (QbD) approach. The approach identifies and helps in controlling critical parameters that affect method performance. Stress degradation and accelerated stability studies were carried out on asiaticoside (triterpenoid component extracted form *C. asiatica*) following the conditions prescribed in the parent drug stability testing guideline (Q_1AR_2) issued by the International Conference on Harmonization. The method employed TLC aluminum plates precoated with silica gel $60F_{254}$ as the stationary phase. The optimized solvent system consisted of toluene: ethyl acetate: methanol:glacial acetic acid 2:7:3:1(v/v/v/v). The derivatization was carried out with 10% methanolic sulfuric acid reagent. This system was found to give compact spots for asiaticoside (R_f value of 0.43). Densitometric analysis of asiaticoside was carried out in the absorbance mode at 595 nm. The linear regression analysis data for the calibration plots showed good linear relationship with $r^2 = 0.982$ and in the concentration range 200–1800 ng/spot. The method was validated for precision, recovery, and robustness using QbD approach. Plackett–Burman design was used for robustness study. The accuracy for the *C. asiatica* plant powder and its formulation was found to be 97.72–104.54% and 100.33–102.67%, respectively. The LoD and LoQ were 51.127 and 154.93 ng/spot, respectively. *P*-value of all selected parameters was higher than 0.05; hence, the method was found to be robust. All the peaks of degraded products were resolved from the standard asiaticoside and its formulation with significantly different R_f values. The obtained result suggested that asiaticoside underwent degradation rapidly when exposed to acidic condition as the hydrolysis of the o-glycoside takes place with removal of water and sugar moieties when this is exposed to acid. As the method could effectively separate the drug with its degradant, it can be employed as a stability indicating one [Figure 1].
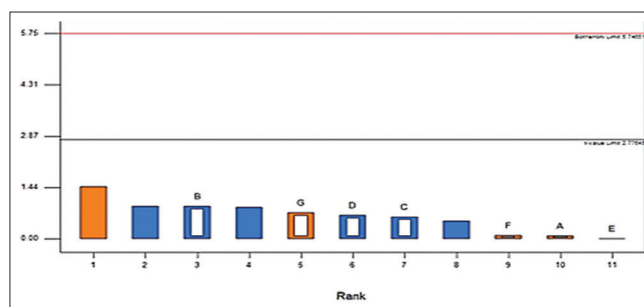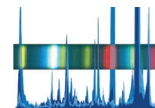

Figure 1: Pareto chart having an effect of factors on developed high-performance thin-layer chromatographic method

Dr. Samir Patel, Univ. of Pune (B. Pharm., 2001 R. G. U. H. S. Bangalore, (M. Pharm., 2004), M. S. Univ. (Ph.D., 2009), Nalsar University of Law (PG Diploma in Patent Law, 2011), Gujarat University (L.L.B, 2017), Associate Professor at Ramanbhai Patel College of Pharmacy, CHARUSAT, Research fields: Development and validation of stability-indicating analytical methods for the estimation of herbal and pharmaceutical drugs, chemometric tools in the development of analytical method and peptide synthesis.

Development of new solvent systems for the analysis of atorvastatin extracted from blood using high-performance thin-layer chromatographic plate
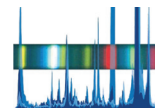
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The drug atorvastatin is a prescription medicine used to treat high cholesterol levels and also to prevent heart disease. The drug is a member of the medication class known as statins and belongs to a group of drugs called HMG-CoA reductase inhibitors. These drugs block an enzyme involved in making of cholesterol within the body. This medication comes in tablet form and is usually taken once a day. It is used to reduce risk for heart attack and stroke. The side effects of atorvastatin are cold symptoms, diarrhea, heartburn, joint pain, confusion, muscle, kidney, and liver problems. There are higher chances of muscle problems if other medicines are also taken along atorvastatin. The drug may also cause liver problems. The drug may prove to be lethal if it is taken as overdose as or more than once a day. An attempt has been made to develop new solvent system (SS) for the analysis of atorvastatin in blood using high-performance thin-layer chromatographic (HPTLC) plates (layer of silica gel 60 F254) by TLC technique. Sample preparation - a tablet of 10 mg was crushed into powder and dissolved into 10 ml of methanol, used as working standard. 5 ml of working standard was spiked into 5 ml of blood, which was further extracted with small volume liquid extraction method. TLC layer - silica gel 60 F254 type was used. Various combinations of solvents were tried. After the TLC, the visualization was done under 254 nm UV light. The retention factor (Rf) value was calculated. The following SS was employed for the analysis of the atorvastatin: (1) Chloroform:toluene:methanol, (2) toluene:acetone, (3) cyclohexane:acetone, (4) chloroform:acetone, (5) benzene:methanol, (6) n-hexane:acetonitrile, (7) n-hexane:methanol, (8) toluene:ethanol, and (9) dichloromethane:methanol. Among all the SS tried, some of the SS, i.e., 1, 2, 3, and 5 gave more suitable Rf was observed in SS 4, 6, 7, 8, and 9, of this four SSs were tried on the spiked blood sample. The Rfs of blood and drug standard were, respectively, as follows: in SS-1 (0.67 and 0.65), SS-2 (0.30 and 0.32), SS-3 (0.35 and 0.35), SS-5 (0.77 and 0.78). Above developed method is simple, rapid, and economical and can be used in any laboratories with simple infrastructure.

Delhi University (B.Sc., 2014), Kurukshetra University, Kurukshetra (M.Sc.2016), UGC - NET qualified, presently working as Assistant professor, Department of Forensic Science and Toxicology, Chandigarh University. Research field: Identification of poisons/drugs of forensic interest by chromatography, extraction of poison from biological matrices, new analytical method for identification, and identification of individuals - forensic anthropology.

PP-30

Simultaneous estimation of drotaverine HCl and nimesulide in pharmaceuticals by high-performance thin-layer chromatographic method

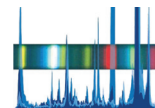**Manoj Shrawan Charde**

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A simple, rapid, precise, and accurate high-performance thin-layer chromatographic (HPTLC) method was developed and validated for simultaneous determination of drotaverine hydrochloride (DRO) and nimesulide (NIM) in pharmaceutical preparations. Separation was achieved on a Merck HPTLC plates (0.2 mm thickness) precoated with 60 F_{254} silica gel on the aluminum sheet as the stationary phase using cyclohexane:methanol:ethyl acetate (5:2.:3v/v/v), as the mobile phase. Densitometric quantification was performed at $\lambda = 295$ nm by reflectance scanning. The R_F values of DRO and NIM were obtained 0.41 and 0.62, respectively. The linearity of the proposed method was investigated in the range of 0.1–0.6 mg/spot and 0.2–0.7 mg/spot for DRO and NIM, respectively. The percentage recoveries for DRO and NIM were 99.91% and 100.19% by area and 99.63% and 99.96% by height, respectively. The developed method was suitably validated for precision, accuracy, specificity, and ruggedness.

Dr. Manoj Shrawan Charde, M. Pharm, Ph. D has completed his B. Pharm (2001), M. Pharm (2003), and Ph. D (2008) from the Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj, Nagpur University, Nagpur, in First Division. At present, he is working as Principal at Government College of Pharmacy, Karad, District Satara, 415124, Maharashtra, India. His research area is Bioanalytical analysis, Phytopharmaceutical and Phytopharmacological evaluation and also keen to work on various drug delivery systems with polymer derivatization. He has total 15 years of academic experience.

PP-31

A validated high-performance thin-layer chromatography method for quantification of kaempferol from rat plasma and its application to pharmacokinetic study using sparse sampling method

Reena Mascarenhas
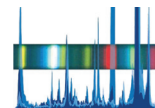
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Genus *Flemingia* belongs to family Fabaceae. Various reported species of *Flemingia* such as *Flemingia tuberosa*, *Flemingia vestita*, *Flemingia strobilifera, Flemingia macrophylla,* and *Flemingia chappar* have been known to comprise flavonoids as the major class of compounds. Of these *F. tuberosa*, Dalzell has been reported to contain kaempferol. As one of the major compounds twith biological activity, kaempferol has been reported to have various pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, antidiabetic, estrogenic, anticancer activities and many more. In this research work, the pharmacokinetics of marker compound kaempferol were studied after the oral administration of the standardized ethanolic extract of aerial parts of *F. tuberosa* in albino Wistar rats using validated high-performance thin-layer chromatography (TLC) method and the pharmacokinetic parameters were evaluated using WinNonlin software. The extract was prepared by sonicating the plant powder in ethanol for 6 h which was then evaporated to dryness after filtration. Chromatographic separation of the phytochemical constituents was achieved on TLC plates (E. Merck) precoated with silica gel $60F_{254}$ (0.2 mm thickness) on aluminum sheet support. A mobile phase, cyclohexane:ethyl acetate:methanol:formic acid in the ratio (6:4.5:05:0.5, v/v/v/v) was used to achieve the separation of kaempferol from the plasma samples. TLC Camag Scanner 4 was used for densitometric scanning with deuterium lamp (365 nm) as a source of radiation. All measurements were performed at 22 ± 1°C. Plate was photodocumented using Reprostar 3 under ultraviolet radiation (254 nm). The method was validated as per the US-FDA guidelines and applied to study the pharmacokinetics of kaempferol from ethanolic extract of *F. tuberosa*.

Reena William Mascarenhas, University of Mumbai (B.Sc., 2013) (M.Sc., 2015). At present, a research scholar under the guidance of Prof. Sunita Shailajan at Ramnarain Ruia Autonomous College, in the subject of Bioanalytical Sciences. Research fields: Phytochemistry, Pharmacognosy, herbal drugs, and pharmacokinetics.

PP-32

Development and validation of stability-indicating high-performance thin-layer chromatography method for simultaneous estimation of ilaprazole and levosulpiride in capsules using design of experiment approach

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High-performance thin-layer chromatography (HPTLC) method has been developed for the separation of pregabalin and nortriptyline using precoated silica gel aluminum plate 60F254, with ultraviolet detection at 488 nm after derivatization using ninhydrin reagent. Box–Behnken design was applied for multivariate optimization of the experimental conditions of the HPTLC method. Effect of three independent factors; methanol content in mobile phase composition, saturation time, and migration distance on Rf and peak area of both drugs were studied using mathematical models. The quadratic model was found to be the best fit for all responses. The developed models were validated and optimized mobile phase was found to be methanol:ethyl acetate:TEA (6:4:0.2, v/v/v) as mobile phase. The method was validated according to the ICH guidelines. Linear responses were observed in the concentration range of 3000–9000 ng/band for pregabalin and 1500–9500 ng/band for nortriptyline hydrochloride. Mean percentage relative standard deviation (RSD) for intraday precision of pregabalin and nortriptyline hydrochloride was found to be 0.19 and 1.18, respectively [Figure 1]. The mean percentage RSD for interday precision of pregabalin and nortriptyline was found to be 0.13 and 1.7, respectively. The limit of detection for pregabalin and nortriptyline hydrochloride was found to be 0.12 ng/band and 0.63 ng/band, respectively, and the limit of quantification for pregabalin and nortriptyline was found to be 0.12 ng/band and 0.63 ng/band, respectively. The amount of pregabalin and nortriptyline was found to be 99.3% and 98.8% from tablets.
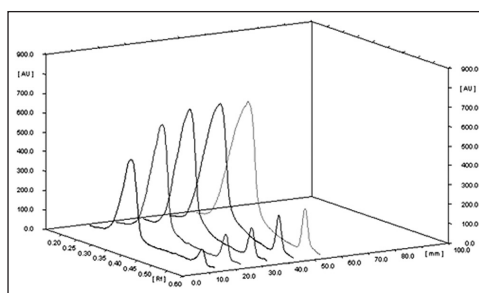
Figure 1: Three-dimensional chromatogram of pregabalin (3000–9000 ng/band) and nortriptyline (1500–9500 ng/band)

Archita Patel - Sardar Patel University (B.Pharm., 2003), S.N.D.T. Women's University (M.Pharm., 2005), and Kadi Sarva Vishwavidyalaya University (Ph.D., 2017), Assistant Professor at K. B. Institute of Pharmaceutical Education and Research. Research fields: Development and validation of stability-indicating analytical methods for the estimation of pharmaceutical compounds, chemometric tools in the development of analytical method.

PP-33

Simultaneous determination and method validation for opioids, cannabinol, and nicotine in postmortem whole blood using high-performance thin-layer chromatography-mass spectrometry
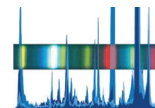
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Opioids, cannabis, and nicotine (NIC) are the oldest and highly abused substance globally. The detection and identification of these drugs are a major challenge for law enforcement agencies as well as forensic chemist, especially, when the cases pertain to crime against women. An attempt has been made to develop and validate the quantification method of morphine (MOR), codeine (COD), thebaine (THB), papaverine (PAPA), cannabinol (CBN), and NIC using high-performance thin-layer chromatography-mass spectrometry (HPTLC-MS) from postmortem whole blood. Quantitative analysis of MOR, COD, THB, PAPA, CBN, and NIC was done using HPTLC-MS (CAMAG-SHIMADZU) operating in selective ion-monitoring mode. Small volume liquid extraction technique using ethyl acetate:hexane (80:20) was used for the extraction of blood. Pre-coated HPTLC (silica gel G 60 F254) plates were developed using mobile phase ethyl acetate:methanol:ammonia (8.5:1:0.5). The screening of HPTLC plates was done by UV light, and the m/z ratio of drugs was obtained by lifting spots from plates using TLC-MS interface. Method validation was done by studying specificity, linearity, precision, accuracy, limit of detection, limit of quantification (LOQ), and recovery from blood samples. Retention factor value of MOR, COD, THB, PAPA, NIC, and CBN was 0.14, 0.20, 0.25, 0.66, 0.36, and 0.83, respectively. Linearity range for MOR, COD, THB, PAPA, NIC, and CBN was 0.5 ug–2.5 ug, 0.5 ug–2.5 ug, 0.25 ug–1.5 ug, 0.1 ug–0.5 ug, 0.1 ug–0.5 ug, and 0.02 ug–0.2 ug and coefficient correlation was 0.999, 0.998, 0.997, 0.995, 0.998, and 0.998, respectively. Repeatability and precision for analyte detected in the mixture was under 5%. LOD for MOR, COD, THB, PAPA, NIC, and CBN was 166.6 ng, 166.6 ng, 83.3 ng, 33.3 ng, 33.3 ng, and 6.6 ng, respectively. LOQ was 500 ng, 500 ng, 250 ng, 250 ng, 100 ng, and 20 ng, respectively. The MS observation showed the m/z ratio of MOR (286), COD (300), THB (312), PAPA (340), NIC (163), and CBN (309). Recovery for MOR, COD, THB, PAPA, NIC, and CBN from blood was in the range of 60–65%. The compound is identified by confirmation of its molecular weight m/z value. The base peak present other than the actual molecular weight and the variation of $\pm$ 1 is due to adduct formation of the drug sample with its solvent. The technique has encouraged the forensic scientists to move toward economic and clean technology. Developed HPTLC-MS method is simple, sensitive, precise, and accurate and can be used for the quantitative analysis of MOR, COD, THB, PAPA, NIC, and CBN in blood.

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PP-34

Comparative evaluation of glass containers and laminated plastic pouches in maintaining chemical integrity of *Ashwagandhadi lehya*

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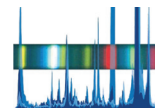
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The studies were aimed to evolve comparative ability of glass containers and packages made up of flexible plastic laminates in protecting chemical integrity of multicomponent *Ayurveda* formulation - *Ashwagandhadi lehya*. *Withania somnifera* is one of the major constituents of this formulation. The comparative observation of fingerprint profile of the formulation, determining the loss on drying, and estimating amount of Withaferin-A, as marker compound, were selected as attributes to be evaluated. Standardized *A. lehya* was packed in glass containers with plastic leads as well as in laminated pouches made up of polyethylene terephthalate:metalized polyethylene terephthalate:metalized cast polypropylene (12 μ:12 μ:25 μ). The packages were placed in stability chamber set at accelerated stability conditions (40 ± 2°C and 75 ± 5% relative humidity). Fingerprint of methanolic extract was developed using high-performance thin-layer chromatography (HPTLC). An analytical method was developed for THE determination of Withaferin-A using HPTLC and validated as per the ICH guidelines. The spot corresponding to Withaferin-A was separated from nearby spots, from methanolic extract of formulation, using precoated silica gel G60 F_{254} plates as stationary phase. Optimized mobile phase comprised toluene:ethyl acetate:methanol:glacial acetic acid (5:5:0.8:0.15, v/v/v/v). The plates were subjected to post-chromatographic derivatization using anisaldehyde-sulfuric acid reagent and quantified using fluorescence reflectance mode at 366 nm. Rf value of spot corresponding to Withaferin-A was found to be 0.4 ± 0.02. The studies ensured that the method was linear (within 50–800 ng/spot), precise, selective, enough sensitive, accurate, and robust to estimate Withaferin-A from sample solution. Amount of Withaferin-A in *A. lehya* was found to be 0.45 ± 0.05 mg % w/w. The studies showed that, during the period of studies, there were no alterations seen in chromatographic profile of the formulation. Amount of Withaferin-A, in the formulation, packed in glass container and that packed in flexible laminate was found comparable too. Value of loss on drying was found increased from 10.49% on 0 day to 29.23% at the end of studies, which was also found comparable to that for the formulation packed in plastic laminates. It was concluded from the studies that flexible plastic laminates might be a substitute alternative of rigid glass containers for such formulations.

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PP-35

Pharmacokinetic evaluation of a polyherbal formulation using high-performance thin-layer chromatography technique

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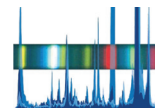
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Ayurveda has been facing constant challenges on the fronts of standardization and pharmacokinetics of the active principles from these formulations. One such formulation is *Pushyanuga churna* which is commonly available OTC in India and is prescribed by Ayurvedic practitioners for various female reproductive disorders. *P. churna* being prepared from 25 plant ingredients and one mineral shows the presence of a myriad of phytoconstituents such as ursolic acid, beta-sitosterol, berberine, and quercetin. These markers are responsible for the overall efficacy of the formulation, and hence, there is a need for the pharmacokinetic profile of these bioactive markers from *P. churna*. Berberine, an isoquinoline derivative alkaloid isolated from *Berberis aristata*, has been reported to be present in *P. churna* and also documented to possess various pharmacological effects including lowering of blood glucose, increasing insulin sensitivity, an excellent potential for the prevention and treatment of metabolic syndrome insulin sensitivity, etc., which form the symptomatic features of many female reproductive disorders including polycystic ovary syndrome (PCOS). Literature survey further reveals that berberine alone can improve the menstrual pattern and ovulation rate, insulin resistance, and total cholesterol, in anovulatory women with PCOS. Hence, in the current research work, a normal phase high-performance thin-layer chromatography (HPTLC) method was developed for evaluating the pharmacokinetic profile of berberine from a polyherbal formulation *P. churna* in albino Wistar rats through oral administration. Processing of plasma involved liquid-liquid extraction technique followed by HPTLC. The mobile phase used for resolving berberine was toluene-ethyl acetate-methanol-formic acid and separation was achieved on silica gel 60 F_{254} HPTLC plates. Densitometric quantitation of berberine was done at 366 nm using CAMAG TLC Scanner 4 and photodocumentation was done using CAMAG Reprostar 3 at 366 nm. Developed method was validated using USFDA guidelines.

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PP-36

Development of validated high-performance thin-layer chromatography method for the estimation of eugenol in the marketed herbal formulation of muscle and joint Hrx pain relieving oil

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A new and simple high-performance thin-layer chromatography (HPTLC) method was developed and validated for the quantitative estimation of eugenol in muscle and joint pain relaxant herbal oil Figure 1. TLC aluminum plates precoated with silica gel 60F-254 (0.2 mm thickness) were used. The linear ascending development was carried out in twin trough glass chamber saturated with mobile phase tolune:ethyl acetate (9.3:0.7) ratio followed by densitometric determination which was carried out by TLC scanner (CAMAG) at 560 nm in reflectance/absorbance mode. The Rf value was found to be 0.58. Linearity was found to be in the concentration range of 24–64 ng. The linear regression data for the calibration plots showed a good linear relationship with $r^2 = 0.99$ for eugenol. According to the ICH guideline, the method was validate for accuracy, precision, specificity and ruggedness. The proposed method is accurate, precise, and reproducible and can be adopted for routine analysis of eugenol from herbal joint and muscle pain relieving oil by HPTLC Figure 2.
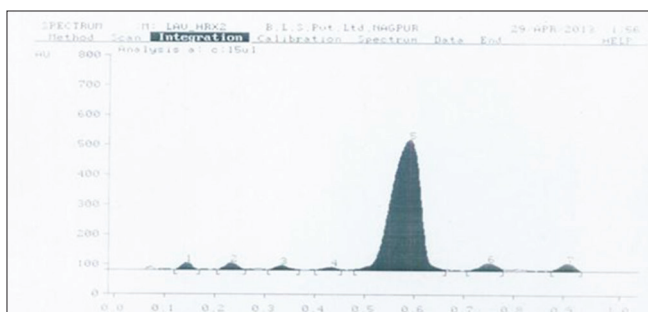
Figure 1: Chromatogram of eugenol in oil formulation
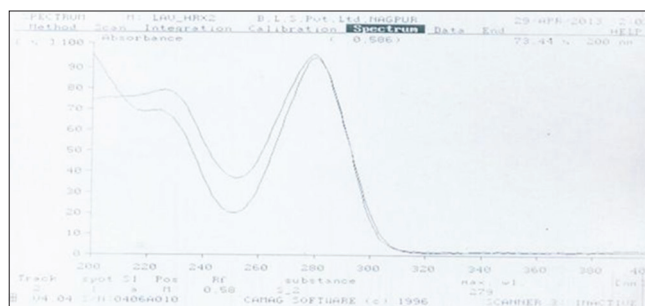
Figure 2: Overlain spectrum of standard eugenol and oil formulation

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PP-37

High-performance thin-layer chromatography densitometry simultaneously determined sesamin and sesamolin in cold pressed sesame oil
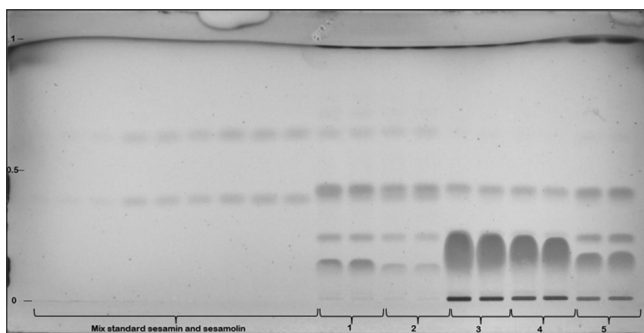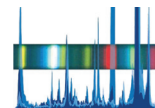
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Cold pressed oil from *Sesamum indicum* L. was prepared from organic sesamum seeds. Sesame oil decreased blood cholesterol, strengthened bone, and improved skin and hair health. The main chemical compositions of sesame oil were sesamin and sesamolin, which were lignans and some fatty acids. High-performance thin-layer chromatography (HPTLC) densitometry was determined the contents of sesamin and sesamolin in cold pressed sesame oil, crude seeds after preparing oil, and sesame seeds. The quantification of sesamin and sesamolin was performed on the HPTLC plate (silica gel60 F_{254}, Merck). Mobile phase composed of ethyl acetate and dichloromethane (1:99). Standard sesamin and sesamolin were prepared in methanol. All samples were extracted in methanol and centrifuged. Then, the supernatant was applied on the HPTLC plate at the band length 7 mm and distance 10 mm. The developed length was 8 cm and scanned at the wavelength of 290 nm. The bands were detected with anisaldehyde TS. The validated method showed the good accuracy of standard sesamin and sesamolin at the concentration ranges 200–1,000 and 250–1,250 ng/spot, respectively. HPTLC fingerprint showed that R_F values of sesamin and sesamolin were 0.40 and 0.65, respectively. Sesamin contents were in the range 3.95–4.43 mg/g in cold pressed sesame oil (1 and 2) compared with 2.31 mg/g of dried sesame seeds (5). In addition, sesamolin contents were in the range 3.03–3.43 mg/g in cold pressed sesame oil and 1.45 mg/g in sesame seeds, respectively. Crude seeds (3 and 4) after preparing oil contained trace amount 0.98–1.03 mg/g of sesamin and 0.51–0.76 mg/g of sesamolin, respectively.

PP-38

Determination of anthraquinones in Japanese knotweed extract by high-performance thin-layer chromatography – mass spectrometry methods

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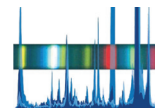
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Japanese knotweed is one of the top 100 world's invasive alien plant species; nevertheless, it represents valuable biomass. Its rhizomes are rich in stilbenes, proanthocyanidins, and anthraquinones, which have positive effects on human health. In the Asian market and to a lesser extent the European market, many formulations and food supplements made from or including Japanese knotweed rhizomes are available. Therefore, it is necessary to develop reliable as well as quick analytical methods that can also be used for quality control of bioactive compounds present in this plant material.

The aim of this study was to develop high-performance thin-layer chromatography (HPTLC)–mass spectrometry (MS)/MS-ESI methods for the determination of anthraquinones in the extracts of Japanese knotweed rhizomes. Analyses were performed on HPTLC diol plates using different developing solvents. All developed HPTLC–MS/MS methods enabled the identification of aloe-emodin, emodin, and their three glycosylated emodin analogs in Japanese knotweed rhizomes.

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PP-39

A high-throughput combinatorial platform of fluorophores for obtaining fingerprints for analyte discrimination

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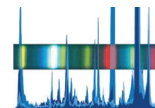
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Development of an array of fluorophores for screening their interaction with analytes in a high-throughput format, using a silica gel-based combinatorial platform is presented here. This system may take advantage of the combined response of its components to create specific pattern responses or fingerprints for analyte discrimination. This is based on a previous paper which demonstrated that practically almost all molecules (potential analytes) produce a response in the emission of fluorophores (either increases or quenchings) when interacting with them in a non-covalent way (either non-specific or specific interactions, respectively) at room temperature. An array was prepared using 49 commercial or freshly synthesized fluorophores with different chemical properties. They were immobilized by spray-on spotting (with ATS4) on high-performance thin-layer chromatography silica gel plates through non-covalent adsorption. Fluorescence measurements of sprayed bands were then performed at five different wavelengths using scanning densitometry. Subsequent spray overspotting of a target analyte on fluorophore bands was performed, and further fluorescence measurements recorded. Cholesterol and sphingomyelin, which are not fluorescent molecules and have poor spectroscopic properties, were used here as examples of analytes. Parameters affecting repeatability of the procedure were studied concerning sample application, scanning densitometry, intra- and inter-plate runs, as well as the effect of time on fluorophore signal. Resulting 254 signals were treated by chemometric techniques, applying different univariate, and multivariate calibration models. A profile per analyte was obtained. Different applications of profiles as analytical signal will be discussed.

Vicente L. Cebolla, PhD in 1987 at CNRS (Institut Recherches sur la Catalyse, France) and University of Zaragoza (Spain). Visiting Prof. in Metz University (2001). Richard H. Glenn Award 2002 from the American Chemical Society (Division of Fuel Chemistry). At present, CSIC Staff Senior Researcher at the CSIC, Instituto de Carboquímica (Zaragoza, Spain). Research interests: HPTLC of complex samples (fuels and lipids); development of fluorescence-based detection techniques for characterization of organic materials.

PP-40

Isolation of bioactive compounds from Japanese knotweed rhizomes using high-performance thin-layer chromatography methods followed by nuclear magnetic resonance characterization

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Japanese knotweed (*Fallopia japonica*), an alien plant species originating in Asia, is nowadays extremely invasive in Europe and North America, where it represents a huge ecological problem due to its fast expansion and destruction of local plant biodiversity. Most of its secondary metabolites, such as flavan-3-ols, their oligomers proanthocyanidins, stilbenes, and anthraquinones, possess a strong antioxidative potential and can therefore in humans prevent different oxidative stress connected diseases. The aim of our work was to isolate some bioactive compounds, especially proanthocyanidins, from Japanese knotweed rhizomes. After fractionation of crude extract of powdered rhizomes on preparative silica gel plates, some fractions rich in bioactive compounds were further separated using high-performance thin-layer chromatography (HPTLC) cellulose plates and different developing solvents. Derivatization of fractions with *p*-(dimethylamino)-cinnamaldehyde (DMACA) reagent (DMACA, specific for flavan-3-ols and proanthocyanidins),[1] HPTLC-densitometric, and HPTLC-mass spectrometry analyses facilitated characterization of compounds and their assignation on the plates. The chromatographic zones of compounds of interest were manually scratched from the plates and extracted with appropriate solvent. Identification of the isolated compounds was confirmed by ^{1}H nuclear magnetic resonance (NMR) and two-dimensional NMR analyses. Although some impurities leaching from the stationary phase were observed in the spectra of isolates, flavan-3-ols ((+)-catechin and (-)-epicatechin), proanthocyanidins, and some anthraquinones were successfully identified in Japanese knotweed rhizomes.

REFERENCE

1.   Glavnik V, Simonovska B, Vovk I. *J Chromatogr A* 2009;1216:4485-91.

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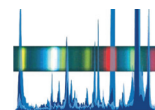
PP-41

Identification of bioactive compounds in *Aristotelia chilensis* by HPTLC-bioassay-mass spectrometry
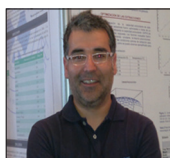
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According to epidemiological evidence, diets rich in fruits and vegetables may reduce the risk of suffering chronic noncommunicable diseases. This beneficial effect is generally associated with the presence of secondary metabolites in foods. One of the most studied groups of secondary metabolites is polyphenols to which several positive effects over human health are ascribed, for example, anti-inflammatory, antioxidant, and antidiabetic. Maqui (*Aristotelia chilensis*) is a Chilean native tree that presents small berries rich in polyphenolic compounds. The objective of this work was to develop high-performance thin-layer chromatographic (HPTLC)-bioassay-MS methods to detect and identify bioactive compounds capable of inhibit acetylcholinesterase (AChE), α-glucosidase (AG), and monoamine oxidase (MAO) in maqui berries. For AChE inhibitors detection, chromatography was carried out on silica gel 60 F_{254} HPTLC plates using a mobile phase composed of dichloromethane:acetonitrile:water:formic acid (5:2:1:2 v/v/v/v) which also contains 1-naphthyl acetate as enzymatic substrate. After separation and drying, the plate was sprayed with an AChE solution (1 U/mL in 0.05 mol/Lof TRIS-hydrochloric acid buffer, pH 7.8) and incubated for 10 min at 37°C. AChE inhibitor compounds were detected as colorless zones over a purple background after atomizing with Fast Blue Salt B solution (1.0 mg/mL). A clear inhibitory band was observed, which was eluted to the mass spectrometer by means of TLC/MS interface, identifying the presence of cyanidin 3-sambubioside-5-glucoside. At present, the detection of AG and MAO inhibitors is under study. It is concluded that the HPTLC/bioassay/MS platform allowed the detection and identification of bioactive molecules with inhibitory activity over AChE present in maqui berries.

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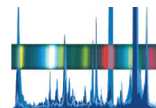
PP-42

HPTLC-ESI-MS/MS determination of fining-related allergen proteins in Chilean wines
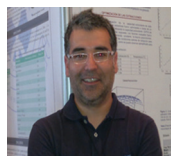
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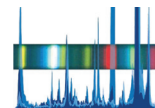
Casein (milk powder) and ovalbumin (egg-white powder) are often used as wine fining agents with the objective of promoting interactions with undesirable compounds, for example, polymeric phenols. Unfortunately, this kind of proteins may trigger allergic reactions in susceptible individuals; therefore, their occurrence in wines could become a human health risk, moreover, when their presence is not reported on the label. The objective of this work was to establish a high-performance thin-layer chromatography (HPTLC)-electrospray ionization (ESI)- mass spectrometry (MS)/MS method to determine casein and ovalbumin in Chilean wines. To the best of our knowledge, the present work reports for the 1ˢᵗ time the detection of these proteins by HPTLC/MS/MS. Proteins were extracted combining the use of ultrafiltration membranes and protein precipitation with organic solvents. Thereafter, proteins were digested with trypsin during 7 hours with a 1:10 enzyme protein ratio. Chromatographic separation was carried out on HPTLC silica gel $60F_{254}$ glass plates using a mixture of 2-propanol:pyridine:ammonia:water (39:34:10:26, v/v/v/v). Peptides were detected by fluorescence applying Fluorescamine reagent. Underivatized peptides were identified by direct coupling with mass spectrometry using the TLC/MS interface. MS was performed in ESI(+) and multiple reaction monitoring modes. The transitions established for confirmation were m/z 742.5→625.2 for b-casein and m/z 764.6→1036.5 for ovalbumin. The percentage of sequence coverage for casein and ovalbumin was 23% and 11%, respectively, which is adequate in terms of yield of the proteolytic digestion. This work demonstrated the feasibility of HPTLC coupled to a mass spectrometer for the identification of peptides resulting from the enzymatic digestion of allergenic proteins used like finning during the winemaking process.

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PP-43

Identification of acetylcholinesterase inhibitors compounds in *in Annona cherimola* Mill. by high-performance thin-layer chromatographic-bioassay-mass spectrometry
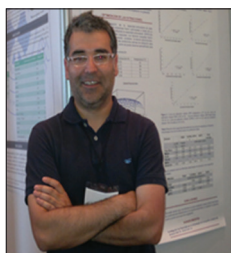
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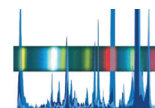
Acetylcholinesterase inhibitors (AChE) compounds become an important strategy in the treatment of neurological disorders such as Alzheimer's disease, senile dementia, ataxia, and myasthenia gravis. Cherimoya (*Annona cherimola* Mill.) is a fruit grown in subtropical areas with only a few studies about its bioactive compounds. For this reason, the objective of this work was to detect and identify AChE inhibitors in cherimoya by high-performance thin-layer chromatographic (HPTLC)-bioassay-mass spectrometry. An optimized enzymatic assay was performed on silica gel plate using a mobile phase composed of chloroform: methanol:ethyl acetate (80:14:6 v/v/v) that contains 1-naphthyl acetate (1.5 mg/mL) as enzymatic substrate. After chromatographic separation, the organic solvents were removed, and the plate was sprayed with an enzymatic solution (1 U/mL in 0.05 mol/Lof TRIS-hydrochloric acid buffer, pH 7.8). The enzymatic reaction was carried out at 37°C for 10 min, and the bands with AChE inhibitor compounds were detected as colorless zones over a purple background after atomizing with Fast Blue Salt B solution (1.0 mg/mL). The methodology was applied to methanol and dichloromethane extracts of pulp, peel, and seeds from cherimoya. Two bands showed inhibitory activity. A fast structural analysis was performed with several reagents showing positive reactions with ninhydrin and Dragendorff reagents. Thereafter, a preliminary identification was done by HPTLC-UV/VIS-electrospray ionization mass spectrometry, showing λ_{max} of 269 and 299 nm, and *m/z* values of 266 and 356, which matched with the alkaloids anonaine and glaucine. To the best of our knowledge, this is the first report about AChE inhibitory activity of these alkaloids.

ACKNOWLEDGMENT

Prof. Dr. Mario Aranda, University of Concepcion, Chile (Pharmacist, 2000), University of Concepcion (M.Sc., 2005), University Hohenheim, Germany (Ph.D., 2008). At present, Prof., University of Concepcion, Faculty of Pharmacy. Research fields: Functional foods, nutraceuticals, and foodomics. His research is focused on detection and evaluation of bioactive molecules with potential use against chronic noncommunicable disease.

PP-44

Analysis of polyphenols in Chilean wine by planar chromatography-mass spectrometry-bioassay

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Malolactic fermentation (MLF) is a critical step to obtain good quality wine. However, there are some factors such as low pH, high alcohol content, and some polyphenols that might alter this fermentative process. Wine polyphenols contribute to its organoleptic features, color, and astringency. There exist studies which indicate that polyphenols have an inhibitory effect on lactic bacteria responsible for MLF. Other authors have reported that polyphenols might also have a stimulating potential. Thus, the herein objective was to evaluate both activities, while identifying bioactive compounds existing on wine [Figure 1]. For this purpose, an high-performance thin-layer chromatography method was proposed which then permitted the separation of nine polyphenols (anthocyanins) existing on wine. Finally, the activity of extracts present in higher concentrations was evaluated on wine using bioassays, for which *Lactobacillus rhamnosus* was employed. Results show that malvidin-3-glucoside possesses a light stimulating activity. Resveratrol presents inhibitory activity. These results are of great importance for the winegrowing industry since using this type of polyphenol might be a solution to improve microbial stability during MLF.
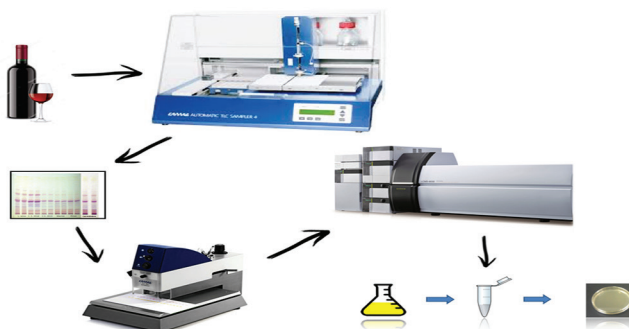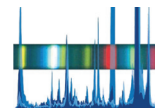

Figure 1: Diagram of analysis of polyphenols present in wine by high-performance thin-layer chromatography -bioassay-MS

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PP-45

Chemical and functional evaluation of fresh and black Chiloe's giant garlic (*Allium ampeloprasum* L.) by high-performance thin-layer chromatography-autography coupled to mass spectrometry
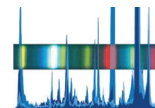
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Garlic is a food and medicinal herb that contains several kind of bioactive molecules to which beneficial effects over human health have been attributed. Chiloe's giant garlic (*Allium ampeloprasum* var. *ampeloprasum*) is botanically related to leek and it is microcultivated in Chiloe island (southern Chile), declared by Food and Agriculture Organization of the United Nations as a Global Importance Agricultural Heritage System. The present work reports for the 1st time a detailed chemical and functional evaluation of Chiloe's giant garlic applying high-performance thin-layer chromatography (HPTLC)-autography-mass spectrometry (MS). After bleaching and methanolic extraction, carbohydrates and organosulfur compound's profiles were determined by HPTLC/Vis with post-chromatographic derivatization with aniline-diphenylamine reagent and ninhydrin reagents, respectively. Sucrose (5.9 ± 0.2 mg/g) and fructose (278.4 ± 19.0 mg/g) showed the higher concentrations in fresh (FG) and black (BG) Chiloe's giant garlic, respectively. Organosulfur compounds alliin (1.7 ± 0.4 mg/g), metiin (46.5 ± 0.6 mg/g alliin equivalents [AE]), and isoalliin (7.1 ± 1.0 mg AE/g) were only detected in FG. The identity of each compound was confirmed by MS through TLC/MS interface. Antioxidant capacity was evaluated by 2,2-diphenylpicrylhydrazyl assay observing that γ-glutamyl-S-allyl-L-cysteine and γ-glutamyl-S-(*trans*-1-propenyl)-L-cysteine showed the highest antioxidant capacity. This activity has not been related o reported previously. Their identities were confirmed by LC/MS and HPTLC/MS. The presence of compounds capable of inhibiting acetylcholinesterase (AChE) and alpha-glucosidase (AG) enzymes was also evaluated. FG and BG extracts showed inhibition zones for AChE observing the following *m/z* 781, 365, 527, and 347. For AG, only BG extract showed inhibition zones. The structural elucidation of these compounds is in process.

Darlene Peterssen, University of Concepcion (analyst chemist, 2014), University of Concepcion (PhD student, Science and Analytical Technology doctorate program, 2015), University of Concepcion (academic collaborator in the Department of Food Science and Technology, Faculty of Pharmacy, 2015 - present). Presently, I work in my doctoral thesis entitled "I worlopment of a scientific and technological platform for the study of giant Chiloe's garlic functionality." Research fields: Bioaccessibility of bioactive compounds; food characterization.

SC-01

High-performance thin-layer chromatography method development - An insight!
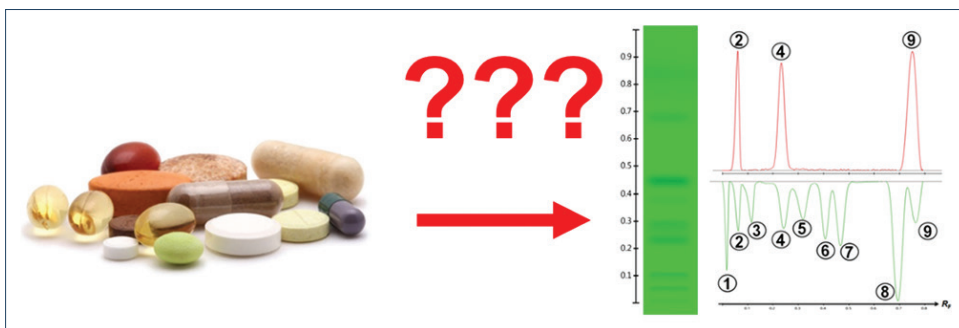
Saikat Subir Mallick
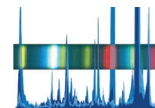
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Saikat Subir Mallick, Pratik Sinalkar

Lab Division, Anchrom Enterprises Pvt., Ltd., Mumbai, Maharashtra, India.

High-performance thin-layer chromatography (HPTLC) is a powerful, rapid, and reproducible method of analysis. It is suitable for qualitative and quantitative analytical tasks in many fields of applications, including identification and quantitation of constituents, impurities, and active substances, process development and optimization, process monitoring, and cleaning validation. As an off-line technique, HPTLC comprises several steps, which are independent in time and location. This offers great flexibility to the analytical process but at the same time requires controlling many parameters for routine methods to be robust and to deliver reliable results. Method development must consider all this. Practicability and fitness for purpose are two other important aspects. This workshop presents a practical strategy for managing various aspects of developing methods with high robustness and reproducibility. Examples of how to optimize all steps of HPTLC in view of the analytical goal of a given method are included in the discussion. Finally, we will introduce approaches to meaningful method validation.

Saikat Subir Mallick, Mumbai University (B.Sc., 2007), Mumbai University (M.Sc., 2009), Mumbai University (Ph.D., 2015), Worked as Analyst at a CRO Therapeutic Drug Monitoring Laboratory., Sion, Mumbai. (2009–2010), Ph.D. Scholar at Anchrom Test Laboratories (2010–2012), worked as Sr. R&D Executive in Sandu Research Foundation Pvt., Ltd. (2014–2017), Presently Assistant Manager at Anchrom Enterprises Pvt., Ltd. (2017–2018). Specialization in applied sciences.

SC-02

Analysis of botanicals in compliance with USP and Ph. Eur

Eike Reich

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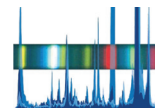**Eike Reich**

CAMAG, Switzerland.

The workshop will include a 30 min lecture explaining the terminology and definition of high-performance thin-layer chromatography (HPTLC) according to the United States Pharmacopeia <203> and European Pharmacopoeia chapter 2.8.25. The concept of HPTLC fingerprints is used to illustrate how compliance of a sample with the specifications in monographs for botanical/herbal drugs can be tested in a fully cross-calibrated multiplatform compliant environment. Furthermore, the lecture will demonstrate how the quality of botanical materials can be related to HPTLC data.

The second part of the workshop will use a live experiment, the identification of common horsetail and limit test for adulteration with marsh horsetail according to Ph. Eur. Monograph 1825, to introduce:

- The practical aspects of standardized HPTLC,
- Proper handling of equipment and software,
- System suitability tests,
- Image and profile comparison, and
- Limit tests based on image evaluation.

Eike Reich received his doctorate degree in Natural Products Chemistry in 1989 from Humboldt University, Berlin. From 1990 to 1998, he was Associate Professor of Chemistry at Longwood College, Virginia, USA. In 1998, he moved to Switzerland to join CAMAG as Head of Laboratory. His research focuses on applications of high-performance thin-layer chromatography, particularly in the field of botanicals. Dr. Reich has published numerous papers on the subject and contributed chapters to textbooks and encyclopedias. He is author of the book "HPTLC for the analysis of medicinal plants." He is founding member and since 2018 President of the International Association for the Advancement of HPTLC.

SC-03

Quantification and validation in high-performance thin-layer chromatography
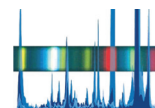
Jean-Marc Roussel

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Jean-Marc Roussel, Pierre Bernard-Savary

Independent Consultant, Mâcon, France, Chromacim sas and Club de CCM, Pommiers la Placette, France.

Since the 80 s and the first texts on analytical methods validation, many things have changed in the way we face this crucial step in our methods life cycle. The most recent recommendations seem to be very different from the "old" ICH Q2 (R1) requirements, and it is time to make a point on these different proposals. All suitable recommendations regarding how to build a quantitative high-performance thin-layer chromatography (HPTLC) plate will be described and explained. Starting from a short review of the different texts and recommendations, we will then focus on the particularities of quantitative HPTLC methods validation. Assessment of the calibration function in HPTLC might be tricky, and care should be taken when choosing the mathematical function, and useful tools such as back-calculated concentration analysis are of great help, and we show, using some examples, how to avoid the calibration pitfalls and judge the quality of the calibration function. Another important point is the misuse of the precision and trueness information, there has been for a long time confusion trueness and accuracy. Accuracy involves a random component (precision) and a systematic component (trueness). We explain and discuss the latest proposals for accuracy assessment and show their interest in the case of HPTLC method validation. We particularly focus on USP <1210> proposal for accuracy determination in the case of drug substances or products and on the use of the so-called beta-expectation tolerance interval, in the case of more complex samples (biological, foodstuff, plant extracts…). We also discuss about uncertainty of measurement and the different ways to extract this information from a modern validation design, either from accuracy or from precision information. We also demonstrate that quantitative HPTLC methods can be validated in a very easy and convenient way, by means of a balanced validation design, using a reasonable number of HPTLC plates and amount of time. Keeping in mind that everything starts and ends with a good quantification on a suitable plate.

Jean-Marc Roussel, Aix-Marseille University (M.Sc. 1984, Ph.D. 1988), At present, Independent Consultant on Assay Methods Development and Validation. President of the French Society for Pharmaceutical Sciences (SFSTP) commission on Uncertainty of Measurement. Codesigner of NeoLiCy®, software for Analytical Methods' Life Cycle Statistical Assessment.

SC-05

New workflows for biological and enzymatic assays combined with planar chromatography

Gertrud Morlock

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Gertrud Morlock

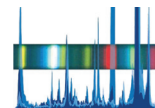*Justus Liebig University Giessen, Chair of Food Science, Giessen, Germany.*

An overview is given on effect-directed analysis and the different techniques reported so far. The proper terminology is clarified, as it is wrongly used in literature here and then. Direct bioautography is focussed in detail, as it is the most straightforward technique of effect-directed analysis. Latest novel workflows for biological and enzymatic assays are reported, which opened up new avenues and shifted the field to modern direct bioautography. Current workflows have led to sharply bounded active zones on a colorful, homogeneous background. These results paved the way for quantitative direct bioautography. Such meaningful autograms were rational to be subjected to densitometric measurements. The trick of inverse scanning is explained and needed to measure zones absorbing less than the background (e.g., bright zones on a colored background). The obtained peaks of active compounds were evaluated and quantified based on external standards. Equivalency calculation with reference to a well-known active compound (e.g., same as positive control) can be an alternative if the active compound is not identified or known. The selection of a positive and negative control is explained as well as the relevance to apply these on each plate. The difference in the results is illustrated when positive control levels for equivalency calculation are only applied versus developed. System blanks are conditio sine qua non and need to be performed as well for each sample set. New application modes of the application of the bioassay suspension or enzymatic solution onto the chromatogram are explained like the peculiarities of piezoelectric spraying. Results of piezoelectric spraying are compared with automated immersion, which is the current status quo. Examples in the field of functional ingredients in food and active compounds in plants are given that illustrate the potential of modern quantitative direct bioautography.

REFERENCE

1. Morlock GE. Bioassays and further effect-directed detections in chromatography. In: Worsfold PJ, Poole C, Townshend A, Miro M editors. Reference Module in Encyclopedia of Analytical Science. 3rd ed. Amsterdam: Elsevier Science; 2018.

Gertrud Morlock is full professor at the Justus Liebig University Giessen in Germany. She holds the Chair of Food Science and, currently, also chairs the Institute of Nutritional Science. She is Director of the TransMIT Center for Effect-Directed Analysis. She has made about 130 peer-reviewed original research paper since 2006, 74 further scientific papers, and 14 book chapters, is editor of the free-of-charge CAMAG CBS journal and its online database CCBS containing ca. 11000 abstracts on TLC/HPTLC, conducted about 70 workshops, presented about 240 posters and 260 lectures at symposia and is active in several scientific committees.

SC-06

High-performance thin-layer chromatography–mass spectrometry for characterization of compounds

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Michaela Oberle[1], Ivan Ng[2]

[1]*Merck Life Science, Frankfurter Straße 250, D-64293 Darmstadt, German,* [2]*Manager, System Solutions Business Development, Southeast Asia, Waters Pacific Pte. Ltd., Singapore.*
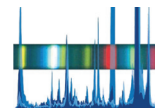
Due to easy handling and less equipment effort, thin-layer chromatography (TLC) is a widely spread analytical method. Direct coupling with mass spectrometry (MS) makes this method successful. This can be shown in various application examples. You are invited to learn more about the advantages of planar chromatography and the principle of the TLC-MS coupling. During the workshop, you will get an introduction to today's modern high-performance TLC (HPTLC), as a robust method, with a focus on HPTLC-MS coupling. You will also learn more about the techniques during an open discussion along a presentation "tips and tricks" for HPTLC-MS coupling. Introduction of the waters Acquity QDa mass detector system. The world's first tool-free maintenance mass detector system. The intuitive software and hardware designs helps user to navigate through the setup as well as operation of the system in a breeze. We will show you how easily it is to operate and as well to maintain the detector which provides mass quality data at the ease of an optical level detector.

Michaela Oberle R&D Project Leader and Specialist for thin-layer chromatography within instrumental analytics at Merck KGaA, Darmstadt, Germany. Bachelor of Science in Business Chemistry (Fresenius Hochschule Idstein, 2014). Joint Merck in 1992 and took over responsibilities in various departments as a specialist for analytical, preparative and flash chromatography; extraction and analysis of natural compounds; cosmetic efficacy screenings including skin and hair applications; product development, TLC application, and international trainings for TLC issues.

Ivan Ng, System Solution Business Development Manager, SEA, joined Waters in 2008 and was responsible for business development in Singapore. As part of his growing portfolio and areas of expertise, he had the additional responsibility to manage Waters local business partners to develop strategies and business plans to promote empower. When he took on the role of Regional LC and Informatics Sales Manager for South East Asia, his focus is to develop initiatives to communicate the value of regulatory compliance and Empower Network. In his current role, heading a team of Business Development Specialists. The team is responsible for the development of strategies and customer education activities on water's informatics and chromatographic and mass spectrometry solution.

MP-01

Thin-layer chromatography/high-performance thin-layer chromatography plates - recent developments and applications

Monika Bäumle
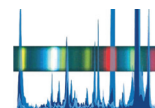
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Monika Bäumle

Merck KGaA, Darmstadt, Sigma-Aldrich Chemie GmbH, Switzerland.

Thin-layer chromatography (TLC) and high-performance TLC (HPTLC) are a very convenient chromatography method due to its robust separation efficiency, high sample throughput, and simple sample preparation. It enables reproducible results and allows multi detection approach. HPTLC combines the advantages of reliable chromatography performance and simple connection to other techniques such as mass spectrometry (MS), Fourier transform infrared, nuclear magnetic resonance, or bioassays. The hyphenation with MS will contribute to the rejuvenated interest in planar chromatography, and TLC/HPTLC-MS is expected to be a field of future growth. With increasing sensitivity, however, any impurities will negativity affect limit of detection. High-quality plates (MS-grade) are prerequisites. To fulfill current and future need of commercially available HPTLC plates of the highest quality, continues development and improvements are key. The lecture will focus on recent improvements in plate development and applications.

Monika Bäumle holds a doctoral degree in chemistry. She has joined Sigma-Aldrich Switzerland in 2004 as Senior Scientist in Research and Development. After 2.5 years' experience as Lab Manager, and Lab Group Manager in the Research and Development, she has started position as global senior product manager for bioanalytics; proteomics and biochemistry at Sigma-Aldrich Merck. Since April 2018, she holds a position as global product manager for thin-layer chromatography.

MP-02

# Are there truly essential rose oils in cosmetics and food?

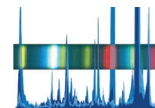**Pia Altenhofer**

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The essential oil of rose is very expensive and target of imitation or adulteration. Many companies claim that their cosmetics or food contains natural ingredients and creates the impression that real rose extract is included. Food, rose extracts, and cosmetics were investigated in comparison with reference terpenoids by high-performance thin-layer chromatography (HPTLC) to determine if real essential rose oil was actually present in the sample. In addition, product information and literature were checked for conformity. Samples were extracted or diluted in toluene, separated on silica-coated HPTLC plates, and developed in the appropriate mobile phases. In addition, two-dimensional separations were done to detect an overlay of substances. The visualization was performed under visible light as well as ultraviolet. Two reagents were used for derivatization. The results showed that essential rose oils vary in their compositions depending on their origin and method of production. The information provided with the products may differ from the HPTLC results as well as literature statements. There are perfumes showing a substance pattern similar to essential oils which leads to the assumption that real rose oil was used in production. Other perfumes seem to be artificial compounded although the manufacturer claims to practice plant cosmetics. In tea compositions containing rose flower leaves, it was possible to detect substances which are also present in different rose extracts, but the major component citronellol as a marker for natural rose oil is nearly completely absent. In rose flower leaf jam from Bulgaria, it was impossible to detect the classical components such as β-damascenone and rose oxide that are responsible for the typical scent of Bulgarian rose oil as mentioned in literature. The jam contained substances that were also found in Rose Absolue. The techniques of HPTLC are perfectly suitable for detecting such challenges.
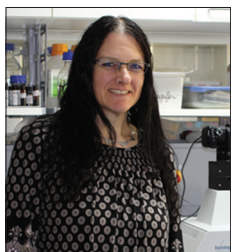
Pia Altenhofer, biostep GmbH Germany, Saarland University (Diploma in biology 1995), Leibniz-Institute for Plant Genetics and Crop Plant Research Gatersleben, Martin-Luther University Halle-Wittenberg. At present, biostep GmbH, Burkhardtsdorf, Germany. biostep GmbH took over the TLC/HPTLC product range from Desaga GmbH. Field of work: Improvement of TLC/HPTLC equipment and separation of essential oil components.

MP-03

Shaping the future of high-performance thin-layer chromatography: The International Association for the Advancement of High-Performance Thin-Layer Chromatography

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Eike Reich

HPTLC Association.

Since 2012, when it was incorporated as a private, non-profit organization according to Swiss law, the High-Performance Thin-Layer Chromatography (HPTLC) Association is actively promoting the use of HPTLC in plant analysis and other analytical fields, bringing together representatives from industry, academia, research, as well as regulatory and standard-setting bodies.
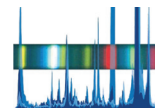
Major accomplishments are collaborations with the US and European Pharmacopoeias resulting in the establishment of the general chapters <203> and 2.8.25, which define HPTLC as highly standardized methodology. Members of the association contributed >200 methods for the identification of herbal drugs, many of which were adopted in pharmacopoeial monographs.

The current focus is on establishing and maintaining the "International Atlas of HPTLC Methods for Identification of Herbal Drugs (ATLAS)" as a globally recognized reference tool for quality control. Pilot projects for setting up collaboration with industry, regulators, and academia are in progress in Thailand and India and the Forum for Harmonization of Herbal Medicines in Asia/Western Pacific Region.

To globally promote the benefits of HPTLC as a routine analytical technique also to other industries, training modules are under development. The HPTLC Association is soliciting applications for membership from interested practitioners and researchers from all over the world.

Eike Reich received his doctorate degree in Natural Products Chemistry in 1989 from Humboldt University Berlin. From 1990 to 1998, he was Associate Professor of Chemistry at Longwood College, Virginia, USA. In 1998, he moved to Switzerland to join CAMAG as the Head of Laboratory. His research focuses on applications of high-performance thin-layer chromatography, particularly in the field of botanicals. Dr. Reich has published numerous papers on the subject and contributed chapters to textbooks and encyclopedias. He is the author of the book "HPTLC for the analysis of medicinal plants." He is a founding member and since 2018 President of the International Association for the Advancement of HPTLC.

www.hptlc.com

MP-04

Mass detection for routine analysis

Ivan Ng

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Ivan Ng

Manager, System Solutions Business Development, Southeast Asia, Waters Pacific Pte. Ltd., Singapore.

Mass detection is widely recognized as a reliable method to derive more meaningful information from your samples, but is not necessarily used by all analytical scientists. Conventionally, mass detection is seen as a costly technique, consuming bench space and needing sample-specific, careful adjustment, and very often requiring training of an expert user to manage its complexity. Hence, mass detection tends to be inaccessible to many laboratories. The Waters QDa Mass Detector is purposefully designed and built to address that problem. It is as intuitive as an optical detector and yet brings about the information-rich data of a mass detector. For the 1ˢᵗ time, any analytical scientist can consistently generate the highest quality mass spectral data routinely.

Ivan Ng, System Solution business development manager, SEA, joined Waters in 2008 and was responsible for business development in Singapore. As part of his growing portfolio and areas of expertise, he had the additional responsibility to manage waters local business partners to develop strategies and business plans to promote empower. When he took on the role of Regional LC and Informatics sales manager for South East Asia, his focus is to develop initiatives to communicate the value of regulatory compliance and Empower Network. In his current role, heading a team of business development specialists. The team is responsible for the development of strategies and customer education activities on water's informatics and chromatographic and mass spectrometry solution.

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