Countercurrent Chromatography Conference 2016

Program & Book of Abstracts

CCC 2016

CCC 2016 | August 1-3, 2016 | Chicago

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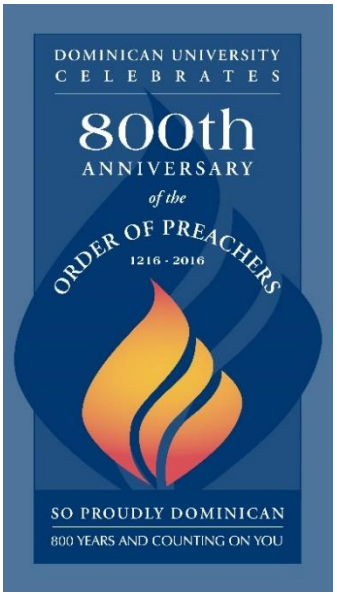
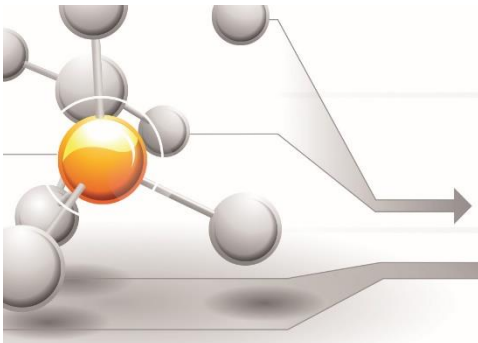


DOMINICAN UNIVERSITY

CENAPT
Center for Natural Products Technologies at UIC



ARMEN INSTRUMENT

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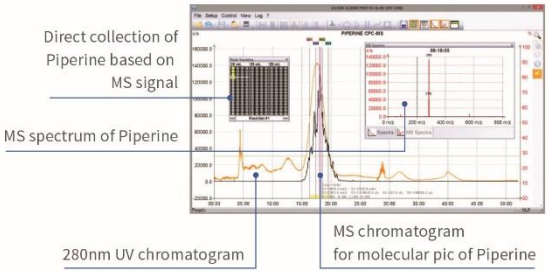
Example of purification of Piperine from crude black pepper extract by SCPC-MS



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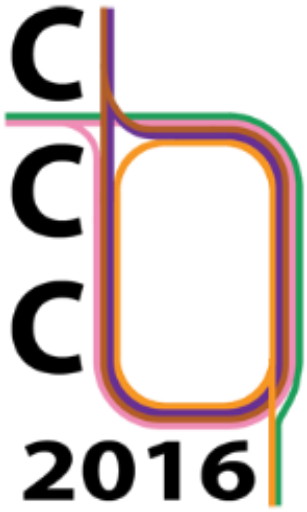
The CCC 2016 Logo



The Loop is the heart of Chicago. Every day, thousands of Chicagoans rely on the efficiency of the public rail system ("The L" for short). More often than not, their trip on The L will take them through The Loop, a set of concentric tracks that facilitates the passage of six train routes. Through the eyes of a chromatographer, the Chicago Loop mirrors the elegance of Countercurrent Chromatography: trains speed by in opposite directions and commuters transfer between routes until ultimately, they reach their respective destinations. We look forward to this exchange of ideas and extend a warm welcome to our great city.

Logo concept by Laura Gauthier, UIC

Contents

Organizers

CHAIR	Brent Friesen (Dominican University)
CO-CHAIR	Guido Pauli (University of Illinois at Chicago)

Local Organizing Committee

Jim McAlpine (University of Illinois at Chicago)
Shao-Nong Chen (University of Illinois at Chicago)
Lucas Chadwick (Bell's Brewery, Michigan)
Jonathan Bisson (University of Illinois at Chicago)
Charlotte Simmler (University of Illinois at Chicago)
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Rasika Phansalkar
Amandine Nachtergael
Yang Liu
Yang Yu
Mary Choules
I-Soo Youn
Edyta Grzelak
Gonzalo Malca

WORKSHOP

Saturday July 30 – Sunday July 31 2016

The workshop will be held in the Physical Science Department facility on the 3rd floor of Parmer Hall. Room 331.

OPENING RECEPTION

Sunday, July 31 at 7:00 PM The opening reception will be held in the Noonan reading room on the second floor of Lewis Hall. (7 on campus map).

CONFERENCE

Monday, August 1 – Wednesday, August 3 2016

The conference, poster sessions, and Exhibition will be held in the Parmer Hall atrium. See (11) on campus map.

LUNCHES

Lunch will be provided for the workshop attendees on both Saturday and Sunday in the Parmer Hall 3rd floor student commons.

On conference days (Monday, Tuesday, & Wednesday) lunch will be served in the Parmer Hall atrium where the poster sessions and Exhibition are being held.

DINNERS

Sunday evening's reception will be held in the Noonan Reading room in Lewis Hall Monday, the dinner will be held in the Dominican University quad (14 on campus map) next to the social hall (9 on campus map).

Tuesday, the dinner will be part of the City of Chicago outing (see SOCIAL PROGRAM).

CONFERENCE DINNER

The conference dinner, Wednesday August 3rd, will be held in the Parmer Hall atrium. The social hour start at 6 PM followed by the dinner at 7 PM at the same location.

SOCIAL PROGRAM

On Tuesday, August 2nd, there will be an evening visit to downtown Chicago. Transport will be provided to take delegates to Chicago and return them to either the Carlton hotel or Dominican University.

Campus Map


Campus Buildings
Parking Locations
Wheelchair Accessible
Handicap Parking
Park Avenue
MAIN ENTRANCE
Division Street
Greenfield Street
Dominican Drive
Timothy Avenue
N
E
S
W

Pre-conference – Sunday July 31, 2016

Opening Reception

(Su) 7:00p Opening reception in Noonan reading room on the 2nd floor of Lewis Hall

Day 1 – Monday August 1, 2016

Conference Opening

(Mo) 08:30 Registration

(Mo) 09:00 **Jeff Breese** - Provost and VP of Academic Affairs of Dominican University
Welcome to Chicago and DominicanUniversity

(Mo) 09:05 **Brent Friesen & Guido Pauli- Cochairs of CCC2016**
What is hot in the CCC Loop?

Session I – Natural Products I

Chairs: Ian Sutherland & Yoichiro Ito

(Mo) 09:35 FO **Gerold Jerz (Braunschweig, Germany)** **#2003**
Mass-spectrometric separation strategies for the guided recovery of genotoxic pyrrolizidine alkaloids from plant and food sources using all-liquid chromatography techniques

(Mo) 10:00 SO **Krystyna Skalinka-Woźniak (Lublin, Poland)** **#2004**
Hyphenated HPCCC and HPLC/DAD/ESI-TOF as
a platform for searching of biologically active coumarins from Apiaceae plants

(Mo) 10:13 FP **Yaoguang Liang (Zhongshan, China)** **#2013**
Rapid purification and scale-up separation of three makamides from *Lepidium meyenii* using high-capacity high-speed counter-current chromatography

10:17 – 10:47 **Tea & Coffee, Posters (odd numbers) & Exhibition**

Session II – Natural Products II

Chairs: Alain Berthod & Peter Hewitson

Session Sponsor **Armen Instrument (St. Avé, France)**

(Mo) 10:47	FO	**Karine Faure (Lyon, France)**	**#2012**
		Comprehensive two-dimensional CPC-LC: providing analytical and preparative separations systems for complex Edelweiss extracts	
(Mo) 11:12	SO	**David Ward (Uxbridge, UK)**	**#2024**
		Purification of monosaccharides from crude hydrolysed sugar beet pulp for the production of sustainable chemical feedstocks	
(Mo) 11:25	SO	**Gregoire Audo (Vannes, France)**	**#2036**
		Preparative separation of marine bioactive compounds by centrifugal partition chromatography	
(Mo) 11:38	FP	**Gerold Jerz (Braunschweig, Germany)**	**#1016**
		Recovery of antibacterial cystobactamids from *Cystobacter* sp. by HPCCC and off-line ESI-MS/MS metabolite profiling	
(Mo) 11:42	FP	**Jizhong Yan (Hangzhou, China)**	**#1013**
		Separation of isomeric monosaccharides by recycling elution-extrusion countercurrent chromatography	
(Mo) 11:46	FP	**Shengqiang Tong (Hangzhou, China)**	**#2011**
		Enantioseparation of aromatic acids by precolumn derivatization countercurrent chromatography	

12:00 – 1:30 **Lunch, Posters & Exhibition**

Session III – Natural Products III

Chairs: Tianyou Zhang & Svetlana Ignatova

(Mo) 01:30 FO **Shihua Wu (Hangzhou, China)** **#2031**
On-demand purification of natural products by counter-current chromatography

(Mo) 01:55 SO **Ian Sutherland (Uxbridge, UK)** **#2023**
The role of counter-current chromatography in the modernisation of traditional chinese medicines: 8 years on

(Mo) 02:08 SO **Xueli Cao (Beijing, China)** **#2032**
Separation and research of anti-tumor active components in *Zanthoxylum ailanthoides* Sieb. & Zucc.

(Mo) 02:21 SO **Xiaohua Jiang, Zhenghong Pan, Xiaojie Yan (Guilin, China)** **#1030**
Preparative Isolation of Flavone *C*-Glycosides from *Peristrophe baphica* by Counter-Current Chromatography Coupled with Other Techniques

(Mo) 02:44 FP **Yun Wei (Beijing, China)** **#2002**
Online enrichment and separation of five flavonoids compounds from *Mikania micrantha* using magnetic nanomaterials coupled with high speed countercurrent chromatography

02:50 – 3:20 **Tea & Coffee, Posters (even numbers) & Exhibition**

Session IV – Natural Products IV

Chairs: Jim McAlpine & Karine Faure

(Mo) 03:20 FO **Qizhen Du (Hangzhou, China)** **#2001**
Preparative separation of sucrose monoesters, diesters and polyesters made of palm oil and sucrose by high-speed countercurrent chromatography

(Mo) 03:45 SO **Tsvetelina Mandova (Paris, France)** **#2015**
Purification of bioactive compounds from *Centaurium erythraea* Rafn. by conventional and new generation designed centrifugal partition chromatography column coupled with MS detector

(Mo) 03:58	SO	**Nektarios Aligiannis (Athens, Greece)** A high-throughput procedure based on CPC-fractionation for the discovery of skin whitening agents from Greek flora extracts	**#1031**
(Mo) 04:11	SO	**Gilda Guimaraes Leitão (Rio de Janeiro, Brazil)** Alternating isocratic and gradient elution CCC for the isolation of minor phenolics from *Ormocarpum kirkii* barks	**#2026**
(Mo) 04:24	SO	**Dalene de Beer (Stellenbosch, South Africa)** comprehensive two-dimensional CCCxLC analysis for improved separation of Rooibos polyphenols	**#2006**
(Mo) 04:50	SO	**Rasika Phansalkar (Chicago, IL USA)** Centrifugal partition chromatography (CPC) enriches dentin-bioactive trimeric and tetrameric proanthocyanidins from medicinal plants	**#1011**
(Mo) 5:03	FP	**Marcela Elizabeth Castro-Benítez (Bogotá, Columbia)** Isolation and characterisation of chlorophylls and xanthophylls in grass by a novel solvent system using countercurrent chromatography	**#1012**
(Mo) 5:07	FP	**Gerold Jerz (Braunschweig, Germany)** Fractionation of bisdesmodic saponins with anti-tumor enhancing activity from *Saponaria officinalis* by HPCCC, use of natural chiral selectors and monitoring by off-line ESI-MS/MS injections	**#1002**
(Mo) 5:11	FP	**Edy Sousa de Brito (Ceará, Brazil)** Value of *K* for application of counter-current chromatography in the isolation of three lipopetide families	**#1018**
5:15		**Group Photo**	
5:30		International Committee Meeting	
6:00		**Reception &**	
7:00		**Dinner in Social Hall**	

Session V – Solvent Systems I

Chairs: Kazufusa Shinomiya & Martha Knight

(Tue) 09:00 FO **Walter Vetter (Stuttgart, Germany)** **#2018**
CCC separation strategies for very nonpolar lipid compounds

(Tue) 09:25 FO **Franziska Bezold (Freising, Germany)** **#2021**
Deep eutectic solvent systems in liquid-liquid chromatography

(Tue) 09:50 SO **Yang Liu (Chicago, IL USA)** **#2010**
Matching sweet spots: refining a TLC-based countercurrent solvent system selection strategy

(Tue) 10:03 SO **Edyta Grzelak (Chicago, IL USA)** **#1010**
Bio-GUESS based countercurrent separation of anti-tuberculosis lead compounds from Actinomycetes

10:17 – 10:47 **Tea & Coffee, Posters (even numbers) & Exhibition**

Session VI –Solvent Systems II

Chairs: Qizhen Du & Guy Harris

(Tue) 10:47 KO **John MacMillan (Dallas TX, USA)** **#3002**
High-Throughput Functional Annotation of Natural Products

(Tue) 11:27 FP **Svetlana Ignatova (Uxbridge, UK)** **#1056**
Development of a counter-current chromatography-based extraction method for emerging contaminants from river water

(Tue) 11:31 FP **Gilda Guimaraes Leitão (Rio de Janeiro, Brazil)** **#2025**
Countercurrent separation of natural products: verbenone from rosemary essential oil

(Tue) 11:35 FP **Duo-Long Di (Lanzhou, China)** **#1054**
Three solvent system CCC combined the use of O-carboxymethyl chitosan as an additive for separation of chemical components in *Lycium barbarum* L.

(Tue) 11:39 FP **Aihua Peng (Chengdu, China)** **#1029**
Quick selection of solvent system for counter-current chromatography separation with one simple HPLC method

(Tue) 11:41 FP **Emma Brace (West Lafayette, IN USA)** **#1019**
Enhancing silymarin fractionation using the conductor-like screening model for real solvents

(Tue) 11:45 FP **Amandine Nachtergael (Chicago, IL USA)** **#1007**
Probing the combinatorial metabolome of flavanolignans in milk thistle (*Silybum marianum* L.)

(Tue) 11:49 FP **Franziska Bezold (Freising, Germany)** **#1020**
Tocopherol separation with deep eutectic solvent-based biphasic systems

12:00 – 1:30 **Lunch, Posters & Exhibition**

Session VII – Large & Industrial Scale

Chairs: Xueli Cao & Gerold Jerz

Session Sponsor **Dynamic Extractions (Gwent, UK)**

(Tue) 01:30	SO	**Laszlo Lorantfy (Dabas, Hungary)** What is Industrial Scale in CPC?	**#2005**
(Tue) 01:43	FO	**Jean-Hugues Renault (Reims, France)** Process intensification and scale-up in pH-zone refining CPC: study case of the purification of alkaloids from *Catharanthus roseus*	**#2029**
(Tue) 02:08	FO	**Guy Harris (Gwent, UK)** Pilot scale purification of xanthophylls for D-Factory using CCC	**#2033**
(Tue) 02:33	SO	**Fernanda das Neves Costa (Rio de Janeiro, Brazil)** *Schinus terebinthifolius* scale-up: CCC method transfer to CPE and CPC	**#2035**
(Tue) 02:46	FP	**Apostolis Angelis (Athens, Greece)** A new process for the analysis of mastic gum and isolation of bioactive triterpens and polymer	**#1026**

02:50 – 03:20 **Tea & Coffee, Posters (odd numbers) & Exhibition**

Session VIII – Applications I

Chairs: Gilda Guimarães Leitão & Yue Hugh Guan

(Tue) 03:20 SO **Raena Morley (Freising, Germany)** **#2022**
Model-based design of a sequential centrifugal partition chromatography process for the preparative batch separation of ternary mixtures

(Tue) 03:33 FP **Shihua Wu (Hangzhou, China)** **#1043**
Strategy for pH-dependent tailing in CCC: alkaloids of *Nelumbo nucifera* GAERTN as examples

(Tue) 03:37 FP **Kyoung Jin Lee (Seoul, Korea)** **#1023**
Linear gradient elution in CCC with average speed of target compounds

(Tue) 03:41 FP **David Ward (London, UK)** **#1032**
Scale up purification of monosaccharides from crude hydrolysed sugar beet pulp

4:30 **Busses leave for Chicago outing**

Session IX – Columns I

Chairs: Shihua Wu & Adrian Weisz

Session Sponsor **Tauto Biotech (Shanghai, China)**

同田生物
TAUTO BIOTECH

(Wed) 09:00 FO **Michael Englert (Stuttgart, Germany)** **#2019**
Advancement of countercurrent chromatography instrumentation by tubing modifications

(Wed) 09:25 FO **Duo-Long Di (Lanzhou, China)** **#2039**
Melamine modified counter-current chromatography column and its separating mechanism

(Wed) 09:50 SO **Aihua Peng (Chengdu, China)** **#2027**
Novel approach to sample injection in counter-current chromatography: case study of honokiol purification

(Wed) 10:03 FP **Martha Knight (Rockville, MD USA)** **#1040**
The Rotify ® bench-top centrifugal precipitation chromatograph

(Wed) 10:07 FP **Yue Hugh Guan (Shanghai, China)** **#1051**
Rational development of conical columns on J-type counter-current chromatography for protein separation using aqueous-two phase systems

10:17 – 10:47 **Tea & Coffee, Posters (odd numbers) & Exhibition**

Session X – Columns II

Chairs: Dalene de Beer & Walter Vetter

(Wed) 10:47 FO **Martha Knight (Rockville, MD USA)** **#2030**
Spiral countercurrent chromatography in its many forms

(Wed) 11:12 FO **Yue Hugh Guan (Shanghai, China)** **#2037**
Connect fundamentals to applications for counter-current chromatography: new rules, third force, virtual column and scaling-up for protein separation

(Wed) 11:37 FP **Peter Hewitson (Uxbridge, UK)** **#1037**
Additive manufacturing: what can it do for the counter-current chromatography researcher

(Wed) 11:41 FP **Yue Hugh Guan (Shanghai, China)** **#2038**
The working mechanism of toroidal columns on J-type counter-current chromatographs

(Wed) 11:45 FP **Shihua Wu (Hangzhou, China)** **#1042**
Concentric coils for counter-current chromatography

(Wed) 11:49 FP **Daniel Kulakowski (Chicago, IL USA)** **#1046**
K-targeted purification of C-glycosylflavones from *Vitex agnus-castus* by orthogonal countercurrent methods

(Wed) 11:53 FP **Hiromitsu Aoki (Hiroshima, Japan)** **#1004**
Development of precipitation couter-current chromatography

(Wed) 11:57 FP **Kazumasa Zaima (Chiba, Japan)** **#1008**
Comparison of partition efficiency between satellite and planetary motions of coil satellite centrifuge for counter-current chromatographic separation of 4-methylumbelliferyl sugar derivatives

12:00 – 1:30 **Lunch, Posters & Exhibition**

Session XI – Applications II

Chairs: Jean-Hugues Renault & Fernanda das Neves Costa

(Wed) 01:30 FO **Peter Hewitson (Uxbridge, UK)** **#2028**
The effect of column aspect ratio on separation in counter-current chromatography

(Wed) 01:55 SO **Marcela Elizabeth Castro-Benítez (Bogotá, Columbia)** **#2009**
Isolation of tocopherols and tocotrienols as constituents of γ oryzanol in baby banana peels with hyperpigmentation by means of spiral coil-LSRCCC

(Wed) 02:08 SO **Adrian Weisz (College Park, MD USA)** **#2016**
Speculations on the formation of a double peak during high-speed counter-current chromatographic separation of an azo dye

(Wed) 02:21 SO **Laura L. Gauthier (Chicago, IL USA)** **#1039**
Designer extracts: targeted depletion of metabolites from botanical extracts using countercurrent separation

(Wed) 02:34 FP **Aihua Peng (Chengdu, China)** **#1036**
Optimization of sample injection in counter-current chromatography

02:50 – 03:20 **Tea & Coffee, Posters (even numbers) & Exhibition**

Session XII – Theory & Modelling

Chairs: Krystyna Skalicka-Woźniak & Hiso Oka

(Wed) 03:20 FO **Gerhard Schembecker (Dortmund, Germany)** **#2007**
Centrifugal partition chromatograph: a continuous multiphase reactor

(Wed) 03:45 FO **Yoichiro Ito (Bethesda, MD USA)** **#2034**
Two-phase motion in hydrodynamic counter-current chromatography: which phase travels toward the head of the coil in hydrodynamic counter-current chromatography?

(Wed) 04:10 SO **Alain Berthod (Lyon, France)** **#2008**
Stationary phase retention in CCC: differences between hydrostatic and hydrodynamic columns

(Wed) 04:23 SO **Léa Marlot (Lyon, France)** **#1003**
K-values in CPC: is prediction reliable?

(Wed) 04:36 FP **Peter Hewitson (Uxbridge, UK)** **#1038**
Computational fluid dynamics modelling of secondary flow in counter-current chromatography instruments

5:00 – 5:30 **Closing Remarks**

5:30 – 6:30 **Cocktail hour**

6:30 – 8:30 **Conference Dinner**

Address by Donna Carroll (President, Dominican University)

Awards Ceremony

♪♫ **Entertainment provided by Roy Schroedl**

PO **Krystyna Skalicka-Woźniak (Lublin, Poland)** **#1001**
Preparative separation of bergapten as a novel compound for the treatment of nicotinism

PO **Gerold Jerz (Braunschweig, Germany)** **#1004**
Metabolite profile of betalains and flavonoids from *Opuntia stricta* var. *Dilleni* by HPCCC and off-line ESI-MS/MS

PO **Gerold Jerz (Braunschweig, Germany)** **#1005**
Recovery of the betacyanin celosianin II and flavonoid glycosides from *Atriplex hortensis var. rubra* by HPCCC and off-line ESI-MS/MS monitoring

PO **Feriel Bouiche (Lyon, France)** **#1006**
In-situ protein determination to monitor CPC contamination

PO **Karine Faure (Lyon, France)** **#1014**
Production of three new antioxidants from edelweiss by multi-heart cutting CPC-LC

PO **Karine Faure (Lyon, France)** **#1015**
Carnosol purification from *Rosmarinus officinalis* by centrifugal partition chromatography, from laboratory to industry

PO **Gerold Jerz (Braunschweig, Germany)** **#1017**
Fractionation of lipophilic components from potatoes (*Solanum phureja*) by HPCCC and monitoring by off-line injections to APCI-MS/MS

PO **Franziska Bezold (Freising, Germany)** **#1020**
Protein separation using a centrifugal partition extractor

PO **Kyoung Jin Lee (Seoul, Korea)** **#1022**
Relationship between the efficiency and rotation speed in the countercurrent chromatography: separation of cytotoxic metabolites by selective enzymatic transformation

PO **Kwang Ho Song (Seoul, Korea)** **#1024**
Application of counter-current chromatography as a powerful fractionation tool. case study: obtaining gram-scale sesquiterpenoids from *Tussilago farfara*

PO **Kwang Ho Song (Seoul, Korea)** **#1025**
Preparative separation of euphorbia factors from *Euphorbia lathyris* by countercurrent chromatography

PO **Raena Morley (Freising, Germany)** **#1027**
Development of a two-dimensional sequential centrifugal partition chromatography process for the preparative separation of ternary mixtures

PO **Raena Morley (Freising, Germany)** **#1028**
Continuous fractionation of multicomponent mixtures with sequential centrifugal partition chromatography

PO **Gilda Guimarães Leitão (Rio de Janeiro, Brazil)** **#1033**
Separation of 5,6-dihydro-α-pyrones from *Hyptis monticola* by high-speed countercurrent chromatography (HSCCC)

PO **Gilda Guimarães Leitão (Rio de Janeiro, Brazil)** **#1034**
Countercurrent chromatography with off-line detection by HPLC-ESI-MS/MS for the separation and identification of saponins from *Ampelozizyphus amazonicus*

PO **Gilda Guimarães Leitão (Rio de Janeiro, Brazil)** **#1035**
Alkaloids from *Triclisia dictyophylla* by pH-zone refining CCC

PO **Shihua Wu (Hangzhou, China)** **#1044**
Room temperature ionic liquids-based salting-in strategy for counter-current chromatography

PO **Simon Röhrer (Freising, Germany)** **#1045**
Novel non-aqueous biphasic solvent systems in centrifugal partition chromatography

PO **Apostolis Angelis (Athens, Greece)** **#1047**
An integrated process for the recovery of high added-value compounds from extra virgin olive oil using solid-support free liquid-liquid extraction and chromatography techniques

PO **Fernanda das Neves Costa (Rio de Janeiro, Brazil)** **#1048**
Isolation of metabolites from mangrove plant *Rhizophora mangle* by countercurrent chromatography

PO **Grégoire Audo (Saint-Avé, France)** **#1049**
Multiple dual-mode CPC as an efficient tool for the purification of caulerpenyne from *Caulerpa taxifolia*

PO **Grégoire Audo (Saint-Avé, France)** **#1050**
Caulerpenyne from *Caulerpa taxifolia*: a comparative study between CPC and classical chromatographic techniques

PO **Duo-Long Di (Lanzhou, China)** **#1052**
Combining several elution modes to separate compounds from complex matrix

PO **Duo-Long Di (Lanzhou, China)** **#1053**
Separation and purification of active components from *Lycium barbarum* L. by HSCCC using dual-mode elution

PO **Nektarios Aligiannis (Athens, Greece)** **#1055**
Separation of saponins from *Silene colorata* by using centrifugal partition chromatography

PO **Douglas Armstrong (Bourbonnais, IL USA)** **#1057**
Countercurrent chromatography fractions of plant extracts with anti-tuberculosis activity

PO **Sri Murhandini (Jakarta, Indonesia)** **#1058**
Isolation and purification of α-mangostin from indonesian *Garcinia mangostana* L. rinds using high performance counter-current chromatography

PO **Adrian Weisz (College Park, MD USA)** **#2017**
Separation and identification of a novel subsidiary color of the color additive FD&C red no. 40 (allura red AC) using spiral high-speed counter-current chromatography

- Xueli Cao, Beijing, China
- Dalene de Beer, Stellenbosch, South Africa
- Fernanda das Neves Costa, Rio de Janeiro
- Qizhen Du, Hangzhou, China
- J Brent Friesen, River Forest, IL, USA
- Karine Faure, Lyon, France
- Svetlana Ignatova, Uxbridge, UK
- Yoichiro Ito, Bethesda, MD, USA
- Gerold Jerz, Braunschweig, Germany
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- Gilda Guimaraes Leitão, Rio de Janeiro, Brazil
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- Adrian Weisz, College Park, MD, USA
- Peter Winterhalter, Braunschweig, Germany
- Shihua Wu, Hangzhou, China

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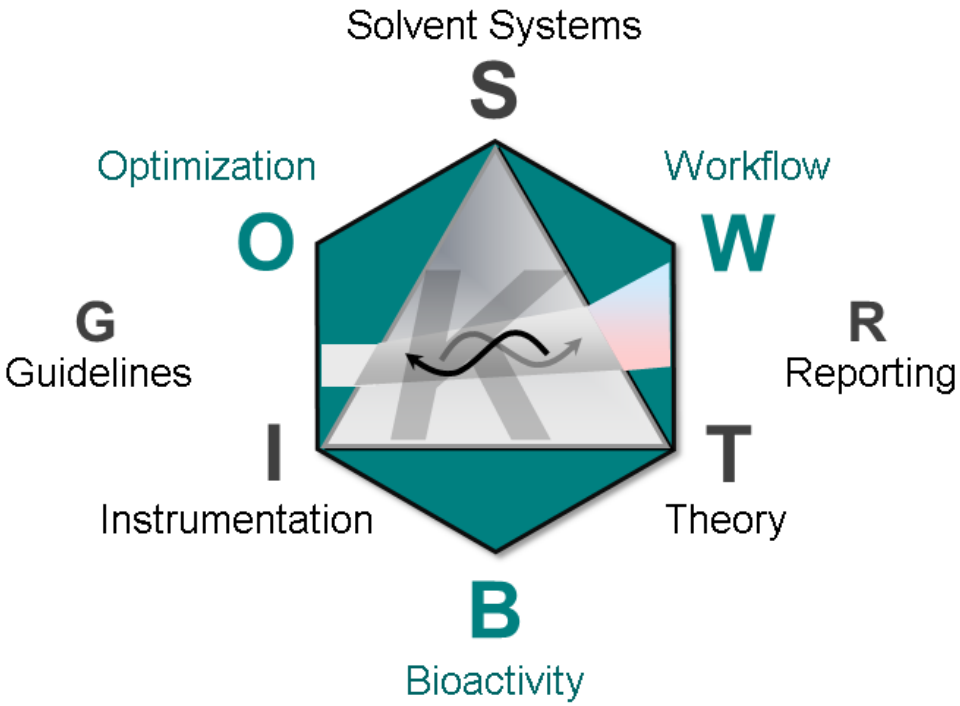
- Guy Harris, Gwent, UK
- Jim McAlpine, Chicago, USA
- Ian Sutherland, Uxbridge, UK
- Tianyou Zhang, Beijing, China
- Yoichi Shibusawa, Nagoya, Japan



KEY QUESTIONS

Q1 **What** are the **scientific innovations** that will drive the field?

Q2 **What** are the **major obstacles** that hinder the scientific development of CCS?

Q3 **Why** has CCS experienced a **low acceptance rate** among the scientific community?

Q4 **How** can the **low acceptance** rate be **overcome**?

Q5 **How** can we **build community and collaboration** among CCS practiotibners?

Q6 **How** can **K be established as an essential physicochemical constant** in the biomedical and other sciences?

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PREPARATIVE SEPARATION OF BERGAPTEN AS A NOVEL COMPOUND FOR THE TREATMENT OF NICOTINISM

1001 PO Session

Krystyna Skalicka-Woźniak[a,*], Barbara Budzynska[b], Malgorzata Wydrzynska–Kuzma[b], Ewelina Kozioł[a], Grazyna Biala[b]

[a*] Department of Pharmacognosy with Medicinal Plants Unit, Medical University of Lublin, Poland, kskalicka@pharmacognosy.org

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Keywords: nicotinism, furanocoumarins, bergapten, psoralen derivatives, memory, depression

Coumarins are a big group of biologically active natural products, known also as important substrates for human cytochrome P-450 2A6 (CYP2A6) and CYP2A13. It was shown that they inhibit CYP2A5-mediated nicotine metabolism *in vivo* in the mice. Hepatic cytochrome CYP2A6 is involved in the 70-80% of the initial metabolism of nicotine and its co-metabolites.

The aim of this study was to investigate whether a known furanocoumarin - bergapten (5-methoxypsoralen) prolongs the behavioral effects of nicotine. Nicotine itself, when administered alone, has the ability to improve memory acquisition and consolidation, as well as exerts antidepressive activity in animal models. These effects are extinguished 48 hours after administration. To investigate the influence of bergapten on the behavioral effects of nicotine the forced swimming test (FST) - animal models of depression, and passive avoidance (PA) test - memory and learning paradigm, were used.

In order to have sufficient quantity of pure bergapten available for pharmacological *in vivo* studies, the dichloromethane extract obtained from fruits of *Heracleum leskovii* Grossh. (Apiaceae) was processed efficiently through high-performance counter-current chromatography (HPCCC) and the process was scaled from analytical to preparative to effect rapid, preparative separation. A two-phase solvent system composed of *n*-heptane/EtOAc/MeOH/H_2O (6:5:6:5, v/v) enables purification of 95 mg of bergapten (purity 99%) after injection of 500 mg of crude extract, in less than 30 min.

Our study revealed that CYP2A6 inhibitor - bergapten (25 mg/kg) prolonged the antidepressive and procognitive effects of nicotine. As nicotine effects were slowed by inhibitors of CYP2A6 this kind of enzymatic inhibition has been proposed as a novel target for smoking cessation, and bergapten may offer a new approach to the treatment of nicotinism.

Acknowledgments: The research was financially supported by Grant No. 2014/13/B/NZ4/01248 from The National Science Centre, Kraków, Poland.

FRACTIONATION OF BISDESMODIC SAPONINS WITH ANTI-TUMOR ENHANCING ACTIVITY FROM *SAPONARIA OFFICINALIS* BY HPCCC AND USE OF NATURAL CHIRAL SELECTORS AND MONITORING BY OFF-LINE ESI-MS/MS INJECTIONS

1002
FP
Session IV

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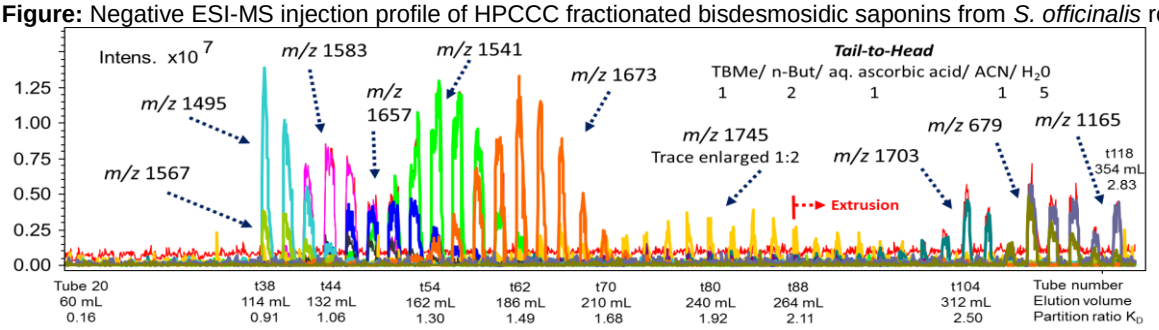
Keywords: Saponaria officinalis, polar anti-tumor saponins, preparative ESI-MS/MS profile

Saponaria officinalis L. (Caryophyllaceae) (soapwort) is a medicinal plant known from Europe to Central Asia. Quillaic acid and gypsogenin bisdesmosidic saponins with specific structural features had been shown to strongly enhance the efficacy of targeted toxins e.g. Sap3-EGF (epidermal growth factor linked to protein toxins) *in-vitro* and *in-vivo* in a synergistic manner (**1**) as a promising novel approach in tumor therapy. Aim of this study was to pre-fractionate and isolate respective target saponins (1300-1800 amu) of similar polarities by HPCCC for further biological evaluation omitting toxic non-volatile reagents. Various biphasic solvent systems with addition of chiral natural selectors had been evaluated to induce specific K_D differences for these saponins (prediction: LC-MS of phases). Strong K_D-value modifying effects on saponins were seen for L-(+)-ascorbic acid (*Asa*). The HPCCC (Spectrum-Dynamic Extractions, column vol. 125 mL) was operated with TBME/ n-butanol/ aq. ascorbic acid (3g–100mL)/ ACN/ H_2O (1:2:1:1:5) (*tail-to-head*, 3.0 mL/min) using *elution-extrusion*. The resulting HPCCC fractions (300 mg injection, t20 - t118) were *off*-line injected in sequence of recovery to an ESI-MS/MS (Bruker HCT Ultra). This combined HPCCC and ESI-MS *guided* isolation procedure (**2**) made use of selective ion traces of the target saponins (cf. Fig.). Elution orders and co-elution effects of minor and major saponins were monitored by selected ion-traces (neg. ESI, *m/z* 100-2500). MS^2 of 5 precursor ions revealed specific sugar substitutions. HPCCC resulted in the fractionation of 9 major saponins, with one very pure saponin (*m/z* 1673.7) (**3**). Saponins with *m/z* 1583, 1673, 1745, 1165 could be directly used for 1D/2D-NMR studies and bio-assays after removal of *Asa*. The mechanism of interaction of *Asa* with saponins could be related to carbonyl-interaction with sugar hydroxy-functions or molecule cavities and seemed not related to molecular weights. Lately, the generation of selected ion trace profile of saponins is a highly versatile tool for accurate tube fractionation and recognition of specific molecules of interest. This method gives access to bio-active saponins for biological evaluation on larger lab-scale.

Figure: Negative ESI-MS injection profile of HPCCC fractionated bisdesmosidic saponins from *S. officinalis* roots.



References

1. A. Weng et al., A convenient method for saponin isolation in tumor therapy. *J. Chromatogr. B* 878 (**2010**), 713–718.
2. G. Jerz et al., Preparative mass-spectrometry profiling of bioactive metabolites in Saudi-Arabian propolis fractionated by high-speed countercurrent chromatography and off-line atmospheric pressure chemical ionization mass-spectrometry injection. *J. Chromatogr.* A, 1347 (2014) 17–29.
3. Y. Lu et al., Antiproliferative quillaic acid and gypsogenin saponins from *Saponaria officinalis* L. roots, *Phytochemistry* 113 (**2015**) 108–120.

K-VALUES IN CPC: IS PREDICTION RELIABLE?

1003
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Session XII

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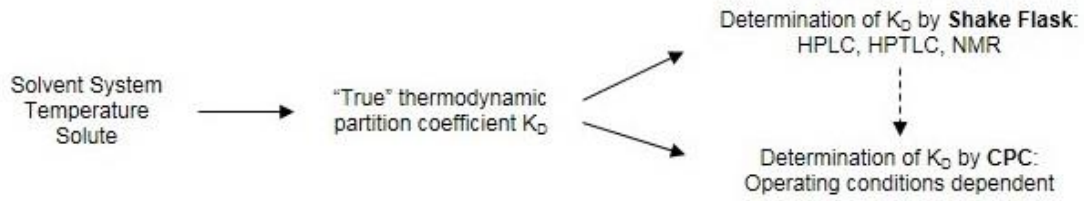
Keywords: K_D prediction, shake flask, K_D deviation

The major issue in method development for CPC consists in finding the best solvent system to attain maximum selectivity between the compounds of interest, based on partition coefficient K_D determination. For one solute in a specific solvent system at a fixed temperature, there should be one true thermodynamic K_D, not easily accessible. To readily obtain the partition coefficient K_D of the compounds in the selected solvent system, the Shake Flask (SF) method is usually performed and the upper and lower phases of the SF are analyzed by a rapid analytical technique such as HPLC, HPTLC or NMR. However, this SF method is only useful if the determination of the K_D-value of a compound allows the accurate prediction of the K_D-value acquired in CPC analysis. Surprisingly, the observation that the K_D-value may vary in CPC was made in the literature (1,2). Similarly, we observed a significant difference of the K_D-value between SF prediction and CPC analysis depending on the CPC operating conditions.

First, the influence of flow rate and centrifugal force have been considered on the K_D-value determination for GUESS compounds in HEMWat 0 solvent system. A significant K_D-values difference was observed depending on these conditions. In the same solvent system, the solute partition coefficients have also been investigated through the use of homologous series (alkylparabens), to exclude chemical discrepancy between solutes. A significant difference was retrieved whatever the retention coefficient of the compound. Interestingly, this deviation between CPC values and SF values was reproducible and not due to experimental errors. Operating conditions were found for which K_D-values determined by CPC are close to predicted K_D-values by SF method (10% deviation). When using HEMWat 0 solvent system, it is now possible to predict the difference between K_D-value obtained by SF and K_D-value obtained by CPC whatever the retention coefficient of the compound and the operating conditions. The influence of solvent system viscosity is currently under investigation using octanol/water solvent system.



References

1. S. Ignatova, D. Hawes, R. van den Heuvel, P. Hewitson, I.A. Sutherland, A new non-synchronous preparative counter-current centrifuge—the next generation of dynamic extraction/chromatography devices with independent mixing and settling control, which offer a step change in efficiency, Journal of Chromatography A, 1217 (**2010**) 34-39.
2. J.B. Friesen, G.F. Pauli, GUESSmix-guided optimization of elution-extrusion counter-current separations, Journal of Chromatography A, 1216 (**2009**) 4225-4231.

METABOLITE PROFILE OF BETALAINS AND FLAVONOIDS FROM *OPUNTIA STRICTA* VAR. *DILLENI BY* HPCCC AND *OFF-LINE* ESI-MS/MS

1004 PO Session

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Keywords: Opuntia stricta var. dillenii, betalain, flavonoid glycosides, ESI-MS/MS metabolite profile

Egyptian *Opuntia* fruits (prickly pears) such as the cultivar *O. stricta var. dilleni* (Cactaceae) were originally spread from Mexico and are hot climate resistant fruits that play an important role as an agricultural crop to enlarge arid areas due to global climatical changes. The profile of secondary natural products, possibly responsible for antioxidant, anti-inflammatory, and anti-cancer effects, and the amount of oligomeric sugars, minerals, vitamins and its high water content classifies *Opuntia* now-a-days as a super-fruit (**1**). Betacyanins and betaxanthins are the responsible pigments for the intensive violet, orange and yellow colors in the *Opuntia* fruits. Betacyanins occur as glycosides with large variations in the substitution with cinnamic acid derivatives (**2**). HPCCC (Spectrum-DE, column 125 mL, 5.0 ml/min, inj. 500 mg, '*head-to-tail*' mode with *elution-extrusion,* TBME/ *n*-BuOH/ ACN/ H_2O (0.7% TFA) [2:2:1:5]) was applied to fractionate the betalains and flavanoid glycosides from a freshly prepared C18 polyphenol fruit extract (**3**). The recovered fractions were injected in sequence to ESI-MS/MS (Bruker HCT Ultra ion-trap, pos. mode, *m/z* 100-1200) to generate a preparative metabolite profile of separated and co-eluting compounds (**2**) using propionic acid in the make-up solvent to omit signal quenching caused by TFA. Selected ion traces visualized that HPCCC well fractionated early eluting pigments (e.g. betanin) and the non-pigment compounds such as flavonoid glycosides. Betanin (*m/z* 551), phyllocactin (*m/z* 637), indicaxanthin (*m/z* 309) and betanidin (*m/z* 389) were recognized as the main pigments, and a minor trace of feruloyl-betanin (*m/z* 727) was found. However, hylocerenin as typical cactus pigment, and organic acids such as piscidic and eucomic acids known from *Opuntia ficus indica* fruits were not detected. This method could be scaled-up for further biological evaluation of these polyphenols.

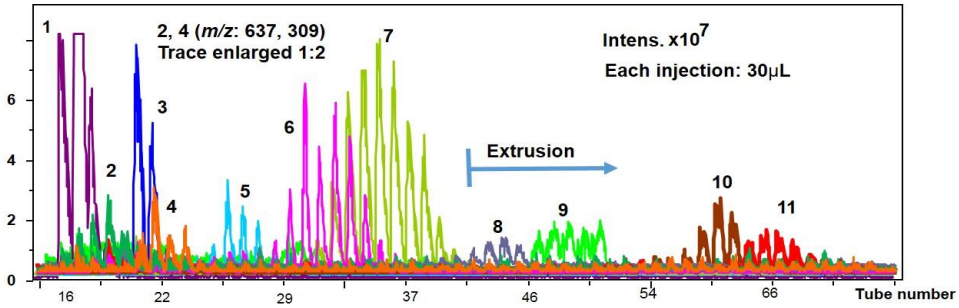

Figure: ESI-MS/MS metabolite injection profile of the HPCCC fractionation of *O. stricta* polyphenol C18 extract from preparative HPCCC coupling with sequential off-line injection to ESI-MS/MS. Selected ion traces of major compounds: **1** betanin (*m/z* 551), **2** phyllocactin (*m/z* 637), **3** not id. (*m/z* 568), **4** indicaxanthin (*m/z* 309), **5** not ident. (*m/z* 689), **6** betanidin (*m/z* 389), **7** isorhamnetin-rutinoside (*m/z* 625), **8** Quercetin-rutinoside (*m/z* 595), **9** not ident. (*m/z* 506), **10** not ident. (*m/z* 620), **11** not ident. (*m/z* 563). One tube fraction represents 5.0 mL CCC elution volume.

References

1. J.M. Feugang et al., *Frontiers in Bioscience*, 11 (2006) 2574-2589. **2.** G. Jerz et al., *J. Chromatogr. A* 1217 (2010) 4544-4554. **3.** G. Jerz et al., *ACS Symp. Series* 1129 (2013) 3-27.

RECOVERY OF THE BETACYANIN CELOSIANIN II AND FLAVONOID GLYCOSIDES FROM *ATRIPLEX HORTENSIS VAR. RUBRA* BY HPCCC AND OFF-LINE ESI-MS/MS MONITORING

1005 PO Session

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Keywords: Atriplex hortensis var. rubra*, celosianin II, flavonoid glycosides, ESI-MS/MS profile*

Atriplex hortensis var. *rubra* (Chenopodiaceae, *engl.* red mountain spinach, red orach) - an ancient vegetable is used for treatment of respiration, digestion and urinary diseases (**1,2**). Rare sulphated flavonoids, kaempferol-, and quercetin-3-*O*-sulphate-7-*O*-α-arabinosides had been elucidated from *A.h.* (**1**). These uncommon structures seem to play a role in plant biological regulation, and possess antiviral and anticoagulant properties (**3**). Preparative HPCCC (Spectrum, Dynamic Extractions U.K., coil vol. 125 mL, *elution-extrusion* approach) was applied using TBME/ *n*-BuOH/ ACN/ H_2O (1/3/1/5; 1% TFA, 4.0 mL/min, *head-to-tail* mode, inj. 460 mg, C18 reversed phase extract) to overview the metabolite profile. The collected fractions were sequentially injected into ESI-MS/MS (Bruker HCT Ultra ion-trap, pos. ESI, *m/z* 100-1200) to monitor target ion traces in the separated fractions (tube 15 – 76) and specific compound co-elutions. To overcome signal quenching caused by TFA, the *make-up* solvent and the tube fractions for MS-detection contained propionic acid. The combination of HPCCC with ESI-MS detection fractionated polyphenols and a principal pigment. The betacyanin 2''-*O*-feruloyl-amaranthine (celosianin II, *m/z* 903*)* was recovered (12 mg)*,* and fractions of flavonoid-glycosides during *elution* and *extrusion*. Quercetin-3-*O*-(malonyl-glc) (*m/z* 551) and kaempferol-3-*O*-(malonyl-glc) (*m/z* 535) were found as main flavonoid glycosides. MS/MS fragmentation of $[M+H]^+$ signals elucidated the potential substitution pattern of flavonoid glycosides by neutral loss differences (Δ*m/z* 86: malonyl; 80: sulphate; 132: arabinosyl, 146: rhamnosyl; cf. Fig.). Major achievement was the recovery of the instable betacyanin celosianin II (*m/z* 903) in a single CCC step which was then fully characterized by 1D/2D-NMR. The HPCCC process induced no pigment degradation as proven by the ESI-MS injection experiment. Selected ion traces clearly guided the accurate process of tube fractionation.

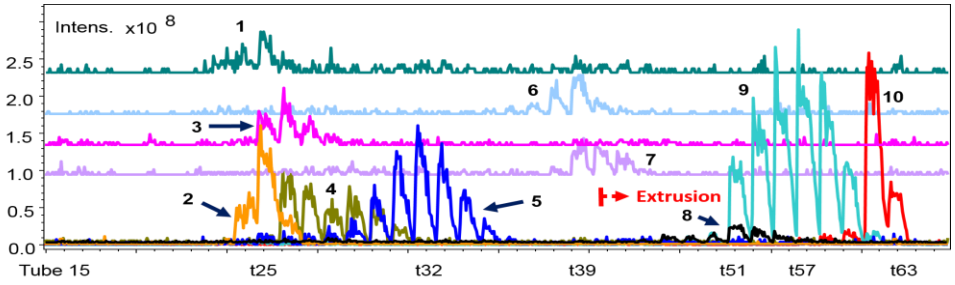

Figure: Sequential *off-line* ESI-MS/MS injections (pos. mode, $[M+H]^+$) of recovered HPCCC fractions with selected ion traces for polyphenol detection in *A. hortensis* leaves (for better visuality, ion traces with graphical off-set were enlarged by a factor 1:3): **1** Kaempferol-ara-SO_4 (*m/z* 499), **2** celosianin II (*m/z* 903), **3** K-glc-malonyl-glc (*m/z* 697), **4** N-feruloyl-putrescine (*m/z* 265), **5** quercetin-rha-glc (*m/z* 611), **6** K-rha-glc (*m/z* 595), **7** Q-ara-glc-malonyl (*m/z* 683), **8** K-ara-glc-malonyl (*m/z* 667), **9** Q-glc-malonyl (*m/z* 551), **10** K-glc-malonyl (*m/z* 535). One tube fraction represents 5.0 mL elution volume. Abbrev.: Q = quercetin, K = kaempferol

References

1. W. Bylka et al., *Acta Physiol. Plantarum*, 23,3 (2001), 285-290.
2. W. Bylka, *Acta Physiol. Plantarum*, 26, 4, (2004), 393-398.
3. Y. C.F. Teles et al., *Molecules,* 20,11 (2015), 20161-20172.
4. G. Jerz et al., *J. Chromatogr. A*, 1344 (2014) 42-50.

IN-SITU PROTEIN DETERMINATION TO MONITOR CPC CONTAMINATION

1006
FP
Session VI

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Keywords: Protein contamination, ADCA, CBB, in situ-method

Over the last years, purification of biomolecules by CPC, especially proteins, regained an increasing interest with the development of the Aqueous Two Phase System (ATPS) (1-2). Thereby with the use of proteins, the contamination risk of the equipment is more important. Indeed proteins can easily adsorb on the rotor material. Thus one of the most important problems for use of CPC in the industry is to ensure the cleanliness of the equipment in order to avoid cross-contamination and also the detection of possible protein contamination. These two issues are highly dependent on rotor material. Thus, in this work we studied two different materials: titanium and stainless steel 316. First of all, a cleaning method was improved to remove protein-metal interaction thanks to a pH switch and an organic solvent combination. Then, a direct method that allows the determination of the effective presence of proteins and the extent of contamination is necessary and was therefore developed.

This in-situ method is derived from Amino Density Estimation by Colorimetric Assay (ADECA) (3). It is based on the affinity of a dye: the Coomassie Brillant Blue (CBB) with protonated amino groups. The procedure was optimized to generate this specific affinity with contaminating proteins. A preliminary study was carried out to limit the non-specific adsorption of the dye on the surface. Then, the ADECA method consisted on three steps. First the fixation of the dye to the amino groups was performed. Afterwards, excess dye and non-specific interactions were removed and finally the dye bounded to proteins was eluted by a pH switch. This method was successfully applied to rotors with various extent of proteins contamination (bovine serum albumin). The eluted dye was quantified and found to respond linearly to proteins contamination up to 60mg. Limit of detection and quantification were recorded and depend on the rotor material. Hence, this method should accurately indicate if a full cleaning and sterilization of the rotor is required whatever the material of the rotor. We hope that this work which allows cleaning and in situ determination of proteins contamination will contribute to the development of proteins purification by CPC.

References

1. Z. Xu, X. Lin, Y. Lu, Separation of Peptides and Proteins by Countercurrent Chromatography. Current Proteomics, 10 (2013), 322-333.
2. N. Mekaoui, et al., Advances in countercurrent chromatography for protein separations, Bioanalysis, 4 (2012) 833-844.
3. 3. G. Coussot, C. Perrin, T. Moreau, M. Dobrijevic, A. Le Postollec, O. Vandenabeele-Trambouze, A rapid and reversible colorimetric assay for the characterization of aminated solid surfaces, Anal Bioanal Chem, 399 (2011) 1061-92.

PROBING THE COMBINATORIAL METABOLOME OF FLAVANOLIGNANS IN MILK THISTLE (*SILYBUM MARIANUM* L.)

1007
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Session

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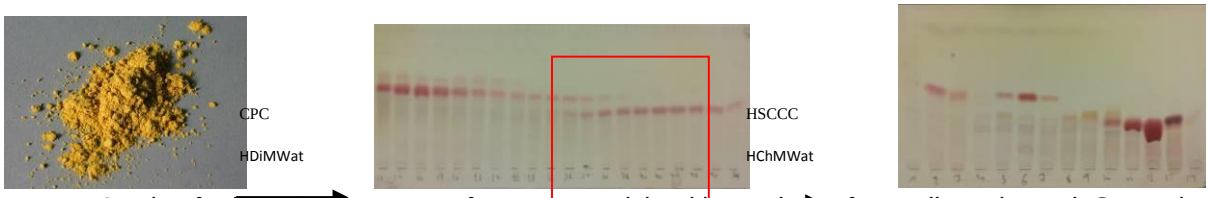
Keywords: Silybum flavanolignan analogues, CPC, HSCCC, orthogonality

Silybum marianum L. (milk thistle) is a medicinal plant that has been used for centuries for liver and gallbladder disorders by protecting liver against snake venom, insect bites, *Amanida phalloides* (mushroom) poisoning, and alcohol abuse [1]. Constituents responsible for the hepatoprotective and anti-hepatotoxicity in standardized milk thistle extract are flavanolignans, which were first characterized in the 1980s and are collectively named "silymarin". Silymarin represents 1.5 to 3% of the milk thistle fruit mass and contains one flavonoid (taxifolin, also known as dihydroquercetin) plus, as reported so far, at least seven flavanolignans (silybin A/B, isosilybins A/B, silychristin, isosilychristin and silydianin) which all share the same molecular weight of 482.44 [2-4]. Our recent preliminary data indicates that *S. marianum* produces a myriad of additional congeneric compounds, tentatively called "*Silybum* Flavanolignan Analogues" (SFAs), with closely resembling structures including stereo- and regio-isomers. Their presence also explains why elucidation of the silymarin complex has been, and continues to be, a phytochemical purification and structural analysis challenge.

As very little is known about the biogenesis and structure of SFAs and their precise biological effects, the objectives of the present work are to (i) develop and optimize new countercurrent chromatography-based methods for the metabolomic fractionation of *S. marianum* extract; (ii) isolate previously unknown SFAs; and (iii) structurally characterize the isolated compounds using MS and 1D/2D NMR. CPC (high capacity) and HSCCC (high resolution) instruments with specifically developed orthogonal two-biphasic solvent systems HChMWat/HDiMWat, (1:22:20:12 (AcOH,0.5%), v/v) were employed. We fractionated the *S. marianum* metaboblome, distinguished the known from the additional compounds, and confirmed that a large number of SFAs remain to be isolated and structurally characterized.



As the further development of new natural health products from silymarin and *S. marianum* depends on the determination and analysis of structure activity relationships of their bioactive constituents, countercurrent separation is likely able to provide new initiatives for the characterization of the *Silybum* flavanolignan metabolome. Furthermore, a better understanding of the metabolomic chemical space of "*Silybum* Flavanolignan Analogues" will provide critical knowledge about the underlying biosynthetic pathways and enhance our ability for their targeted analysis and production.

References

[1] G. Karimi, M. Vahabzadeh, P. Lari, M. Rashedinia, M. Moshiri, "Silymarin", a promising pharmacological agent for treatment of diseases, Iran J Basic Med Sci. 14 (2011) 308-317.

[2] N.-C. Kim, T.N. Graf, C.M. Sparacino, M.C. Wani, M.E. Wall, Complete isolation and characterization of silybins and isosilybins from milk thistle (Silybum marianum), Org Biomol Chem. 1 (2003) 1684-1689.

[3] D.J. Kroll, H.S. Shaw, N.H. Oberlies, Milk thistle nomenclature: why it matters in cancer research and pharmacokinetic studies, Integr Cancer Ther. 6 (2007) 110-119.

[4] .G. Napolitano, D.C. Lankin, T.N. Graf, J.B. Friesen, S.-N. Chen, J.B. McAlpine, N.H. Oberlies, G.F. Pauli, HiFSA fingerprinting applied to isomers with near-identical NMR spectra: the silybin/isosilybin case, J Org Chem. 78 (2013) 2827-2839.

COMPARISON OF PARTITION EFFICIENCY BETWEEN SATELLITE AND PLANETARY MOTIONS OF COIL SATELLITE CENTRIFUGE FOR COUNTER-CURRENT CHROMATOGRAPHIC SEPARATION OF 4-METHYLUMBELLIFERYL SUGAR DERIVATIVES

1008
FP
Session X

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Keywords: Coil satellite centrifuge, satellite motion, planetary motion, partition efficiency.

The coil satellite centrifuge (CSC) produces the satellite motion such as the triplicate rotation of coiled column around three axes including the sun axis (the angular velocity, ω_1), the planet axis (ω_2) and the satellite axis (the central axis of the column) (ω_3) according to the following formula: $\omega_1 = \omega_2 + \omega_3$. Improved separation for 4-methylumbelliferyl sugar derivatives were achieved using the common multilayer coiled column with ethyl acetate/1-butanol/water (3 : 2 : 5) for lower phase mobile at the combination of the rotation speeds (ω_1, ω_2, ω_3) = (300, 150, 150 rpm), and (1 : 4 : 5) for upper phase mobile at (300, 100, 200 rpm).

In order to reveal the effect of the satellite motion on the partition efficiency, the peak resolution and the stationary phase retention were measured for each separation with the different rotation speed of ω_2 and ω_3 under the constant revolution speed at ω_1 = 300 rpm. The lower phase mobile produced almost similar results on the peak resolution and the stationary phase retention regardless of the change of ω_2 and ω_3, while the upper phase mobile offered each different result on these two values by varying the rotation speeds of ω_2 and ω_3. Especially, two rotation speeds of ω_2, 147 and 200 rpm, did not retain any lower stationary phase in th coiled column while 150 rpm retains enough volume of stationary phase. On the other hand, the planetary motion providing at (ω_1, ω_2, ω_3) = (300, 300, 0 rpm) or (300, 0, 300 rpm) produced insufficient peak resolution with both lower and upper phase mobiles except (300, 0, 300 rpm) with upper phase mobile, where these results may be caused from the lack of rotation speeds. At lower rotation speed, better partition efficiencies were obtained by the satellite motion than by the planetary motion.

The effect of the hydrophobicity of two-phase solvent systems on the stationary phase retention was further examined using the *n*-hexane/ethyl acetate/1-butanol/methanol/water system at different volume ratios. In the satellite motion at (ω_1, ω_2, ω_3) = (300, 150, 150 rpm), the lower phase mobile obtained almost constant stationary phase retention regardless of the change of the hydrophobicity whereas the upper phase mobile brought different stationary phase retention according to the volume ratio of the two-phase solvent system. However, the planetary motion showed stable stationary phase retention in either phase used as the mobile phase. The overall results indicate that the satellite motion is seriously affected by the combination of rotation speeds and the hydrophobicity of the two-phase solvent system if the upper phase was used as the mobile phase.

DEVELOPMENT OF PRECIPITATION COUTER-CURRENT CHROMATOGRAPHY

1009
FP
Session X

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Keywords: precipitation, solubility, heparin, molecular weight

A (HS)CCC column is typically prepared by winding of PTFE tubing, and there is not any solid support, which enable to separate crude samples. We have developed new separation method for water soluble polymers using this CCC advantage. In this study, heparin was selected as a water soluble polymer. First, heparin was intentionally precipitated in the CCC column using organic solvents. The separated heparin from the solvent would be retained in the column under centrifugal force field. Then, mixture of water and organic solvent was pumped into CCC column as a mobile phase to re-solve the precipitated heparin. If precipitation and dissolution is repeated, the analytes will be separated in order of solubility. We named this method as “precipitation CCC (pCCC)”.

CCC (Type J, column volume 100 mL) was filled with organic solvents, such as methanol, ethanol, isopropyl alcohol or acetone, and rotated at 100 – 850 rpm. Heparin aqueous solution was injected and mobile phase was pumped at 1.0 mL/min. Mobile phase was altered in steps by addition of water to the reservoir containing the organic solvent. Fractionated pCCC samples were subjected to GPC analysis.

Figs 1 and 2 are examples of pCCC chromatograms. The line was represented as the peak area and the dot was retention time obtained by GPC analysis. They were plotted against the retention volume of CCC. When methanol or ethanol was used as solvent, almost of heparin were eluted as solvent front (100 – 150 mL). On the one hand, when IPA or acetone was used, the heparin was strongly hold in the column, and extruded at 250 – 300 mL. Fig 1 shows that heparin was separated into three fractions using MeOH/IPA = 1/3, and the retention time gradually decreased, indicating that heparin was eluted in order of increasing molecular weight. The mixture of heparin and low molecular weight heparin (LMWH) has been successfully separated based on the molecular weight by pCCC method as shown in Fig 2. The pCCC method will be applied to separation of various water soluble polymer according to the molecular weight by choice of solvent combinations and rotation speed.

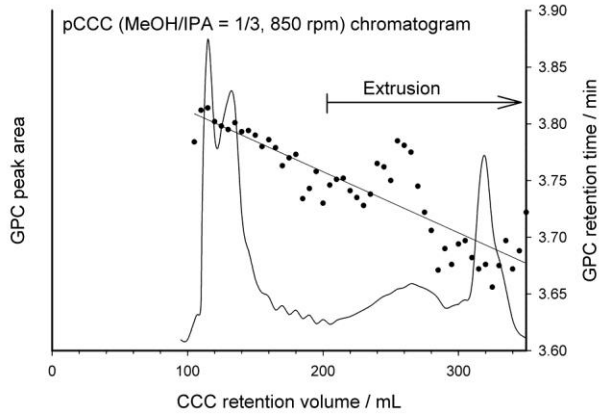

Fig. 1. pCCC chromatogram of heparin

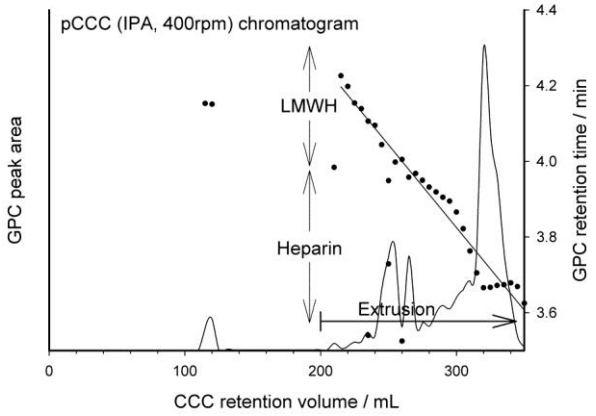

Fig 2. pCCC chromatogram of Heparin & LMWH

BIO-GUESS BASED COUNTERCURRENT SEPARATION OF ANTI-TUBERCULOSIS LEAD COMPOUNDS FROM ACTINOMYCETES

1010
SO
Session IV

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Keywords: anti-tuberculosis, bio-GUESS, thin-layer chromatography direct bioautography, Generally Useful Estimate of Solvent Systems (GUESS).

Actinomycetes are known as an important source for various antibacterial secondary metabolites. This study describes a novel approach to the targeted isolation of anti-tuberculosis active lead compounds from extracts of the actinomycete strain *Streptomyces* sp. "Anti-TB bioautography" (1) is combined with countercurrent separation (CCS), which in turn is based on the "Generally Useful Estimate of Solvent Systems" (GUESS) (2), to establish the new Bio-GUESS approach. The anti-TB direct bioautography method was previously developed in our lab for the avirulent, bioluminescent *Mycobacterium tuberculosis* (*Mtb*) strain mc^{2}7000 luxABCDE. This approach provides information on anti-TB activity of the sample within only 24 hours.

The combination of the GUESS method with anti-TB bioautography constitutes a truly targeted isolation procedure of potential anti-TB leads, because the choice of the CCS solvent system is tailored exclusively to the active compounds. Criteria for this selection were (i) the location of the inhibition zone on the TLC, which needed to indicate the CCS „sweet spot" (R*f*-values ~ 0.5), and (ii) the near equal distribution of the active compounds between upper and lower phase. The direct bioautography method was used throughout the entire isolation process to monitor fractions. The anti-TB active compounds were detected in fractions with a K value in CCS "sweet spot", which confirms the validity of the Bio-GUESS concept. The activity of all fractions was confirmed with the Microplate Alamar Blue Assay using using*Mtb* H37Rv (3) and resulted in a good correlation with the bioautography results.

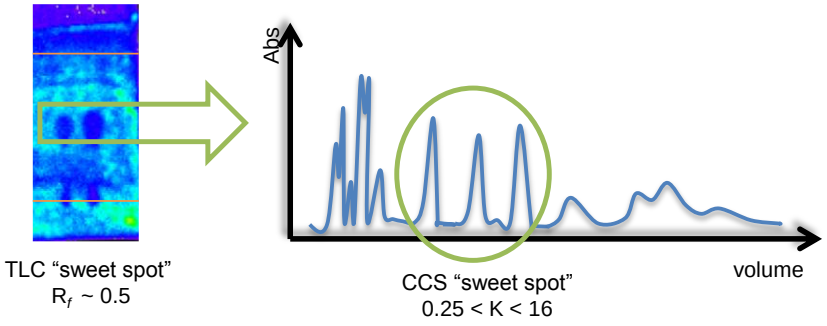

Figure 1. The conc ept of Bio-GUESS based counter-current separation.

References

(1) E.M. Grzelak et al. *ACS Infect. Dis.* 2 (2016) 294-301.
(2) B. Friesen and G. Pauli *J. Liq. Chromatogr. R. T.* 28 (2005) 2777-2806.
(3) L. Collins and S.G. Franzblau *Antimicrob. Agents Chemother.* 14 (1997) 1004-1009.

Acknowledgement

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CENTRIFUGAL PARTITION CHROMATOGRAPHY (CPC) ENRICHES DENTIN-BIOACTIVE TRIMERIC AND TETRAMERIC PROANTHOCYANIDINS FROM MEDICINAL PLANTS

1011
SO
Session IV

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Keywords: Oligomeric proanthocyanidns (OPACs), Degree of Polymerization (DP), enrichment, dentin biomodification

Proanthocyanidins (PACs) are oligomers/polymers of flavan-3-ol monomers, commonly known as the "catechins". PACs have been tested in a multitude of in-vitro and in-vivo biological assays, most commonly for their anti-oxidant, anti-inflammatory and cardio-protective properties. For the same reason, they are promoted by the dietary supplement as well as the food industry. Our recent collaborative studies have shown that oligomeric PACs (OPACs) have potent dentin biomodifying properties and excellent application potential as restorative dental biomaterials.

One of the common obstacles in any PAC related research is the challenging chromatographic separation. PACs are known to irreversibly adsorb on to the solid phase chromatographic systems leading to poor recovery and subsequent low yield. Thus a liquid-liquid based separation method is the best suited for the separation of PACs. Mid-size OPACs with a degree of polymerization (DP) 3-5 have shown the optimum dentin stiffness and bond strength in the dentin biomodification assays. Since OPACs are highly polar molecules, they need solvent systems with higher polarity and such solvent systems are known to retain poorly on the traditional HSCCC instruments, mainly due to longer settling times or smaller density differences between the two phases. Hence, centrifugal partition chromatography was chosen for the enrichment of trimeric and tetrameric PACs. Additionally, CPC offers a much higher loading capacity which was one of the requirements of the present study. In this study, we have developed CPC methods using elution-extrusion for the enrichment of selected OPACs with DP 3 to 4(5) from four different plants - grape seed extract, cocoa extract, pine bark extract and cinnamon bark extract. The DP of the CPC fractions was monitored by TLC, MS, and NP-HPLC with diol column. CPC separation proved to be an efficient chromatographic tool to sort dentin bioactive OPACs by their DP. The different methods are summarized in the following table. All the methods in general follow the monomer knock-out (KO), polymer KO, and further enrichment approach.

Source	Starting material	Method
Grape (*Vitis vinifera* L.) seed extract	Methyl acetate (Me) layer of the methyl acetate/water partition →polymer KO	1) HEMeWat 0.8/4/1/4; descending mode → monomer KO 2) HEMeWat 0.4/4/1/4; ascending mode → dimer KO
Cocoa (*Theobroma cacao* L.) extract	Methyl acetate (Me) layer of the methyl acetate/water partition →polymer KO	1) HEMWat 1/10/1/10; ascending mode → monomer KO 2) HEMeWat 0.4/4/1/4; ascending mode → dimer and higher oligomer KO
Pine (*Pinus massoniana*) bark extract	EtOAc partition →polymer KO	HEMWat 0.5/4/1/4; ascending mode → monomer KO
Cinnamon (*Cinnamomum verum*) bark	70% acetone extract	EtOAc/EtOH/Water=6:1:5; ascending mode →trimer + tetramer enriched fraction

ISOLATION AND CHARACTERISATION OF CHLOROPHYLLS AND XANTHOPHYLLS IN GRASS BY A NOVEL SOLVENT SYSTEM USING COUNTERCURRENT CHROMATOGRAPHY

1012
FP
Session IV

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Keywords: chlorophylls, xanthophylls, grass, isolation, novel solvent system

In order to isolate chlorophyll from plant extract by means of High Speed Countercurrent Chromatography (HSCCC) excluding the use of acetone as part of the stationary phase, a novel solvent system composed of hexane/dichloromethane/ethanol/water 4:2:6:2 (v/v/v/v) was applied. The isolation of chlorophylls a, b and pheophytins a, b was successfully performed in the case of grass when dichloromethane was part of the solvent system **(Figure 1/left).** Comparatively, when chloroform was applied as part of the stationary phase, the xanthophyll separation showed better resolution compared to chlorophylls **(Figure 1/right).**

Dichloromethane and chloroform are non-polar solvents. Although, they have similar density (1.49 and 1.32 g/mL), the dielectric constant is higher for dichloromethane (9.1) in comparison with chloroform (4.81). Therefore the polarity of dichloromethane is higher than of chloroform **(1).** Additionally, the Hansen solubility parameters values show that δ P (Polar bonds) and δ H (Polar hydrogen bonding) are meaningfully different. The δ P and δ H values for dichloromethane are 7.3 and 7.1, respectively, whereas for chloroform they are 3.1 and 5.7, respectively **(2).** Consequently, dichloromethane is more polar than chloroform. Therefore, dichloromethane is a polar aprotic solvent and chloroform a non-polar solvent. Therefore, hexane/dichloromethane/ethanol/water 4:2:6:2 (v/v/v/v) is adequate for chlorophyll separation because it changes the system to yield more polarity. This hypothesis is supported by the fact that the elution mode length was 10 hours and the extrusion mode 2 hours **(Figure 1/left),** whereas with chloroform the elution mode length was 7 hours and the extrusion mode 4 hours. Structure elucidation of chlorophylls, pheophytins and xanthophylls was done by modern spectroscopy techniques including LC-APCI-MS/MS and Nuclear Magnetic Resonance (NMR) 1D/2D-NMR experiments (^{13}C, $^{1}H/^{1}H$-COSY, HSQC, HMBC) **(3).**

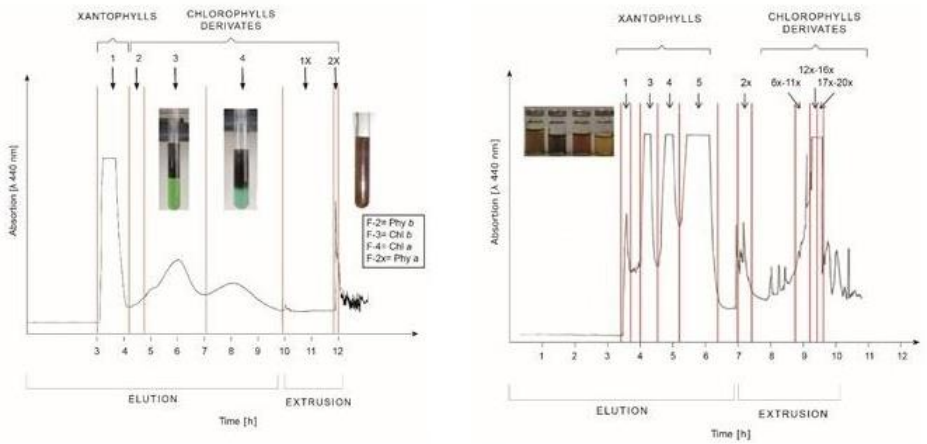

Figure 1. HSCCC chromatogram from grass extract with elution and extrusion mode. **Left**: solvent system: hexane/dichloromethane/ethanol/water (4/2/6/2). Chlorophyll separation showed better resolution in comparison to xanthophylls in elution and extrusion mode. **Right**: solvent system: hexane/chloroform/ethanol/water (4/2/6/2). Xanthophyll separation showed higher resolution in elution in comparison with chlorophylls in extrusion mode.

References

1. E. M. Kosower, An Introduction to Physical Organic Chemistry, John Wiley and Sons, New York (1968).
2. C. M. Hansen, Hansen Solubility Parameters: A user's handbook, second ed., CRC Press, Boca Raton (2007).
3. M.E. Castro-Benítez. Phytochemical analysis of Baby Banana peels (*Musa acuminata*) in relation with a hyperpigmentation phenomenon. Ph.D. Thesis. Cuvillier, Göttingen, Germany (2015).

SEPARATION OF ISOMERIC MONOSACCHARIDES BY RECYCLING ELUTION-EXTRUSION COUNTERCURRENT CHROMATOGRAPHY

1013
FP
Session II

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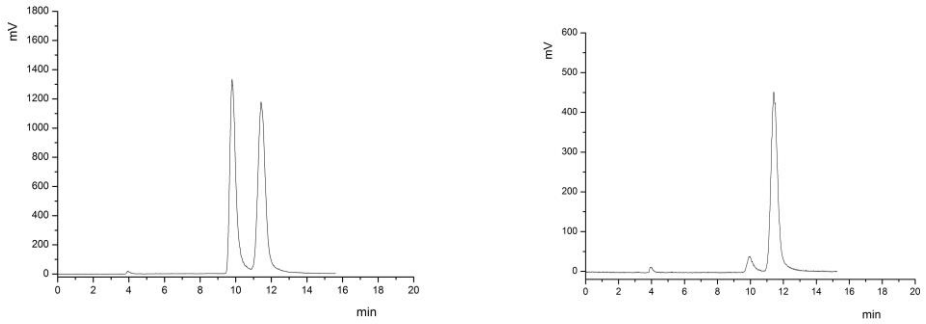
Keywords: Isomeric monosaccharides; Elution-extrusion countercurrent chromatography; Separation

Monosaccharides are difficult to separate and analyze by conventional methods. Traditionally, precolumn derivatization is necessary for separation of monosaccharides by liquid chromatography or gas chromatography due to their high polarity. Elution-extrusion counter-current chromatography is especially useful for separation of components, in which sufficiently large differences in polarity are involved based on their chemical structures. However, in our present work, elution-extrusion countercurrent chromatography was investigated for separation of two isomeric monosaccharides, including fructose and glucose. Both of them are highly polar and soluble in water. Generally, aqueous-aqueous solvent system could be selected for separation of monosaccharides, but here the biphasic solvent system water-butanol (1:1, v/v) was selected, in which a very low partition coefficient of <0.1, was observed for both monosaccharides. The aqueous phase was used as the stationary phase and the organic phase was used as the mobile phase. It was impossible to elute the monosaccharide from the stationary aqueous phase by conventional elution method if organic phase was used. Therefore, recycling elution mode was used after the sample was injected. Almost baseline separation could be achieved for the two monosaccharides by recycling elution-extrusion chromatography, as shown in the HPLC analysis (Figure 1). A mathematic model for separation of isomeric monosaccharides by recycling elution-extrusion countercurrent chromatography was proposed, in which recycling time, extrusion time and peak resolution would be discussed.



(a) (b)

Figure 1. HPLC chromatograms of (a) two isomeric monosaccharides and (b) fractions from modified elution-extrusion counter-current chromatography.

References

1. Pauli, Guido F; Pro, Samuel M; Chadwick, Lucas R; Burdick, Thomas; Pro, Luke; Friedl, Warren; Novak, Nick; Maltby, John; Qiu, Feng; Friesen, J. Brent. Real-Time Volumetric Phase Monitoring: Advancing Chemical Analysis by Countercurrent Separation, Anal. Chem, 87 (2015): 7418-7425
2. Paek, Ji Hun; Lim, Soon Sung. Preparative isolation of aldose reductase inhibitory compounds from *Nardostachys chinensis* by elution-extrusion counter-current chromatography, Arch. Pharm. Res, 37 (2014):1271-1279
3. Y.Wang, S. Guan, R.Feng, J. Zhang, Q. Li, X.Chen, K. Bi, D. Guo. Elution-extrusion counter-current chromatography separation of two new benzyl ester glucosides and three other high-polarity compounds from the tubers of *Pleione bulbocodioides*, Phytochem. Analysis, 24 (2013):671-676

PRODUCTION OF THREE NEW ANTIOXIDANTS FROM EDELWEISS BY MULTI-HEART CUTTING CPC-LC

1014 PO Session

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Keywords: Edelweiss, antioxidants, CPC-LC, prepLC, scale-up

Leontopodium alpinum, commonly known as Edelweiss, is one of the most famous plants of the European Alps. In folk medicine, extracts of Edelweiss are used for the therapy of several diseases such as bronchitis or cancer. Wild Edelweiss is protected by the law but the plant is now cultivated in large numbers and extracts of the aerial parts are used for their anti-oxidative properties (1,2). In order to improve clinical research and provide high quality standards, the production of three new antioxidants from Edelweiss plant is required, namely: leontopodic acid A, leontopodic acid B and 3,5-dicaffeoylquinic acid. HPLC analysis exhibits the complexity of this plant extract (1,2). Unfortunately, the compounds of interest have very close chemical structures which prevents their isolation by HPLC at preparative scale due to insufficient resolution. The CPC technique was found to be the unique complementary technique to LC in order to combine two different selectivities through two different mechanisms of separation.

As the CPC separation did not provide sufficient resolution for the separation of the three compounds of interest, the technique was combined to LC technique to recover the antioxidants from Edelweiss plant with the required purity. Thereby, the CPC technique led to a first separation of the three compounds according to their partition coefficients in the solvent system and the LC technique was performed on the recovered fractions to lead to a second separation. The multi-heart cutting CPC-LC was enforced at laboratory scale which represents an innovative separation for the purification of the Edelweiss plant. To produce these three antioxidants, the CPC and LC methods were then transferred at industrial scale. The separation qualities were preserved. Up to two gram of the full Edelweiss plant extract was injected and the three antioxidants were recovered with the required purity, as checked by analytical HPLC and MS.

The complementary use of CPC and LC techniques for the production of three new antioxidants from Edelweiss plant is a strategic combination which represents an innovative separation. This multi-heart cutting CPC-LC allows a gain of time and helps recovering the antioxidants with a high purity. The implementation of the multi-heart cutting CPC-LC method for preparative purposes in industry is achieved.

References

1. S. Schwaiger, R. Cervellati, C. Seger, E.P. Ellmerer, N. About, I. Renimel, C. Godenir, P. Andre, F. Gafner, H. Stuppner, Leontopodic acid – a novel highly substituted glucaric acid derivative from Edelweiss (Leontopodium Cass.) and its antioxidative and DNA protecting properties, Tetrahedron, 61 (2005) 4621-4630.
2. S. Schwaiger, C. Seger, B. Wiesbauer, P.Schneider, E.P. Ellmerer, S. Sturm, H. Stuppner, Development of an HPLC-PAD-MS assay for the identification and quantification of major phenolic edelweiss (*Leontopodium alpium* Cass.) constituents, Phytochemical Analysis, 17 (2006) 291-298.

CARNOSOL PURIFICATION FROM ROSMARINUS OFFICINALIS BY CENTRIFUGAL PARTITION CHROMATOGRAPHY, FROM LABORATORY TO INDUSTRY

1015 PO Session

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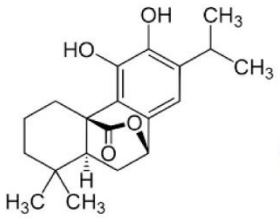
Keywords: rosemary, scale-up



Rosemary (*Rosmarinus officinalis*) is an aromatic herbal plant belonging to the Lamiaceae family and known for its medicinal and taste properties. Recent studies have shown its pharmacologic activities for cancer chemoprevention and therapy due to phenolic compound presence such as carnosol, carnosic acid and rosmarinic acid. **Carnosol** was more specifically evaluated for anti-cancer properties in prostate, breast, skin, leukemia and colon cancer showing promising results. Its purification is required at lab-scale for toxicology studies and at industrial scale for production as an active ingredient. In this context, we present the centrifugal partition chromatography (CPC) method development and carnosol purification from a Rosemary leaves extract on a lab-scale instrument, highlighting the advantages of the CPC technique on natural products purification.

After a rapid method development on a small-scale 35-mL CPC instrument that allowed for the determination of the solvent system and maximum sample concentration and volume, the purification was transferred on an industrial-scale 1-liter instrument using the "free space between peaks" method. The method takes into account the technical limitations of the larger instrument, such as pressure and/or maximum centrifugal field, and allows, by simply running an analytical-sized injection on the large scale rotor, to give an accurate prediction of the maximum sample load and best throughput. The 0.27 g of rosemary extract maximum load on the 35-mL CPC was transferred as 9 g load (33 times bigger load) on the 1000-mL CPC (28 times larger column volume). If the scaling-up in CPC instruments is not directly homothetic, the described protocol makes it highly predictable through few simple experiments.

RECOVERY OF ANTIBACTERIAL CYSTOBACTAMIDS FROM *CYSTOBACTER* SP. BY HPCCC AND OFF-LINE ESI-MS/MS METABOLITE PROFILING

1016
FP
Session II

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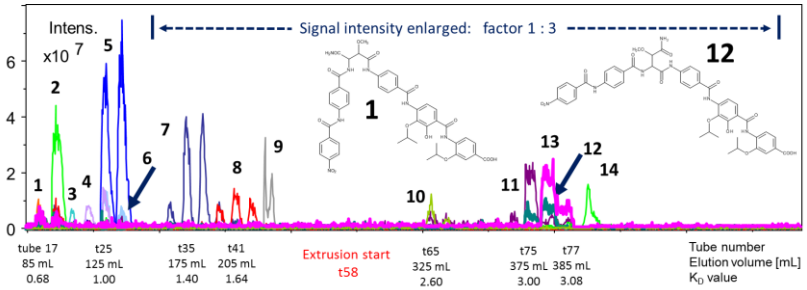
Keywords: Cystobacter sp., cystobactamide, antibiotics, preparative ESI-MS/MS profile

Myxobacteria are known for the production of unique antibacterial antibiotics, such as the myxo- and corallopyronins and the disciformycins (**1,2,3**). Recently, the cystobactamids, which constitute a new class of myxobacterial topoisomerase inhibitors with strong broad-spectrum activity against Gram-negative multiresistant pathogens, have been discovered (**4**). This compound class is of great interest for development as a remedy to combat the multiresistant human pathogens. However, downstream procession is hampered by the fact that enrichment of cystobactamids from crude extracts via flash-chromatography leads to substantial loss of compounds and an economical process for their recovery remains to be established. In this study we have investigated HPCCC as a novel method for the recovery of cystobactamids from crude extracts of the producer *Cystobacter* sp. (**4**). Our model substances for the preparative fractionation were the two cystobactamids (M_r 919-1 and 919-2, structures cf. Fig. - ion signals **1** and **12**, ESI neg. $[M-H]^-$ *m/z* 918) showing minimum inhibitory concentrations in the low µg/mL range (**4**). Suitable solvent systems for recovery were evaluated by LC-ESI-MS analysis. Respective K_D-values of targets in the phase layers were used for prediction of compound elution and occurrence of potential co-elution effects with other metabolites. The HPCCC (Spectrum-DE, column vol. 125 mL) was operated with *n*-hexane/EtOAc/MeOH/H_2O (1:2:1:2) (*head-to-tail, flow* 5.0 mL/min) using *elution-extrusion* (start at tube 58). The resulting HPCCC fractions from a 100 mg sample (tube 17-97) were injected *off*-line in sequence of recovery to an ESI-MS/MS (Bruker HCT Ultra) to project a mass spectrometry metabolite profile (**5**), and the target cystobactamids based on selected ion traces (neg. ESI, *m/z* 100-2500). (cf. Fig.). Elution orders and co-elution effects of minor and major congeners were monitored and MS^2 of 5 precursor ions delivered fragment ions for structural identification and confirmation. HPCCC resulted in the fractionation of the two principal cystobactamids visualized by selected ion traces (ESI neg) with low signal intensity in close eluting tubes (t17-t19 and t53-57). Major ion signals of good purity were seen for metabolites **5**, **7** **8**, **9**, **10**, **14** (cf. Fig.). The elution order of the two isobars **1** Mr 919-1, and **12** 919-2 was as expected, but interestingly the HPCCC K_D value of 3.1 for **12** was twice as high as in the LC-ESI-MS K_D prediction. HPCCC removed a majority of metabolites and concentrated the cystobactamids for fine purification steps. As all CCC methods, the recovery process could be extended to larger lab-scale.

Figure: ESI-MS (neg. mode) injection profile with principal $[M-H]^-$ ions of *Cystobacter* metabolites fractionated by HPCCC: *Cystobactamid* 1: *m/z* 918, 2: 372, 3: 475, 4: 371, 5: 403, 6: 417, 7: 260, 8: 387, 9: 363, 10: 221, 11: 590, *cystobactamid* 12: 918, 13: 860, 14: 526. Injection of every 2nd fraction into the ESI-MS.



References **1.** W. Kohl *et al.*, Liebigs Ann. Chem. **1983,** 1665-1667. **2.** R. Jansen *et al.*, Liebigs Ann. Chem., **1985**, 822-836. **3.** F. Surup *et al.*, Ang. Chem. Int. Ed., **2014**, 13588-13591. **4.** S. Baumann *et al*., Angew. Chem. Int. Ed., **2014**, 14605-14609. 5**.** G. Jerz *et al.*, *J. Chromatogr. A.*, **2014**, 17–29.

FRACTIONATION OF LIPOPHILIC COMPONENTS FROM POTATOES (*SOLANUM PHUREJA*) BY HPCCC AND MONITORING BY *OFF-LINE* INJECTIONS TO APCI-MS/MS

1017 PO Session

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Keywords: HPCCC, APCI-MS/MS, carotenoids, glucocerebroside, cycloartenol

Potatoes, breeding variety Mayan Gold - *(Solanum phureja*) contain a number of lipophilic compounds (unsaponifiable matters). Generally, non-polars such as carotenoid pigments are known to positively influence human health. Intake by food is inversely correlated to the appearance of degenerative diseases such as age-related macular degeneration (**1**). Non-colored membrane components such as sphingolipids play a key-role in eukaryotic cells. Their metabolites, e.g. ceramide, sphingosine-1-phosphate and long chain base phosphates are regulators in apoptotic-like programed cell death (**2**). Aim of this study was to fractionate bioactive metabolites from *S.p.* for spectroscopic analysis (APCI-MS and NMR). A number of biphasic solvent systems had been evaluated by HPLC-DAD to achieve appropriate K_D-values for an optimized separation. The HPCCC (Spectrum-DE, column vol. 125 mL) was operated with *n-hexane/ EtOAc/ EtOH/ H_2O* (5:5:7:4) (*head-to-tail*, 4.0 mL/min) using the *elution - back extrusion* procedure.

The HPCCC fractions (non-polar saponified crude extract: 300 mg injection, t14 – t100) were *off-line* injected in sequence of elution to an APCI-MS/MS (**3**) where 8 principal components were detected. Ion signals at *m/z* 601, 585, 551, and 569 were carotenoid pigments. Violaxanthin (*m/z* 601) was directly recovered and identified by APCI-MS/MS, ^{1}H- and ^{13}C-NMR data. Some further ion signals were tentatively correlated to lutein-epoxide or antheraxanthin (*m/z* 585), and *m/z* 569 for lutein or zeaxanthin. Almost all carotenoid signals gave Δ*m/z* 18 neutral loss cleavages indicating APCI *in-source* fragmentation related to hydroxylation of xanthophylls (**4**). The CCC-fraction amounts were not sufficient in purity for NMR carotenoid identification. The tube 22 (cf. Fig.) contained the glucocerebroside d18:2-C16:0h-Glc as principal compound ($[M+H]^+$ *m/z* 714). The structure was confirmed by APCI-MS/MS (neg./pos.) (**5**,**6**), and 1D/2D-NMR. Further minor concentrated glucocerebrosides of slightly differing substitution were detected in the HPCCC-APCI-MS profile (not displayed here). Tube fractions 95-96 contained the cyclopropane-type steroid cycloartenol (*m/z* 427) confirmed by MS/MS fragmentation (**7**), and ^{13}C-NMR data.

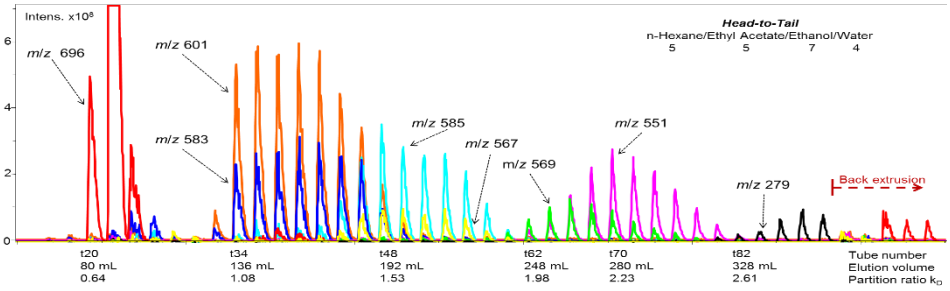

Figure: Positive APCI-MS/MS injection profile of HPCCC fractionated non-polars from *S. phureja* potatoes with selected ion traces $[M+H]^+$. Injection of every second tube fraction.

References

1. R. Aman et al., J. Chromatogr. A, 1074 (2005) 99.
2. K. P. Alden et al., Biochem. Biophys. Res. Com., 410 (2011), 574.
3. G. Jerz et al., J. Chromatogr. A., 1347 (2014), 17–29.
4. T. Maoka et al., J. Oleo. Sci., 51 (2002), 1.
5. F. Fang et al., J. Food Lipids, 12 (2005) 327.
6. N. Bartke et al., Mol. Nutr. Food Res. 50 (2006) 1201-1211.
7. S. Mo et al., Lipids 48 (2013) 949–956.

VALUE OF *K* FOR APPLICATION OF COUNTER-CURRENT CHROMATOGRAPHY IN THE ISOLATION OF THREE LIPOPETIDE FAMILIES

1018
FP
Session IV

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Keywords: Iturin; Fengycin; Surfactin.

The interest for biological control programs against pests in the world has grown substantially. *In vitro* experiments have shown that the corn-isolated endophytic bacterial strain CNPMS 22 (*Bacillus subtilis*), produces three families of lipopeptides (Iturin, fengycin and surfactin) with antifungal activity [1]. However, the production of these compounds in large-scale requires innovative technologies for their maximum recovery. The counter-current chromatography (CCC) is an efficient technique for the separation and purification of various organic substances. Nevertheless, no lipopeptide separation by CCC has been reported in the literature up to now. In this context, the objective of this study was to evaluate the best solvent system to be used in CCC for separation of three families of CNPMS22 lipopeptides (iturin, fengycin and surfactin), through the assessment of their partition coefficients (*K*). The 9 × 9 map-based solvent selection strategy [2] was chosen to perform those experiments. Initially, 2 mg of the lipopeptide extract, obtained by acid precipitation of the fermentation broth, were added to test tubes. The tested solvents were *n*-hexane-ethyl acetate-methanol-water in the following ratios: (3:7:3:7), (5:5:5:5), (7:3:7:3), (1:9:2:8), (1:9:3:7), (2:8:2:8) and (2:8:3:7) (v/v/v/v), respectively. After the two phase separation, the upper and lower phases were completely separated. The phases were filtered through PTFE filter (0.22µm) and injected on UPLC-ESI-QTOF-MS/MS. The partition coefficients (*k*) were determined with the ratio A_U/A_L where A_U and A_L are the peak areas of the compounds at the upper and lower phase, respectively. The results showed that the ratio of 1:9:3:7 is promising for the separation of iturins and fengycins (Table 1) and the ratio of 5:5:5:5 is the most appropriate for the separation of surfactins (Table 2). This is a first study about solvent system selection for isolation of these three families of lipopeptides to be applied in counter-current chromatography. Hence, further information is required for process optimization and being an applicable method.

Table 1. Value of K for solvent ratio (1:9:3:7)

Compounds	Molecular mass $[M-H]^+$	value of K
Iturin A2	1043.5588	0.2731
Iturin A3-A5	1057.5729	0.5822
Iturin A6-A7	1071.5889	0.6449
Fengycin A	1435.7742	0.0727
Fengycin A	1449.7961	0.1081
Fengycin B	1477.8228	0.7967
Fengycin A	1463.8141	0.2259
Fengycin B	1491.8446	0.7004
Fengycin B	1505.8540	1.1138

Table 2. Value of K for solvent ratio (5:5:5:5)

Compounds	Molecular mass $[M-H]^+$	value of K
surfactin	994.6481	1.6452
surfactin	1008.6709	2.1608
surfactin	1022.6839	2.9309
surfactin	1036.6935	3.2209
surfactin	1044.6576	1.3186

References

1. Patel S.; Ahmed, S.; Eswari, J.S. Therapeutic cyclic lipopeptides mining from microbes: latest strides and hurdles, World J Microbiol Biotechnol, 31, 1177–1193, August 2015.

2. Liang, J.; Meng, J.; Wu, D.; Guo, M.; Wu, S. A novel 9 × 9 map-based solvent selection strategy for targeted counter-current chromatography isolation of natural products, J Chromatogr A, 1400, 27-39, June 2015.

ENHANCING SILYMARIN FRACTIONATION USING THE CONDUCTOR-LIKE SCREENING MODEL FOR REAL SOLVENTS

1019
FP
Session VI

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Keywords: bioseparations, COSMO-RS, flavonoids, silymarins, nutraceuticals

The market for bio-based products from plant sources is on the rise. There is a global challenge to implement environmentally clean practices for the production of fuels and pharmaceuticals from sustainable resources. A significant hurdle for discovery of comparable plant-derived products is the extensive volume of trial-and-error experimentation required. To alleviate the experimental burden, a quantum mechanics-based molecular modeling approach known as the COnductor-like Screening MOdel for Real Solvents (COSMO-RS) [1] was used to predict the best two-phase solvent system to purify six silymarins from an aqueous mixture. Silymarins are a class of flavonolignans present in milk thistle (*Silybum marianum* L.), which has been used in traditional eastern medicine to treat liver disease. Previous research has shown that these compounds can be fractionated using centrifugal partition chromatography (CPC), but not to an acceptable level of purity [2,3]. Due to previous incomplete fractionation, the silymarins are ideal compounds to assess use of a molecular modeling approach to predict partitioning in a CPC separation. Utilization of the COSMO-RS via the software programs HyperChem, TurbomoleX, and COSMOthermX in order to calculate partition coefficients for compounds in CPC solvent systems was first illustrated by Hopmann et al. [4]. In this study, the methods have been applied to silymarins to evaluate the effectiveness of COSMO-RS in screening solvent systems for separation of biomolecules in a bi-phasic solvent system using CPC. COSMOthermX was used to calculate the activity coefficients of the silymarins in each solvent system, based on the molecular structure of the compounds and phase partitioning data gathered from gas chromatography. The activity coefficient was then used to calculate a partition coefficient for each silymarin in each solvent system. The partition coefficient was verified by experimentation and compared to the results of the model. Use of the COSMO-RS method allowed the range of possible solvent systems to be quickly narrowed down, reducing the quantity of trial-and-error tests and the time required to achieve results.

References

[1] A. Klamt, Conductor-like Screening Model for Real Solvents: A New Approach to the Quantitative Calculation of Solvation Phenomena, J. Phys. Chem. 99 (1995) 2224–2235. doi:10.1021/j100007a062.
[2] Q. Du, W. Cai, Y. Ito, Preparative Separation of Fruit Extract of *Silybum marianum* Using a High-Speed Countercurrent Chromatograph with Scale-up Columns, J. Liq. Chromatogr. Relat. Technol. (2002). doi:10.1081/JLC-120014271.
[3] A.S. Engelberth, D.J. Carrier, E.C. Clausen, Separation of Silymarins from Milk Thistle (Silybum Marianum L.) Extracted with Pressurized Hot Water using Fast Centrifugal Partition Chromatography, J. Liq. Chromatogr. Relat. Technol. (2008). doi:10.1080/10826070802424907.
[4] E. Hopmann, W. Arlt, M. Minceva, Solvent system selection in counter-current chromatography using conductor-like screening model for real solvents, J. Chromatogr. A. (2011). doi:10.1016/j.chroma.2010.11.018.

TOCOPHEROL SEPARATION WITH DEEP EUTECTIC SOLVENT-BASED BIPHASIC SYSTEMS

1020
FP
Session V

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Keywords: vitamin E, centrifugal partition extraction, water-free solvent system

Tocopherols are a class of molecules which are known for their vitamin E activity. Among these, α-tocopherol is the most important vitamin E source in the human diet. Hence, there is a great interest in separation methods that can provide purified α-tocopherol. The purification of tocopherols involving biphasic liquid systems can be challenging since these vitamins are poorly soluble in water.

In this work, a mixture of tocopherols mainly consisting of α- and γ-tocopherol has been separated using centrifugal partition extraction. For this purpose a biphasic system composed of organic solvents and a deep eutectic solvent (DES) was used. DES are formed when a hydrogen bond acceptor, in this case a quaternary ammonium salt, and a hydrogen bond donor are combined. The mixture has a significantly lower melting point than the pure components and therefore remains liquid at room temperature (1). The tocopherol constituents show different affinity for hydrogen bonding, resulting in different partitioning between the two liquid phases of the solvent system (2). A solvent system screening was performed using the predictive thermodynamic model COSMO-RS to find an appropriate DES-based biphasic system. The most promising biphasic solvent system was selected to perform the separation. For the liquid-liquid chromatographic separation a centrifugal partition extractor (SCPE-250-BIO from Armen Instrument, France) with a column volume of 250 ml was used. Although the DES-rich phase has a higher viscosity than conventional solvents, such as water or methanol, high stationary phase retention was obtained. Approximately 70 % of stationary phase were retained in the column at a flow rate of 20 ml/min and approximately 60 % at 40 ml/min, both at a rotation of 2000 rpm.

The results of this work show the potential of DES-based biphasic systems for the purification of water insoluble natural compounds.

References

1. A.P. Abbott, G. Capper, D.L. Davies, R.K. Rasheed, V. Tambyrajah, Novel solventproperties of choline chloride/urea mixtures, Chem. Commun. 1 (2003) 70–71.
2. Yang, Q., Xing, H., Cao, Y., Su, B., Yang, Y., & Ren, Q., Selective separation of tocopherol homologues by liquid−liquid extraction using ionic liquids, Ind. Eng. Chem. Res, 48(13) (2009) 6417-6422.

PROTEIN SEPARATION USING A CENTRIFUGAL PARTITION EXTRACTOR

1021
PO
Session

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Keywords: ionic liquids, aqueous two-phase systems, protein separation

Aqueous two-phase systems (ATPS) provide a mild environment for the separation of biomolecules, such as proteins and peptides, with liquid-liquid extraction or liquid-liquid chromatography (1). Conventional ATPS are composed of two polymers or a polymer and inorganic salt in water. In 2003, Rogers et al. introduced biphasic systems containing imidazolium-based ionic liquids, inorganic salts and water (2). Ionic liquids show high solvation capacity for many compounds. Since proteins partition almost exclusively into the ionic liquid-rich phase, these ATPS are well suited for extraction processes. (3) However, for liquid-liquid chromatographic separations, moderate partition coefficients of the solutes are desirable. Pereira et al. proposed to use ionic liquids as adjuvants to tailor the extraction capacity of biomolecules in polyethylene glycol (PEG)- and salt-based ATPS. Our measurements demonstrate that the addition of ionic liquids significantly influences the partition coefficients of proteins in biphasic systems composed of PEG 1000 and inorganic salts. (3) In these systems the partition coefficients are more suitable for an application in liquid-liquid chromatography. However, it is reasonable to use the minimal possible amount of modifier to tune the partition coefficient to keep the separation and material costs low. The influence of ionic-liquids on protein partitioning in such PEG-based ATPS was determined in shake flask experiments and compared with the influence of sodium chloride, which is commonly used as an additive in biphasic systems for the separation of biomolecules. A mixture of the two proteins lysozyme and myoglobin was separated using PEG-based biphasic systems in a centrifugal extractor (SCPE-250-BIO from Armen Instrument, France).

References

1. R. Hatti-Kaul, Aqueous two-phase systems,in: R. Hatti-Kaul (Ed.), Aqueous Two-phase Systems: Methods and Protocols, Humana Press, 2000, pp. 1–10.
2. K.E. Gutowski, G.A. Broker, H.D. Willauer, J.G. Huddleston, R.P. Swatloski, J.D. Holbrey, R.D. Rogers, Controlling the aqueous miscibility of ionic liquids: aqueous biphasic systems of water–miscible ionic liquids and water-structuring salts for recycle, metathesis, and separations, J. Am. Chem. Soc., 125 (2003), pp. 6632–6633
3. F. Bezold, J. Goll, M. Minceva, Study of the applicability of non-conventional aqueous two-phase systems in counter-current and centrifugal partition chromatography, J. Chromatogr. A, 1388 (2015), pp. 126-132
4. J.F.B. Pereira, A.S. Lima, M.G. Freire, J.A.P. Coutinho, Ionic liquids as adjuvants for the tailored extraction of biomolecules in aqueous biphasic systems, Green Chem., 12 (2010), pp. 1661–1669

RELATIONSHIP BETWEEN THE EFFICIENCY AND ROTATION SPEED IN THE COUNTERCURRENT CHROMATOGRAPHY: SEPARATION OF CYTOTOXIC METABOLITES BY SELECTIVE ENZYMATIC TRANSFORMATION

1022 PO Session

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Keywords: prosaikogenins, rotation speed, stationary phase retention

Countercurrent separation (CS) is one of the efficient methods to separate the pure compound from the diverse natural resources. Establishing an optimal condition is the most important procedure for the efficient separation. The retention of stationary phase (S_f) strongly affects the resolution of peaks. *Du* (1) and *Wood* (2) demonstrate the relationship between the stationary phase retention and various parameters. More recently, Berthod et al. shed light on the relation of resolution and stationary phase retention (3). The rotation speed of countercurrent chromatography machine is also greatly affects the resolution of peaks. With the given solvent system and countercurrent apparatus, the optimal rotation speed was speculated by several preliminary experimental results and calculations. The speculation was further demonstrated by the actual experiments.

Saikosaponins are bioactive compounds from the roots of *Bupleurum falcatum*. Despite of various pharmacological benefits, the application of those compounds is restrained due to their lower bioavailability and the lack of large-scale separation method. The separation method for the metabolites of those compounds was developed with the application of enzymatic transformation. By the enzymatic transformation, glucose at the C-3 position of structure was eliminated. As a result, the polarity and molecular weight were decreased. The converted fraction was then separated by countercurrent chromatography on the preparative scale. Through the investigation on the cytotoxicity of the separated compounds, the converted compounds seem to be more promising for the candidate of anticancer agent.

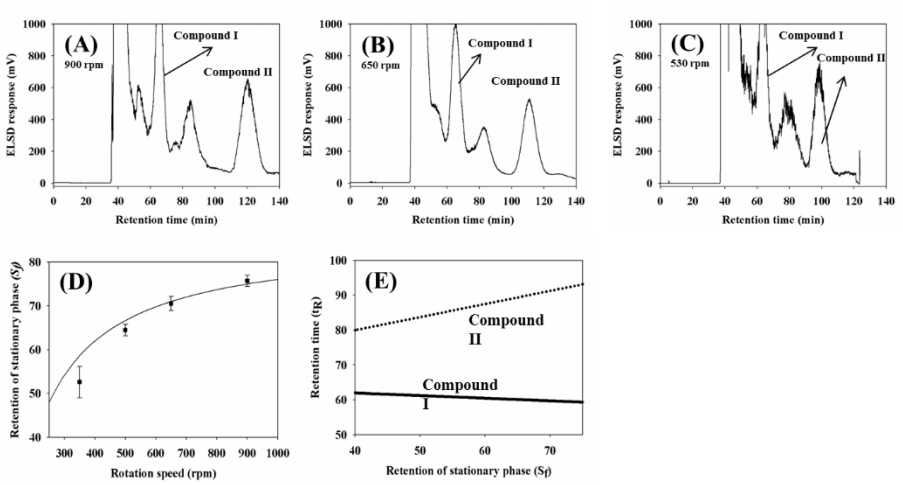

Figure 1. Relationship between the resolution and the rotation speed.

References

(1) Du, Q., Wu, C., Qian, G., Wu, P., Ito, Y., Relationship between the flow-rate of the mobile phase and retention of the stationary phase in countercurrent chromatography, J. Chromatogr. A, 835 (1999) 231-235.

(2) Wood, P., Janaway, L., Hawes, D., and P., Janaway, L., Hawes, D., Sutherland, I., A., Stationary phase retention in contercurrent chromatography: Modeling the J-type centrifuge as a constant pressure drop pump, J. Liq. Chrom. & Rel. Technol., 26 (2003) 1373-1396.

(3) Berthod, A., Faure, K., Revisiting resolution in hydrodynamic countercurrent chromatography: Tubing bore effect, J. Chromatogr. A, 1390 (2015) 71-77.

LINEAR GRADIENT ELUTION IN COUNTERCURRET CHROMATOGRAPHY WITH AVERAGE SPEED OF TARGET COMPOUNDS

1023
FP
Session VIII

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Keywords: linear gradient, average speed, Pulsatilla koreana

Natural products extracts are mixture of compounds of wide polarity range. Gradient elution in countercurrent separation (CS) allows the wide polarity range to be handled and co-eluting compounds to be separated. Several modeling studies (1) have illustrated that various parameters have to be considered for gradient elution. In the present study, solvent systems for the gradient elution were pre-evaluated on the basis of several criteria (e.g. partition coefficients, settling time, and the retention of stationary phase). The linear-gradient change was settled down by speculating average speeds (|*V*|) of target compounds. The important issues in linear gradient elution are the starting point and the duration of the second mobile phase. Those conditions were speculated by the location (*L*) of target compounds.

Pulsatilla koreana (Ranuculaceae) is a perennial herb from the hillocks in South Korea. The root of this plant has long been used as a traditional herbal medicine to treat amoebic dysentery, malaria, chills, and fevers. The major components of *P. koreana* are triterpenoid saponins. Because of their pharmacological activities, a preparative separation method for obtaining pure saponins from *P. koreana* needs to be developed. To date, several triterpenoid saponins of lupane or oleanane-type have been isolated using conventional chromatographic methods despite of low recovery. In the present study, four compounds were separated by linear gradient elution, including hederacolchiside E, which has recently been highlighted as an agent for the treatment of Alzheimer's disease.

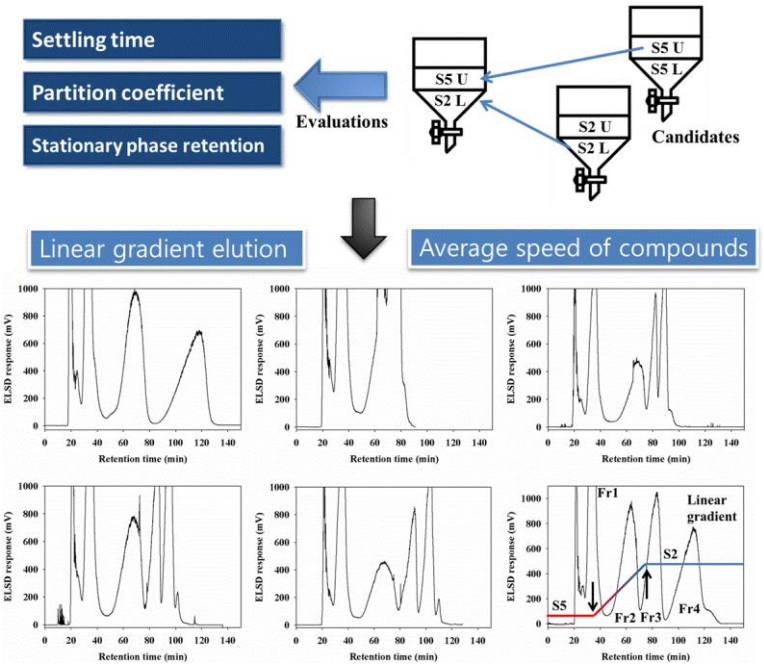

Figure 1. Schematic explanation of linear gradient countercurrent separation.

References

(1) S. Wu, D. Wu, J. Liang, A. Berthod, Modeling gradient elution in countercurrent chromatography: efficient separation of tanshinones from *Salvia miltiorrhiza* Bunge. *J. Sep. Sci.*, (2012) 35, 964-976

APPLICATION OF COUNTER-CURRENT CHROMATOGRAPHY AS A POWERFUL FRACTIONATION TOOL. CASE STUDY: OBTAINING GRAM-SCALE SESQUITERPENOIDS FROM *TUSSILAGO FARFARA*

1024 PO Session

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Keywords: Gram-scale fractionation, Tussilago farfara*, Sesquiterpenoids*

Natural extracts from plants, source of biologically active metabolites, are exploited for drug or health supplement developments. However, its chemical complexity and diversity requires time-consuming multi-step purification procedures. *Tussilago farfara*, one of the medicinal plants of the family Asteraceae, contains diverse sesquiterpenoids, which show pharmacological activities. Minor sesquiterpenoids with similar chemical properties make pure compound isolation difficult in one-step CCC separation. Also, low contents of the sesquiterpenoids in raw material require efficient fractionation procedure.
In this study, new approach for enriching sesquiterpenoids from the buds of *Tussilago farfara* was developed. CCC operation was performed by HSCCC (TBE-1000A, Tauto Corp., China: coil volume: 1000 mL; 3.0 mm tube i.d.). 1.8 g of sesquiterpenoids-enriched fraction was obtained from 66.8 g crude extract of *Tussilago farfara* in a single CCC operation, with separation time of 7.5 hrs. For enriching target mixture, only 0.95 L water and 2.95 L organic solvents in total was used including extraction. Also, quantification studies of three major sesquiterpenoids in each fraction of different fractionation methods were conducted. CCC operation results shows the best efficiency compared to the conventional multi-step fractionations processed in series: solvent partition and open column chromatography. Considering its lab scale CCC device, solvent consumption, and processing time, this method enables powerful product recovery with high quality enrichment.

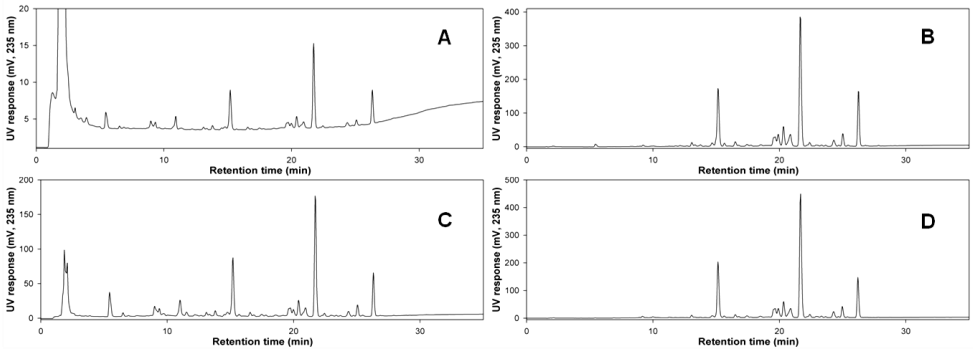

Figure 1. HPLC-UV chromatogram. (A) Crude extract; (B) CCC, (C) *n*-hexane, and (D) 100 % methanol fraction.

Table 1. Efficiency of three fractionation methods in recovery and solvent consumption.

Extract (66800 mg) from 200 g of raw material; 3.3, 6.7 and 2.5 mg/g of compounds 1, 2, and 3, resp.					
Fractionation tool		**CCC**	**Solvent partition (n-hexane)**	**Open column (Diaion HP-20)**	
				90 % MeOH	**100 % MeOH**
Yield (mg)		1784	3827	220	930
Compound 1 (mg/g), (% recovery)		119.3 (96.7)	55.2 (96.0)	54.7 (5.5)	125.3 (53.0)
Compound 2 (mg/g), (% recovery)		248.9 (99.0)	106.1 (90.5)	103.7 (5.1)	272.2 (56.4)
Compound 3 (mg/g), (% recovery)		93.8 (98.8)	35.1 (79.3)	34.3 (4.5)	82.2 (45.1)
Solvent consumption (mL) (including extraction)	**Aqueous**	950	2300	3500	
	Organic	2950	2300	5100	

PREPARATIVE SEPARATION OF EUPHORBIA FACTORS FROM *EUPHORBIA LATHYRIS* BY COUNTERCURRENT CHROMATOGRAPHY

1025 PO Session

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Keywords: Euphorbia *factors,* Euphorbia lathyris*, One-step separation*

This work demonstrates preparative one-step separation of *Euphorbia* factors from the seeds of *Euphorbia lathyris* using countercurrent chromatography with low cost. CCC operation was performed by HSCCC (TBE-1000A, Tauto Corp., China: coil volume: 1000 mL; 3.0 mm tube i.d.). Four major euphorbia factors: euphorbia factor L8 (19.6 mg), euphorbia factor L1 (128.8 mg), euphorbia factor L2 (56.2 mg), and euphorbia factor L3 (118.9 mg) were obtained from 8.8 g of the crude extract of *Euphorbia lathyris* in a single CCC operation. Including the extraction process, only 0.5 L water and 1.9 L organic solvents in total was used to isolate the target compounds. Isolated compounds were above 95 % in purity, as determined by HPLC (280 nm) and LC-MS analysis. This is the first report that euphorbia factors from the seeds of *Euphorbia lathyris* were successfully separated by CCC.

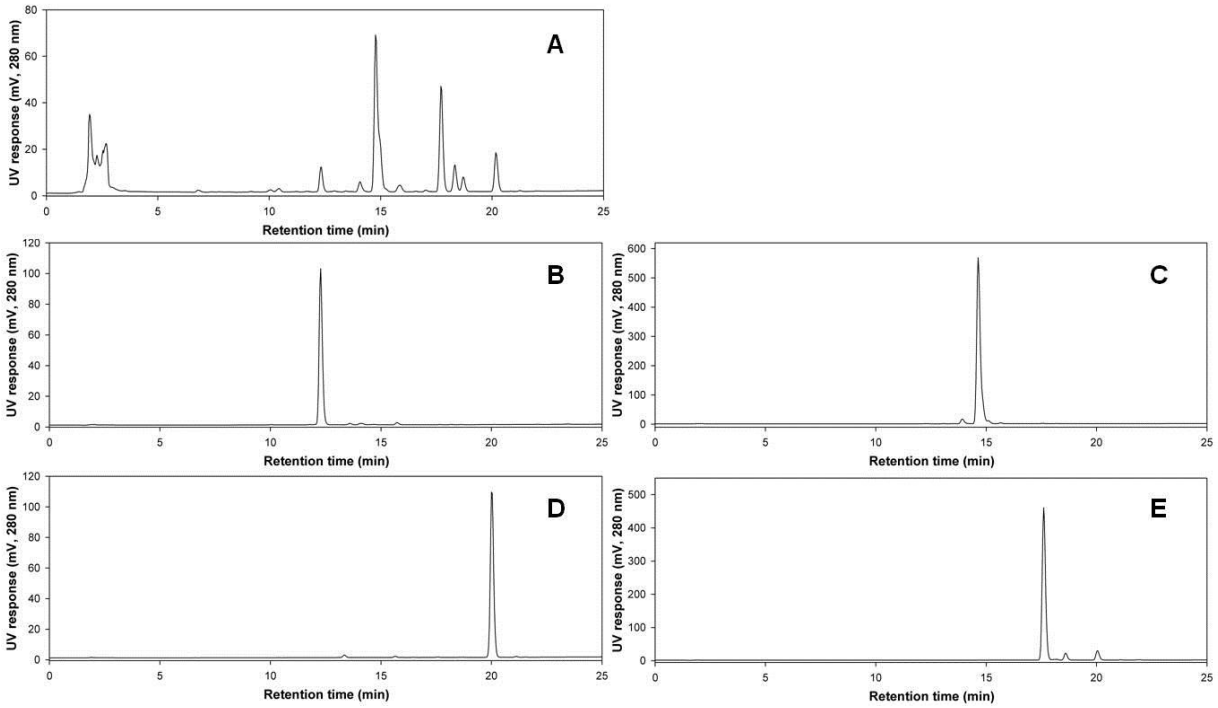

Figure 1. HPLC-UV chromatogram. (A) Crude extract; (B) Euphorbia factor L8; (C) Euphorbia factor L1; (D) Euphorbia factor L2; (E) Euphorbia factor L3

A NEW PROCESS FOR THE ANALYSIS OF MASTIC GUM AND ISOLATION OF BIOACTIVE TRITERPENS AND POLYMER

1026
FP
Session VII

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Keywords: mastic gum, pH zone step gradient, ^{13}C NMR dereplication, supercritical fluid chromatography

Mastic gum is a natural resin obtained from *Pistacia lentiscus* L. var Chia after "hurting" the trunk and branches. This high-value product is collected for more than 2,500 years and used in traditional medicine for various gastrointestinal disorders as well as in perfumery, dentistry and as a spice and flavoring in Mediterranean cuisine (1). Chemically, mastic gum consists of acidic and neutral triterpenes (about 75%) and polymers (25%). Due to the high complexity of this material the purification of the bioactive compounds is time consuming and requires multi-stage separation procedures to usually recover small amounts of compounds [2]. The present work aims to develop a rapid and effective process for the isolation of the main mastic gum constituents. The first step consists in the fractionation of crude mastic gum extract by using a novel CCC method which combines in the same run the pH zone refining and step gradient elution modes. The experiment starts by treating the row material with the biphasic system *n*-hexane/EtOAc/EtOH/H_2O 8:2:5:5 (v/v/v/v) in pH zone refining mode in order to fractionate the acidic triterpenes. Thereafter, a step gradient elution mode takes place by passing the lower phases of the same biphasic system in ratios 8:2:7:3, 8:2:8:2 and 8:2:9:1 (v/v/v/v) in order to separate the neutral triterpenes. Finally the column is extruded with *n*-hexane, resulting in the recovery of pure polymers. The separation was performed in a 300 mL CPE column leading to the effective fractionation of 7 g of mastic gum in only 2 hours. The chemical composition of each CPE fraction was established by ^{13}C NMR dereplication and 2D NMR analyses. Further analysis of enriched CPE fractions by supercritical fluid chromatography SFC-CO_2 using a chiral column led to the purification of the main triterpenic isomers of the mastic gum. This process can be considered as a new approach for the isolation of bioactive compounds from mastic gum. Furthermore, the proposed CCC methodology could be also applied for the effective fractionation of numerous plant extracts containing complex mixtures of neutral and acidic or basic components.

References

1. Al Said, M., A. M. Ageel, N. S. Parmar, and M. Tariq, Evaluation of mastic, a crude drug obtained from *Pistacia lentiscus* for gastric and duodenal anti-ulcer activity, J. Ethnopharmacol. 15, (1986), 271–278.
2. S. Paraschos, P. Magiatis, S. Mitaku, K. Petraki, A. Kaliaropoulos, P. Maragoudakis, In vitro and in vivo activity of chios mastic gum extracts and constituents against Helicobacter pylori, Antimicrobial Agents Chemotherapy, 51, (2007), 551–559.

Acknowledgment

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DEVELOPMENT OF A TWO-DIMENSIONAL SEQUENTIAL CENTRIFUGAL PARTITION CHROMATOGRAPHY PROCESS FOR THE PREPARATIVE SEPARATION OF TERNARY MIXTURES

1027 PO Session

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Keywords: two-dimensional separation, ternary mixture, continuous separation, sequential centrifugal partition chromatography (sCPC)

Preparative liquid-liquid chromatographic processes often require the isolation of components of intermediate elution speed from complex mixtures. When performing batch injections, peak cutting is usually necessary to obtain pure fractions of these target components, resulting in decreased productivity and yield. “Trapping” an intermediate component inside the column while eluting the neighboring compounds during multiple cycles of ascending and descending steps (1,2) can be used to achieve higher system throughput. However, this operating mode cannot be run continuously, since the process must eventually be stopped for collection of the non-eluted component. Continuous processes are often preferred in industrial settings for their high productivities and ability to be highly automated. An option for continuous separation of ternary mixtures is the two-dimensional liquid-liquid chromatography process reported by Couillard et al. in 2005 (3). In the proposed set-up, two two-column units are connected in series. In the first unit, a ternary mixture (A, B, and C) is continuously injected and fractionated into two product streams eluted sequentially during multiple cycles of ascending and descending steps. One product stream contains one component (A), while the other contains two (B and C). The two-component product stream (B and C) is fed directly to the second unit. Two product streams are again obtained, this time containing one component each (B or C).

The aim of this study was to extend an existing model-based design approach for the separation of binary mixtures using sequential centrifugal partition chromatography (sCPC) (4) for selection of the operating parameters in two sCPC units for the two-dimensional separation of (pseudo-)ternary mixtures. Before developing the continuous separation process, each sCPC step was evaluated individually. A short-cut design method was used along with simulations based on the equilibrium cell model for selection of the operating parameters such as feed and mobile phase flow rate, duration of the ascending and descending steps, and feed concentration. Several options for integraton of the two sCPC steps for continuous operation were explored, and strategies for adapting the product stream from the first unit to the feed stream of the second unit were addressed. The parameter selection approach was validated experimentally for each of the sCPC steps using a model mixture of three components of similar molecular structure (ethyl paraben, propyl paraben, and butyl paraben).

References

1. P. Hewitson, S. Ignatova, H. Ye, L. Chen, I. Sutherland, J. Chromatogr. A 1216 (2009) 4187.
2. N. Mekaoui, J. Chamieh, V. Dugas, C. Demesmay, A. Berthod, J. Chromatogr. A 1232 (2012) 134.
3. F. Couillard, A. Foucault, D. Durand, French Pat. FR2856933 (2005).
4. E. Hopmann, M. Minceva, J. Chromatogr. A 1229 (2012) 140.

CONTINUOUS FRACTIONATION OF MULTICOMPONENT MIXTURES WITH SEQUENTIAL CENTRIFUGAL PARTITION CHROMATOGRAPHY

1028 PO Session

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Keywords: continuous separation, sequential centrifugal partition chromatography (sCPC), multicomponent mixture

Sequential centrifugal partition chromatography (sCPC) is a cyclic liquid-liquid chromatographic process in which the feed stream is continuously introduced between two columns connected in series. The products are collected sequentially at opposite ends of the two-column set-up during multiple cycles consisting of two elution steps (ascending and descending). This concept was first reported by Couillard et al. in 2005 (1). A model-based design approach for the selection of the biphasic system and unit operating parameters for the separation of two-component mixtures using sCPC was previously described (2,3). The objective of this work was to extend this design approach to continuous pseudo-binary separations of multicomponent mixtures. Additionally, a strategy for maximizing system throughput while maintaining stable, predictable operation was developed (4).

The model mixture used in this investigation consisted of four components with similar molecular structure: methyl paraben (A), ethyl paraben (B), propyl paraben (C), and butyl paraben (D). Three separation tasks were performed as proof-of-concept of pseudo-binary fractionation: production of pure A, production of pure D, and separation of A and B from C and D. The durations of the ascending and descending steps were selected under ideal conditions (no dispersion effects) using a short-cut design method (2) so as to obtain the desired pure product streams from the quaternary mixture. Simulations based on the equilibrium cell model were used to verify that high purities could be obtained with the selected unit operating parameters under non-ideal conditions as well. The three pseudo-binary fractionations were performed with a global feed concentration of 8 mg/mL. Purities over 99% were achieved in all experiments.

A strategy for increasing the system throughput was then explored. In sCPC, higher throughput can be attained by increasing the feed concentration and/or the feed flow rate. When determining the maximum feed concentration, it must be considered that high solute concentrations inside the column can lead to changes of the initial volume ratio and compositions of the phases of the biphasic system. Partition coefficients of the solutes may be affected as well. Under such conditions, stable and predictable operation becomes impossible. Therefore, physical properties and partition coefficient measurements were performed at varying global concentrations of the paraben mixture in the biphasic system. The linear ranges of the partition coefficients were limited at a global concentration of 20 mg/mL. This corresponded to the concentration above which changes in the phase volume ratio were observed. The maximum feed concentrations allowing for operation in the linear range of the partition isotherm were determined by simulations. For production of pure D, this was found to be a global feed concentration of 18 mg/mL. As predicted by simulations, experimental product purities of ≥ 99% were obtained for this separation. Throughput was three times greater and solvent consumption three times lower than in the corresponding proof-of-concept experiment. The results of this study show that after determining the linear ranges of the partition coefficients, the short-cut method can be used in combination with the equilibrium cell model for improvement of sCPC unit separation performance.

References

1. F. Couillard, A. Foucault, D. Durand, French Pat. FR2856933 (2005).
2. J. Völkl, W. Arlt, M. Minceva, AIChE J. 59 (2013) 241.
3. J. Goll, A. Frey, M. Minceva, J. Chromatogr. A 1284 (2013) 59
4. J. Goll, M. Minceva, submitted manuscript

QUICK SELECTION OF SOLVENT SYSTEM FOR COUNTER-CURRENT CHROMATOGRAPHY SEPARATION WITH ONE SIMPLE HPLC METHOD

1029
FP
Session VI

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Keywords: solvent systems, HPLC method, GUESSmix, relationship

Counter-current chromatography (CCC) is widely used in the separation and enrichment of bioactive agents from complex samples, such as Chinese herbal medicines, fermentation and synthetic crude (1-3). The choice of an appropriate solvent system for CCC is a critical step in the purification of complex samples. However, it is still very laborious to find a suitable solvent system. The classical approach is to choose solvent system by K values or directly with analytic CCC (4). Previously, the authors discovered that an initial relationship could be established between HPLC elution system and the HEMWat system for CCC. This study is taking much broader approach to investigate the relationship between the gradually increasing of MeOH in MeOH/H_2O elution system for HPLC and HEMWat system S-8~S+8 (5) with GUESSmix, a mixture of 21 commercially available natural products and based on that, a quick selection of CCC solvent system for complex sample separation could be obtained by HPLC chromatogram. Furthermore, three complex herbal medicine sample types were also tested in this work to prove the generality of the method.

Acknowledgments

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References

1. I.A. Sutherland, D. Fisher. J Chromatogr A. 1216 (2009) 740
2. J. Wang, Y. Wen, X. Chen, Y. Lin, J. Zhou, Y. Xie, H. Wang, H. Jiang, W. Zheng. J Chromatogr A. 1217 (2010) 5687
3. A. Weisz, J.J. Witten, Y. Zeng, E.P. Mazzola, Y. Ito. J Chromatogr A. 1237 (2012) 106
4. Y. Ito. J. Chromatogr A. 1065 (2005) 145
5. J.B. Friesen, G.F. Pauli. J. Chromatogr. A 1151 (2007) 51

PREPARATIVE ISOLATION OF FLAVONE *C*-GLYCOSIDES FROM *PERISTROPHE BAPHICA* BY COUNTER-CURRENT CHROMATOGRAPHY COUPLED WITH OTHER TECHNIQUES

1030
FP
Session III

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Keywords: C-glycosides, solvent system selection, HSCCC

Preparative separation method using counter current chromatography (CCC) was developed for the purification of flavone C-glycosides from the dried aerial part of *P. baphica*. The crude sample was prepared by extract with ethanol and fractionated with EtOAc. The aqueous layer was adsorbed by polyamide column. Elution was carried out with H_2O, followed by 50% EtOH. There are six major flavone C-glycosides in the 50% EtOH fraction, and five compounds have same molecular weights and similar structures. Because of their similarities, separation of these isomeric flavone C-glycosides is relatively difficult and laborious. To improve their separation procedure, the two phase solvent system was applied for purification composed of system: ethyl acetate/ethanol/n-butanol/acetic acid/water at a volume ratio of 16:4:4:1:20. The purity of separated compounds was greater than 90% assessed by high-performance liquid chromatography-UV. Finally, preparative high performance liquid chromatography (pre-HPLC) was used to improve the purity of these compounds. Structures of compounds were characterized by HPLC-ESI-MS and 1D/2D-NMR experiments (^1H, ^{13}C, ^1H-^1H COSY, HSQC, HMBC). This separation yield flavone C-glycosides: vicenin-2 (1), neoschaftoside (3), schaftoside (4), and 6-*C*- β -D-glucopyranosyl-8-*C*- β -D-arabinofuranosyl-apigenin (5), neoisochaftoside (6). This is the first report that flavone C-glycosides from *P. baphica* have been successfully isolated and purified by CCC.

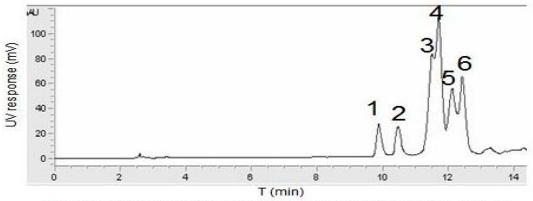

Figure1. HPLC chromatogram of the aqueous fraction of *P.*baphica. Compounds 1, Vicenin-2; 3, Neoschaftoside; 4, Schaftoside; 5, 6-C-β-D-glucopyranosyl-8-C-β-D-arabinofuranosyl-apigenin; 6, Neoisochaftoside. Detection wavelength: 280 nm.

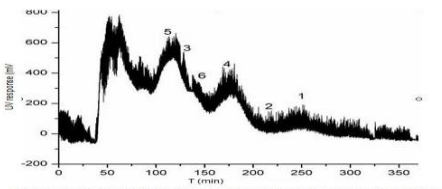

Figure 2. CCC chromatogram of aqueous fraction of *P. baphica*. The column volume was 325ml(TBE -300C,Tauto). Compounds 1, Vicenin-2; 3, Neoschaftoside; 4, Schaftoside; 5 6-C-β-D-glucopyranosyl-8-C-β -D-arabinofuranosyl-apigenin; 6, Neoisochaftoside. CCC condition: tail-to-head mode; flow rate: 3ml/min; revolution speed: 900 rpm; temperature: 25° C; retention of the stationary phase: 63%; sample amount: 160mg; injection volume: 12 ml; detection wavelength: 280nm.

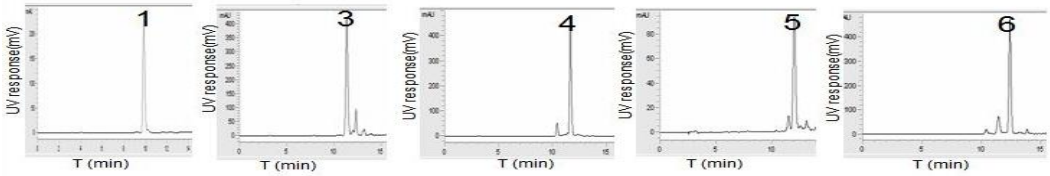

Figure 3. HPLC chromatogram of the aqueous fraction of *P.*baphica. Compounds 1, Vicenin-2; 3, Neoschaftoside; 4, Schaftoside; 5, 6-C-β-D-glucopyranosyl-8-C-β-D-arabinofuranosyl-apigenin; 6, Neoisochaftoside. Detection wavelength: 280 nm.

Acknowledgments
The authors would like to gratefully acknowledge the Bagui Scholar Program of Guangxi for providing financial support.

A HIGH-THROUGHPUT PROCEDURE BASED ON CPC-FRACTIONATION FOR THE DISCOVERY OF SKIN WHITENING AGENTS FROM GREEK FLORA EXTRACTS

1031
SO
Session IV

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Keywords: multivariate data analysis, heterocovariance approach, skin-whitening agents, Greek flora, bioactive natural compounds

Greece has an exceptionally rich flora which forms, geographically and historically, a link between the plant-life of Europe and that of Asia [1]. It is characterized by high biodiversity, consisting of 6300 species of which 950 are endemic and thus could be an ideal source for the discovery of bioactive compounds [2]. The aim of this project is to discover natural compounds with skin whitening properties via the establishment of a high-throughput platform relying on CPC, HPTLC and NMR, for their direct detection and identification prior to any isolation. 600 plants existing in a unique plant-library comprising around 32% of the Greek flora were selected and the plant extracts were produced by Accelerated Solvent Extraction. The extracts were *in vitro* investigated for their tyrosinase inhibition activity and the most promising ones were selected for further elaboration. More specifically, the extracts of *Morus alba, Veratrum album*, *Paeonia mascula, Cercis siliquastrum, Pistacia terebinthus* and others were fractionated by a CPC gradient step-wise methodology involving five biphasic solvent systems consisted of heptane, ethyl acetate, butanol, methanol and water. The resulted fractions (45 per extract) were evaluated for tyrosinase inhibition potential and further analyzed by HPTLC and NMR. An integrated HPTLC-based procedure for the tracing of compounds that contributed to tyrosinase inhibitory effect in active fractions was established with the use of multivariate data analysis. Additionally, NMR spectral data were correlated with the activity towards tyrosinase resulting in the identification of bioactive compounds through the combination of the Heterocovariance approach (HetCA) and the statistical total correlation spectroscopy (STOCSY). The combined data deriving from NMR and HPTLC correlated to the results of the biological activity by the statistically driven approach, revealed flavans, flavonols, phenolic compounds and stilbenoids as the most promising whitening agents, providing a major reduction of analysis time in comparison with conventional bio-guided procedures. Furthermore, the step-gradient elution-extrusion method is suitable to perform separations of compounds in complex mixtures with large differences in hydrophobicity. However, it is also of great importance the fact that CPC is characterized by easy scaling up and higher loading capacity in comparison to other conventional chromatographic techniques, allowing to receive fractions in sufficient amount for their biological evaluation and isolation of the bioactive constituents.

References

1. S. Kokkini, V. Karagiannakidou, E. Hanlidou, D. Vokou. Geographical and altitudinal distribution of the family Lamiaceae in Greece. Phyton (Austria) 28 (1988) 215-228.
2. P. Dimopoulos, Th. Raus, E. Bergmeier, Th. Constantinidis, G. Iatrou, S. Kokkini, A. Strid, D. Tzanoudakis. Vascular plants of Greece: an annotated checklist. – Berlin: Botanischer Garten und Botanisches Museum Berlin-Dahlem, Freie Universität Berlin; Athens: Hellenic Botanical Society. Englera 31 (2013) 1-367.

SCALE UP PURIFICATION OF MONOSACCHARIDES FROM CRUDE HYDROLYSED SUGAR BEET PULP

1032
FP
Session VIII

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Keywords: monosaccharaides, sugar beet pulp, biorefinery

The isolation of component sugars from biomass represents an important step in the bioprocessing of sustainable feedstocks such as sugar beet pulp. Centrifugal partition chromatography (CPC) is proposed to be used as an alternative to multiple resin chromatography steps to isolate component monosaccharides from a hydrolysed sugar beet pulp pectin fraction (1). An ethanol - ammonium sulphate (300 g/L) phase system (0.8:1.8 v/v) was used in ascending mode to fractionate three sugar fractions (L-rhamnose, L-arabinose and D-galactose, and D-galacturonic acid) from a synthetic crude in a single step. Sample was prepared in the stationary phase to prevent solubility issues but was not shown to have a detrimental effect on separation performance based on synthetic crude separations. The optimised conditions were then scaled up from 200ml Kromaton CPC instrument to 2L RotaChrom CPC unit. The latter has different cell design allowing to achieve much higher flow rates with no loss in separation performance. To match the *g*-field level the rotational speed was dropped to 450rpm; while the mobile phase flow rate was increased up to 150ml/min providing 70% of initial stationary phase retention. The separation time was shortened by 50% with better resolution between target peaks. The separation, therefore, improved and further increase in scale and throughput are possible.

The present study is a proof of concept and the throughput is below the maximum loading capacity of the 2L instrument used.

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References

1. D.P. Ward, M. Cárdenas-Fernández, P. Hewitson, S. Ignatova, G.J. Lye, Centrifugal Partition Chromatography in a Biorefinery Context: Separation of Monosaccharides from Hydrolysed Sugar Beet Pulp, J. Chromatogr. A 1411 (2015) 84-91

SEPARATION OF 5,6-DIHYDRO-A-PYRONES FROM *HYPTIS MONTICOLA* BY HIGH-SPEED COUNTRCURRENT CHROMATOGRAPHY (HSCCC)

1033 PO Session

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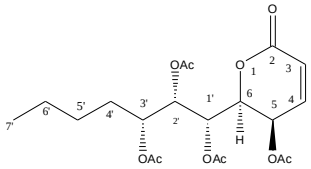
Keywords: Hyptis monticola*, Lamiaceae, alpha-pyrones, monticolides A-G*

Hyptis monticola Mart. ex Benth (Lamiaceae) is an endemic high-altitude grassland Brazilian species. Currently, about 580 species of *Hyptis* are known and the main phytochemicals described in the literature are terpenoids, alpha-pyrones, flavonoids and lignans. Alpha-pyrones are secondary metabolites endowed with cytotoxic properties towards a series of cancer cell lines [1]. To date, to the best of our knowledge, there is no record of the separation of this class of substances by countercurrent chromatography. Thus, the aim of this study was to develop a rapid method to isolate these compounds from the dichloromethane (DCM) extract from leaves of *H. monticola*. The plant was collected in Vale das Videiras, Rio de Janeiro, Brazil at 1.239m altitude. Leaves were dried, powdered and extracted with EtOH: H_2O (7:3). The resulting extract was subjected to liquid-liquid partitions with solvents of increasing polarity: hexane, CH_2Cl_2, EtOAc and *n*-BuOH. For the selection of the two-phase solvent system for HSCCC, eleven different ratios of the HEMWat solvent system (hexane: EtOAc: MeOH: H_2O) were tested due to the versatility of this quaternary system and because of its range of polarities. The following proportions were tested: **1** (3:1:1:0.5); **2** (3:2:1:0.5); **3** (2:3:1:0.5); **4** (1:1:1:1); **5** (2:1:1:1); **6** (3:1:1:1); **7** (1:2:1:1); **8** (0.8:1:0.8:1); **9** (1:0.8:1:0.8), **10** (0.5:1:0.5:1) and **11** (1:0,5:1:0.5). The results were visualized by TLC and solvent system **8** was chosen. The distribution coefficient (*k*) of the main compounds in the DCM extract were calculated by HPLC, from their peak areas in the chromatogram, and ranged from 0.6 to 10.8. The DCM extract was fractionated using the 95 mL coil of a Quattro HTPrep apparatus, with a flow rate of 3 mL/min (upper phase as mobile), 860 rpm. A total of 65 fractions (3 mL) were collected with the rotation on. Afterwards, 30 more fractions were collected pumping out the mobile phase. Fractions 14-21 afforded a tetracetylated 5,6-dihydro-alpha-pyrone which was named Monticolide A. Fractions 40-49 afforded a mixture of two triacetates named Monticolides B and C, which interconvert into each other by transesterification reactions. Fractions 56-67 afforded a mixture of Monticolide C and a diacetate, named Monticolide D. Frations 77-84 contained a mixture of three diacetates, with a free hydroxyl in C-5 of the lactone ring. They were named Monticolides E-G. Scale-up studies aimed at obtaining larger amounts of these compounds which allowed for the preparation of their Mosher esters and determination the absolute stereochemistry of Monticolides B-G.



Monticolide A

References

1. Pereda-Miranda, R. et al, J Nat Prod, 56 (4): 583-593, 1993.

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COUNTERCURRENT CHROMATOGRAPHY WITH OFF-LINE DETECTION BY HPLC-ESI-MS/MS FOR THE SEPARATION AND IDENTIFICATION OF SAPONINS FROM *AMPELOZIZYPHUS AMAZONICUS*

1034 PO Session

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Keywords: Ampelozizyphus amazonicus, Rhamnaceae, triterpene saponins

Ampelozizyphus amazonicus Ducke (Rhamnaceae) is a climbing shrub native to the Amazonian region, where its bark and roots are used in the folk medicine to prepare a beverage to cure and prevent malaria, as well as a tonic and fortifier, among other uses (1). The main compounds cited in the literature are jujubogenin glycosides saponins (2,3,4). The crude extract of the saponins is too complex for any kind of structural identification and HPLC separation was not sufficient enough to resolve this issue. Therefore, the aim of this work was to obtain saponin concentrates from bark ethanol extract of *Ampelozizyphus amazonicus* by Countercurrent Chromatography (CCC) and to identify them by HPLC-HRMS and MS^n.

The bark ethanol extract was partitioned between water and in hexane, ethylacetate and butanol in the sequence. The butanol-rich phase was then fractionated by CCC with hexane - ethyl acetate - butanol - ethanol - water (1:6:1:1:6; v/v) solvent system yielding 5 group fractions. The first and third groups were further separated by CCC with dichloromethane - isopropanol - metanol - water (6:3:2:4; v/v) and ethylacetate - ethanol - water (1:0.2:1; v/v) solvent systems, respectively. The collected fractions from these two runs were analyzed for structural identification by HPLC-HRMS and MS^n. Group 1 contained mostly saponins with α- and β-amyrin skeletons. In Group 3 jujubogenin glycosides and keto-dammarane-type triterpene saponins with a C31 skeleton were the main compounds (Figure 1), indicanting that CCC was able to sort the saponins according to their skeletons. Thereby less complex samples could be analyzed by HPLC-ESI-MS/MS. Studies are in course to the complete elucidation of their structures.

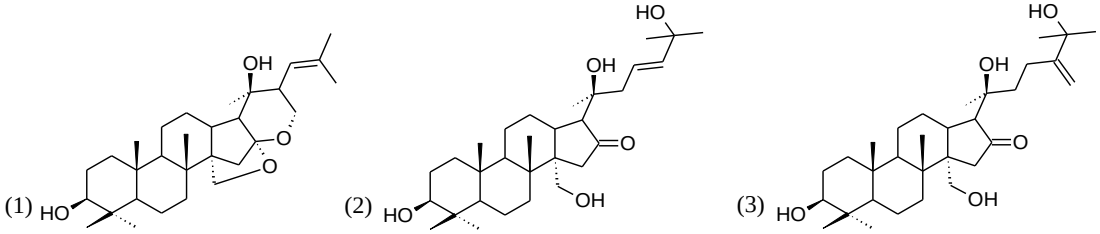

Figure 1. Saponin aglycone skeleton types present in group 3 fractions: (1) jujubogenin; (2;3) keto-dammarane.

References

1. D.R. Oliveria et al. Acta Amazonica 41 (2011) 383-392.
2. M.G.L. Brandão et al. Phytochemistry 31 (1992) 352-354.
3. M.G.L. Brandão et al. Phytochemistry 34 (1993) 1123-1127.
4. L.M.T. Peçanha et al. Journal of Biomedicine and Biotechnology (2013) 1-11.

Acknowledgements

FAPERJ, CAPES, CNPq

ALKALOIDS FROM TRICLISIA DICTYOPHYLLA BY PH-ZONE REFINING CCC

1035 PO Session

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Keywords: Triclisia dictyophylla*, alkaloids, Plasmodium, pH zone refinement*

Triclisia dictyophylla Diels (Menispermaceae) is a climbing plant or scrambling shrub of the lowland dense rain-forest with stems that can be up to 30 m long and 10 cm in diameter. The plant occurs from Liberia to West Cameroon and in East Cameroon to Zaïre and Angola. The plant is harvested from the wild for local medicinal use [1]. A methanol extract of the stem bark of *T. dictyophylla* showed strong activity against *Plasmodium falciparum in vitro* and significant effect against *Trypanosoma brucei* [2]. Previous phytochemical studies on this plant revealed the presence of bisbenzylisoquinolines phaeanthine, *N*,*N*'-dimethyl phaeanthine, tetrandrine, trigilletine, cocsuline and trigilletimine from roots and stems. Oxo-isoquinoline *O*-methylmoschatoline and indenoisoquinoline triclisine were further obtained from stems. Analyses of the leaves showed bisbenzylisoquinolines stebisimine, obamegine, gilletine and isogilletine-*N*-oxide togheter with a morphinan alkaloid tridictyophylline [3-5].

The aim of this study was to develop rapid methodology for the isolation of tertiary and quaternary alkaloids from *T. dyctyophylla* by using pH zone refining CCC. A series of test tube partitioning tests were initially performed in order to select the best solvent system for the fractionation of the crude ethanol extract from stems of *T. dictyophylla* and then, the concentration of the retainer trimethylamine (TEA) and the eluter HCl were studied. The first solvent system family tested was HEMWat in various ratios but the results showed that a more polar solvent system was necessary. So, EBuWat was tested instead. A series of ratios (x:y:10) were investigated varying from x:y = 9:1 to 5:5. EBuWat 5:5:10 was chosen for optimizing the concentrations of TEA and HCl. After testing several concentrations of TEA in the upper organic phase and HCl in the lower aqueous mobile phase, 1g of the crude ethanol extract was injected in the 98mL coil of the Quattro HTPrep instrument, with the solvent system ethyl acetate-butanol-water 5:5:10, upper organic phase with 60mM TEA as stationary phase and aqueous lower phase with 5mM HCl as mobile phase, 2mL/min. Fractions of 4mL were collected. The present investigation led to the isolation of four major compounds from the stems of *T. dictyophylla*. Identification of the isolated compounds is being carried out by ^{1}H and ^{13}C NMR and by ESI-MS spectral data.

References

1. H.D., Neuwinger. 2000. African Traditional Medicine. A Dictionary of Plant Use and Applications. Medipharm Scientific Publishers, Stuttgart (p. 533).
2. S.J. Partridge *et al*. 1989. In vitro antimalarial activity of *Triclisia dictyophylla* and some of its constituent alkaloids. *J. Pharm. Pharmacol.* Suppl. 41, 92.
3. A.I., Spiff *et al*. 1981. Constituents of West African medicinal plants. XXX. Tridictyophylline, a new morphinan alkaloid from *Triclisia dictyophylla*. *J. Nat. Prod.* 44,160-165.
4. A.N., Tackie *et al*. 1974. Constituents of West African medicinal plants. II. The isolation of alkaloids from selected Triclisia species. *Lloydia* 37, 1-5.
5. D., Dwuma-Badu,*et al.* 1975. Trigilletimine: a new bisbenzylisoquinoline alkaloid from Triclisia species. *Experientia* 31, 1251-1252.

Acknowledgements to FAPERJ for finantial support and to CNPq/TWAS for a pos-doctoral fellowship (E.L.D.Kamto).

OPTIMIZATION OF SAMPLE INJECTION IN COUNTER-CURRENT CHROMATOGRAPHY

1036
FP
Session XI

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Keywords: sample injection, GUESS mix, pharmaceuticals mix, sample loading, throughput

Counter-current chromatography (CCC) is well known for its high capacity of sample loading (1). However, it is still very much trial & error method when it comes to loading studies. The classical approach is to dissolve sample in one or both phases of two-phase solvent system and then inject sample solution without adjusting flow rate. Recently the authors developed a novel strategy, which allowed to increase loading by up to 40% by using “the best solvent” approach for preparing sample solution and by splitting sample injection into three stages. This strategy was well demonstrated on a separation of pre-purified natural product extract (2).

This study is taking much broader approach to investigate how solvents miscibility in the system and sample type affects the flexibility of the proposed injection strategy. Heptane-ethyl acetate-alcohol-water solvent systems, where alcohol is either methanol (HEMWat) or ethanol (HEEtWat), were compared as starting point. Methanol, as the aqueous phase modifier, is not miscible with heptane while ethanol is.ihua

Two sample types were tested in this work. One is mimicking a natural product extract – multicomponent mix within a wide range of polarity with an active compound (or two-three actives) at the content no more than 15%. The model sample was based on GUESS mixture of natural product molecules (3). Whereas the second model sample was mimicking a synthetic mix containing 2-5 components in a close polarity range with the main compound up to 70%. The latter sample was based on pharmaceutical molecules used by the authors in their previous study on method development (4).

Acknowledgments

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References

1. C. DeAmicis, N. Edwards, M. Giles, G. Harris, P. Hewitson, L. Janaway, S. Ignatova, S., J. Chromatogr. A. 1218 (2011) 6122-6127
2. A. Peng, P. Hewitson, H. Ye, L. Zu, I. Garrard, I. Sutherland, L. Chen, S. Ignatova, J. Chromatogr. A, submitted (2016)
3. J.B. Friesen, G.F. Pauli, J. Liq. Chromatogr. Rel. Technol. 28 (2005) 2777–2806
4. S. Marsden-Jones, N. Colclough, I. Garrard, N. Sumner, S. Ignatova, J. Chromatogr. A, 1398 (2015) 66–72

ADDITIVE MANUFACTURING: WHAT CAN IT DO FOR THE COUNTER-CURRENT CHROMATOGRAPHY RESEARCHER

1037
FP
Session X

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Keywords: Additive manufacturing, columns, rapid prototyping, 3D printing

Additive manufacturing (3D printers) is becoming widespread with sales of over 200,000 printers in 2015 and annual growth rates of over 20%. 3D printing is being widely used for making prototypes in various research fields (1), which reduces production time tremendously in comparison with using traditional engineering approaches. Thus, it would be advantageous to apply this new technology in the counter-current chromatography (CCC) research environment to rapidly produce different bobbin geometries and change column materials.

Therefore, a variety of components required for making a CCC column have been produced using a stereolithography (SLA) 3D printer (3Dsytems Inc. Viper SI2) from a photopolymer UV curing epoxy resin (Accura® Xtreme). This resin has similar properties to CNC machined polypropylene parts of CCC analytical columns at the Advanced Bioprocessing Centre.

Parts manufactured include snap-fit column spools and union fittings, splined interchangeable bobbins, flying lead moulds, threaded flying lead fittings, and counterweights for an analytical scale column. The advantages and disadvantages of the resin in rapid prototyping of parts for use in high cyclic *g*-fields will be discussed in detail. Advantages include reduced overall column weight, very short build times, and the ability to incorporate standard HPLC tubing fittings without modification due to snap fit plastic moulding. These parts have been tested on a standard analytical CCC one-bobbin instrument at the ABC labs and shown to be robust enough for testing of new column designs including standard sets of retention/resolution studies.

The potential to extend the design space into direct metal laser sintering, whereby highly robust lightweight metal parts can be produced, will also be discussed.

Acknowledgments

The authors would like to thank Krishna Burugapalli for assistance operating the SLA 3D printer.

References

1. Gross, B. C., Erkal, J. L., Lockwood, S. Y., Chen, C., & Spence, D. M. (2014). Evaluation of 3D printing and its potential impact on biotechnology and the chemical sciences. Analytical chemistry, 86(7), 3240-3253.

COMPUTATIONAL FLUID DYNAMICS MODELLING OF SECONDARY FLOW IN COUNTER-CURRENT CHROMATOGRAPHY INSTRUMENTS

1038
FP
Session XII

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Keywords: computational fluid dynamics (CFD), column shape, aspect ratio, secondary flow

Recent studies have used counter-current chromatography instruments for field-flow fractionation (FFF) in a cyclic *g*-field (1, 2), generally with a single liquid carrier and in a variety of column shapes. As a consequence of the curved tubing, a secondary flow is generated in the liquid carrier which enhances the mixing within the column.

To improve understanding of this phenomena and to enable its control depending on a tubing shape, computational fluid dynamics (CFD; Ansys CFX and Fluent software) was used to model the flow in rectangular columns with different aspect ratios (0.32 and 3.1). The secondary flows observed in these models were compared to those present in standard circular tubing of the same cross-sectional area. The secondary flow in case of the rectangular tubing being aligned horizontally to the *g*-field was seen to have similar velocities to that of the round tubing (0.157 m/s cf. 0.154 m/s), whereas the rectangular tubing aligned vertically to the *g*-field showed increased secondary flow velocities (0.20 m/s). Furthermore, the high velocity area in the vertically aligned tubing was far more uniform than in the other tubing shapes.

The simulation models were setup for standard fluids used in FFF experiments, including water and alcohols. The obtained results allow to draw some conclusions in regard to tubing selection for the fractionation process.

Analysis is at present being extending to include a range of other tubing shapes, and solvents.

References

1. Hewitson, P., et. al.; “A new dynamic fractionation method and column design for size separation of bio-polymer particles”; 15th International Symposium on Preparative and Industrial Chromatography and Allied Techniques SPICA2014, Basel, Switzerland; 5th – 8th Oct 2014
2. Fedotov, P. S., Ermolin, M. S., & Katasonova, O. N. (2015). Field-flow fractionation of nano-and microparticles in rotating coiled columns. Journal of Chromatography A, 1381, 202-209.

DESIGNER EXTRACTS: TARGETED DEPLETION OF METABOLITES FROM BOTANICAL EXTRACTS USING COUNTERCURRENT SEPARATION

1039
SO
Sesssion XI

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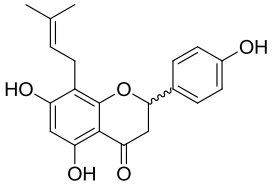
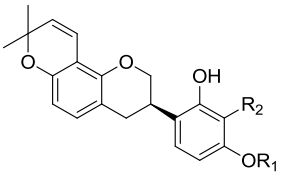
Keywords: DESIGNER extracts, Knockout, Licorice, Glycyrrhiza glabra, Hops, Humulus lupulus

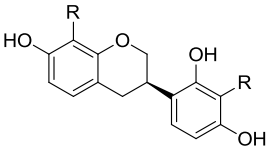
Counter**c**urrent **S**eparation (**CCS**) is an ideal technique to **D**eplete and **E**nrich **S**elect **I**ngredients to **G**enerate **N**ormalized **E**xtract **R**esources (DESIGNER) in a targeted, loss-free and reproducible manner (1). Analogous to gene knockout models, **DESIGNER** extracts have been presented as a tool to unravel the biological effects of individual compounds in complex metabolic mixtures (2). The proof of concept is demonstrated first by the K-targeted depletion of 8-prenylnaringenin **1** from spent hops (*Humulus lupulus* L.) extract for the creation of a single compound knockout extract. The methodology is expanded for the generation of a multi-knockout licorice (*Glycyrrhiza glabra* L.) extract depleted of glabridin **2** and four congeneric metabolites **3-6**. In each case, multiple CCS steps were used for the fractionation and assembly of the DESIGNER products. All products, including isolated compounds and knock out extracts were assessed by quantitative ^{1}H-NMR and UHPLC-UV profiling. The results highlight the utility of CCS for the simultaneous and targeted enrichment and depletion of structurally related metabolites in two different complex botanical matrices.

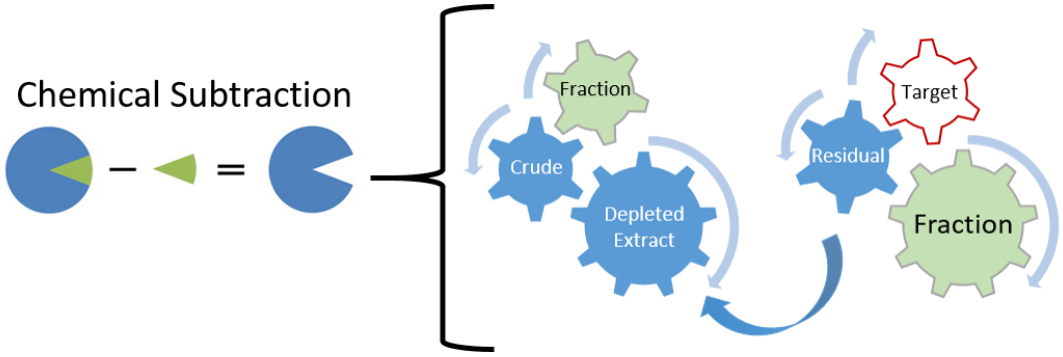


(1) 8-prenylnaringenin



(2) $R_1 = R_2 = H$
(3) $R_1 = CH_3$, $R_2 = H$
(4) $R_1 = CH_3$, $R_2 = OH$
(5) $R_1 = H$, R_2 = prenyl
(6) R = prenyl





References

1. R.F. Ramos, J.B. Friesen, D. Nikolic, C. Simmler, J.G. Napolitano, R. van Breemen, D.C. Lankin, J. McAlpine, G.F. Pauli, S.N. Chen. K-Targeted Metabolomic Analysis Extends Chemical Substraction to DESIGNER Extracts: Selective Depletion of Extracts of Hops (*Humulus* lupulus), J. Nat. Prod. 77 (2014) 2595-604.
2. S.N. Chen, A. Turner, B.U. Jake, D. Nikolic, R.B. van Breemen, J.B. Friesen, G.F. Pauli, An Experimental Implementation of Chemical Subtraction, J. Pharm Biomed. Anal. 46 (2008) 692-8.

THE ROTIFY ® BENCH-TOP CENTRIFUGAL PRECIPITATION CHROMATOGRAPH

1040
FP
Session IX

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Keywords: Centrifugal precipitation chromatography, proteins, carbon nanotubes

A prototype of the centrifugal precipitation chromatograph has been fabricated and more recently modified to improve the flow tubing arrangement. The versatile protein fractionation device has been used and shown capabilities to separate proteins out of complex extracts, and perform affinity and immunochemistry procedures (1). A sample can be passed through the system simply to perform buffer exchange or dialysis in just 75 min. Two high density polyethylene plates clamp a dialysis membrane (6000-8000 MW cutoff) between a spiral flow channel that has continuous flow on the top and flow in reverse direction on the underside. The sample flow is through tubing that is clamped above the center of the rotor and passes through a side rod (black in photo) that also holds the outflow tubing and in-flow and out-flow of the gradient solution that passes on the other side of the membrane. In the photo, the tubing passes through gears underneath the rotor and up into the center of the rotor to connect with the spiral channels within the disk. When the system is centrifuged at 2000rpm, a protein mixture is passed in one channel at 50ul/min and a decreasing gradient of a precipitating agent is pumped at 5 or 1 ml/min entering at the opposite end of the channel. The small MW molecules pass through the membrane and in time the high MW molecules precipitate and move, re-dissolve and precipitate again at individual rates, such that they are separated and are eluted out the other end and collected. The process and structure of the device will be described. We have passed samples of carbon nanotubes and have removed other high MW constituents. The method should be useful also for purifying protein conjugates.

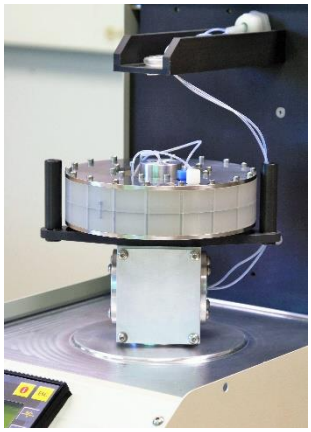

Figure 1. Close up of the Rotify®

Table 1. Gradient Solvent Systems

Analyte	Sample channel	Gradient channel
Proteins	Aq. buffers	95% sat. $AmSO_4$ – 0% Decreasing acetone concentration (2)
Carbon nanotubes	1% SDS	33% Am acetate – 0%

References

1. Y. Ito, *Anal. Biochem*. 277:143-153 (2000).
2. S. Baldermann *et al. J. Sep. Sci.* 34:1-6 (2011) DOI:10.1002/jssc.-201100508B.

CONCENTRIC COILS FOR COUNTER-CURRENT CHROMATOGRAPHY

1042
FP
Session X

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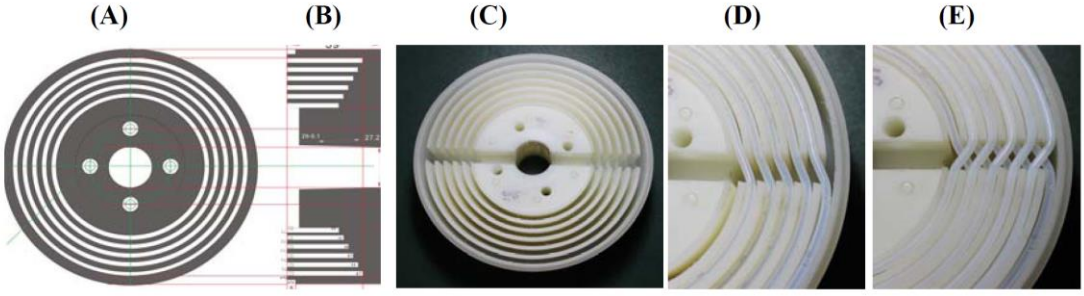
Keywords: Concentric coils; Helical coils; Spiral coils; Natural Products isolation; CCC column

Countercurrent chromatography (CCC) is an efficient separation technique in which two immiscible liquid phases are used and hold in the CCC column under high centrifugal force. Due to lack of the complications resulted from the support matrix, CCC has been widely used for the preparative purification of natural and synthetic products. Historically, modern CCC originated from the helical coil planet centrifuge in early 1970s by Yoichiro Ito [1, 2]. The helical coiled tube was found to be efficient to be used as a CCC column including type I and type J flow-through CPC, multi-layer CPC, eccentric multi-layer CPC, high-speed CCC, cross-axis CPC, low-speed CCC, and even high-performance CCC. Usually, helical coils can provide stable axial driving force in the centrifugal field but lack efficient pressure gradient of centrifugal force, especially in type-J high-speed CCC, which attain the unilateral hydrodynamic equilibrium in the coiled tube. Recently, spiral coils or disk assemblies [3, 4] were found to improve the retention of the stationary phase and enhance the partition efficiency for high-speed and low-speed CCC in the isolation of peptide and proteins. However, the spiral coils still lack the axial gradient of the centrifugal field for type J CCC, although it can attain an extra centrifugal gradient in the radial direction. More recently, we built conical coils to form centrifugal force gradients in both axial and radial directions [5]. Compared with helical and spiral coil CCC, conical coil CCC not only put the CCC column in a two-dimensional centrifugal field, but also provided a potential centrifugal force gradient both in axial and radial directions. The extra centrifugal gradient made mobile phase move faster and enabled higher retention of stationary phase and better resolution. However, above coiled columns were still difficult for fractionation and machine balance. Therefore, in this work, we developed a simple concentric coil or disk assembly as CCC column. This new assembly was shown to be efficient in the separation and purification of several natural products.



References
1. Y. Ito, M. Weinstein, I. Aoki, R. Harada, E. Kimura, K. Nunogaki, The coil planet centrifuge. Nature 212 (1966) 985-987.
2. Y. Ito, R.L. Bowman, Countercurrent Chromatography . Liquid-Liquid Partition Chromatography without Solid Support. Science 167 (1970) 281-&.
3. Y. Ito, F. Yang, P. Fitze, J. Powell, D. Ide, Improved spiral disk assembly for high-speed counter-current chromatography. J Chromatogr A 1017 (2003) 71-81.
4. Y. Ito, F.Q. Yang, P.E. Fitze, J.V. Sullivan, Spiral disk assembly for HSCCC: Column design and basic studies on chromatographic resolution and stationary phase retention. Journal of Liquid Chromatography & Related Technologies 26 (2003) 1355-1372.
5. J. Liang, J. Meng, M. Guo, Z. Yang, S. Wu, Conical coils counter-current chromatography for preparative isolation and purification of tanshinones from Salvia miltiorrhiza Bunge. Journal of chromatography. A 1288 (2013) 35-39.

STRATEGY FOR PH-DEPENDENT TAILING IN COUNTER-CURRENT CHROMATOGRAPHY: ALKALOIDS OF *NELUMBO NUCIFERA* GAERTN AS EXAMPLES

1043
FP
Session VIII

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Keywords: pH-dependent tailing, leading peak; alkaloids; liensinine; supermolecular separation

Counter-current chromatography (CCC), as a unique liquid-liquid partition chromatography, has been found to be a very efficient separation technique for resolving the complex natural products. Due to lack of support matrix, it eliminates some complications resulted from solid supports such as in irreversible solute adsorption, contamination, reaction and deactivation in the process of common chromatographic separation [1]. In addition, CCC may also eliminate tailing of solute peaks although the tailings of basic compounds in reversed-phase and normal liquid chromatography is a major problem that commonly occurs in the separations of pharmaceutical, peptides and proteins [2]. Usually, tailing has been linked with residual silanol groups on the surface of the solid supports such as silica stationary phase that remain after surface modification with reversed-phase groups. However, little is known about peak tailing or leading in CCC [3-5]. In this work, we found that in some cases, CCC can also produce significant tailing or leading peaks, as illustrated in the following figure. Therefore, this work aimed to developed several strategies to resolve the peak tailing in the separation of alkaloids of *Nelumbo nucifera* GAERTN.

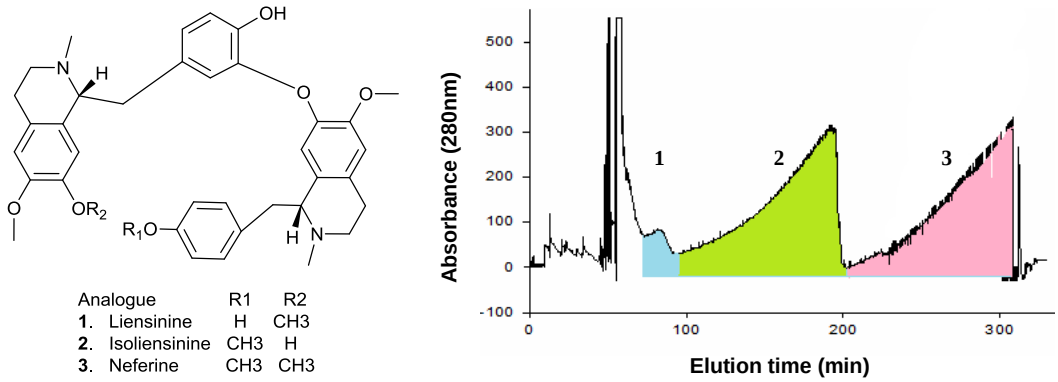

Figure 1. Leading peaks in the separation of alkaloids of *Nelumbo nucifera* GAERTN using lower phase of HEMWat systems as mobile phase.

References

1. J.B. Friesen, J.B. McAlpine, S.-N. Chen, G.F. Pauli, Countercurrent Separation of Natural Products: An Update. Journal of Natural Products 78 (2015) 1765-1796.
2. E.A. Smith, M.J. Wirth, pH dependence of tailing in reversed-phase chromatography of a cationic dye: measurement of the strong adsorption site surface density. Journal of Chromatography A 1060 (2004) 127-134.
3. S. Carda-Broch, A. Berthod, pH dependence of the hydrophobicity of beta-blocker amine compounds measured by counter-current chromatography. Journal of Chromatography A 995 (2003) 55-66.
4. Yuan Gao, Rapid isolating and screening several natural alkaloids with antitumor acitivity by countercurrent chromatography and following cytotoxic assay. Master dissertation in Zhejiang University, 2008
5. A. Berthod, N. Mekaoui, Distribution ratio, distribution constant and partition coefficient. Countercurrent chromatography retention of benzoic acid. Journal of chromatography. A 1218 (2011) 6024-6030.

ROOM TEMPERATURE IONIC LIQUIDS-BASED SALTING-IN STRATEGY FOR COUNTER-CURRENT CHROMATOGRAPHY

1044 PO Session

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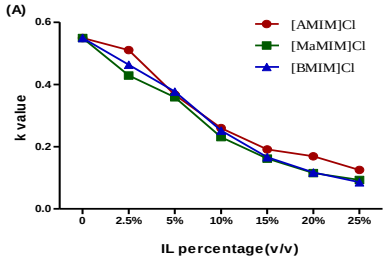
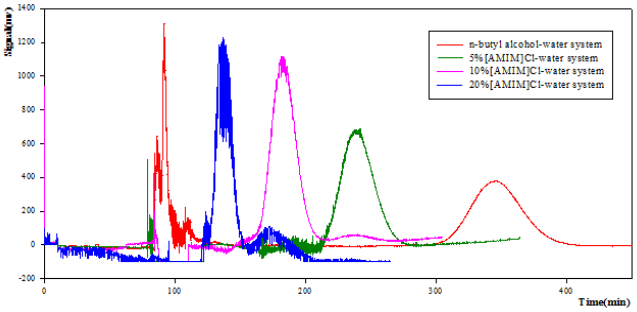
Keywords: Room temperature ionic liquids; Salting in; Salting out; Solvent system selection; Solvent systems.

Counter-current chromatography (CCC) is an unique liquid-liquid partition chromatography without complications caused by solid-support matrix and has been shown to be very efficient for the separation and purification of natural products. However, CCC separation is still a challenge. Usually, a common CCC separation process involves several steps such as the selection of solvent systems, instrumentation and separation theory mode. The selection of appropriate solvent system for target compound(s) is the first and the most important step in CCC separation, which probably accounts for 90% of the whole work [1]. Salting-out is a very common but not simple physical phenomenon extensively exploited by biopolymer science, ion-exchange chromatography, and counter-current separations. It has been proposed and used for the partition study of protein, amino acid, and hydrophilic natural products. Previous studies indicated salting-out was efficient method for separation of several natural products using some one-component inorganic/salt-containing systems such as NaCl, KCl, $(NH_4)_2SO_4$, KNO_3 as salting-out agents [2]. Different from the inorganic salts, we recently found that some organic salts such as sodium dodecyl sulfate (SDS) may play a “salting-in” role and make the partition coefficients of the solutes decrease with the increase of its concentrations [3]. Room temperature ionic liquids (RTILs) are well known organic salts being made up of cations and anions. Unlike classical viscous organic liquids, they are actually molten salts with melting point close or below room temperature. Their liquid state allows them as both the mobile and stationary phases in CCC [4]. Recent study [5] showed a similar salting-in properties with SDS. In this work, we report several RTILs-based salting-in strategy for CCC based isolation of natural products.





References

1. Y. Ito, Golden rules and pitfalls in selecting optimum conditions for high-speed counter-current chromatography. Journal of Chromatography A 1065 (2005) 145-168.
2. M. Guo, J. Liang, S. Wu, On-line coupling of counter-current chromatography and macroporous resin chromatography for continuous isolation of arctiin from the fruit of Arctium lappa L. Journal of Chromatography A 1217 (2010) 5398-5406.
3. Z. Yang, X. Hu, S. Wu, Preparative isolation of a cytotoxic principle of a forest mushroom Suillus luteus by sodium dodecyl sulfate based "salting-in" countercurrent chromatography. Journal of Separation Science 39 (2016) 732-740.
4. A. Berthod, M.J. Ruiz-Angel, S. Carda-Broch, Ionic liquids in separation techniques. J Chromatogr A 1184 (2008) 6-18.
5. F. Bezold, J. Goll, M. Minceva, Study of the applicability of non-conventional aqueous two-phase systems in counter-current and centrifugal partition chromatography. Journal of Chromatography A 1388 (2015) 126-132.

NOVEL NON-AQUEOUS BIPHASIC SOLVENT SYSTEMS IN CENTRIFUGAL PARTITION CHROMATOGRAPHY

1045 PO Session

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Keywords: deep eutectic solvents, non-aqueous biphasic systems, hydrophobic compounds

In Centrifugal Partition Chromatography and Countercurrent Chromatography, the most frequently used biphasic solvent systems are composed of three to four solvents of different polarity, such as heptane, hexane, ethyl acetate, methanol, ethanol and water. Even though these systems cover a wide range of polarities, they are often unsuitable for the separation of highly hydrophobic and hydrophilic compounds. The partition coefficients are very high or very low for such compounds because they preferentially dissolve in one of the two phases. For the purification of active compounds from plant extracts and biotechnological products, there is a strong need for new biphasic solvent systems especially for the separation of hydrophobic mixtures (1).

Deep Eutectic Solvents consist of two (or three) compounds capable of associating with each other to form a eutectic mixture with a melting point substantially lower than that of each individual compound (2).

In this work, the applicability of Deep Eutectic Solvents (DES) as solvents in liquid-liquid chromatography was evaluated. For this purpose, the partition coefficients of several natural compounds of different hydrophobicity in heptane/ethanol/DES biphasic systems were determined. Since many DES are hygroscopic, the influence of the DES composition and the presence of water in the biphasic system on the partition coefficient was also examined. In addition, several process-relevant biphasic system physical properties, such as the density and viscosity of the phases, were evaluated (3).

Finally, the suitability of DESs as solvents in liquid-liquid chromatography was demonstrated at pilot scale using a Centrifugal Partition Extractor column (SCPE-250-BIO from Armen Instrument, France).

References

1. J.B. Friesen, J.B. McAlpine, S.-N. Chen, G.F. Pauli, Countercurrent separation of natural products: an update, J. Nat. Prod. 78 (2015) 1765–1796
2. A.P. Abbott, D. Boothby, G. Capper, D.L. Davies, R.K. Rasheed, Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids, J. Am. Chem. Soc. 126 (2004) 9142–9147.
3. S. Roehrer, F. Bezold, E.M. García, M. Minceva, Deep Eutectic Solvents in Countercurrent and Centrifugal Partition Chromatography, J. Chromatogr. A 1434 (2016) 102-110.

K-TARGETED PURIFICATION OF C-GLYCOSYLFLAVONES FROM *VITEX AGNUS-CASTUS* BY ORTHOGONAL COUNTERCURRENT METHODS

1046
FP
Session X

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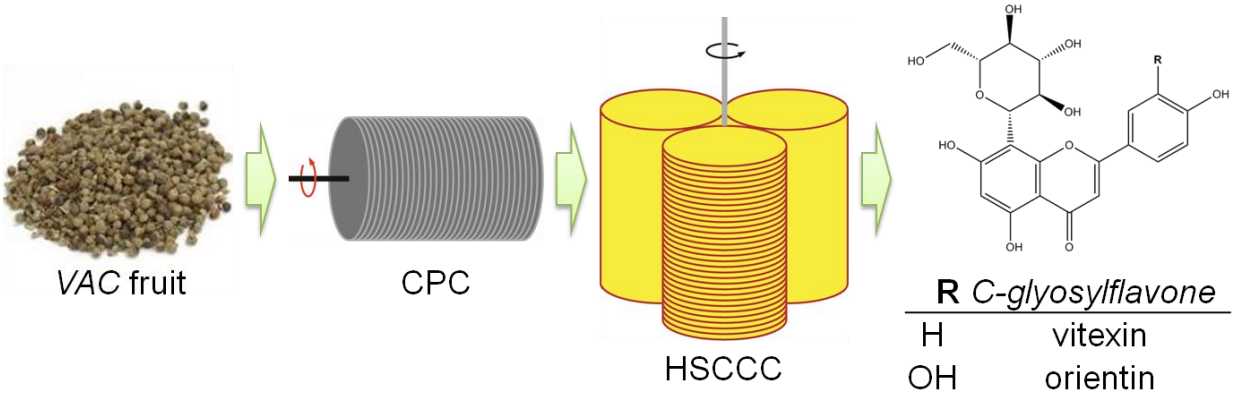
Keywords: CPC, HSCCC, LC-MS, shake flask, vitexin, orientin

C-glycosylated flavones, including orientin, isoorientin, vitexin and isovitexin, are minor but biologically significant constituents of chaste-tree (*Vitex agnus-castus* L.) fruits, which are used as a botanical supplement to treat PMS and postmenopausal symptoms. The partition coefficient, or *K*-value, is the ratio of the concentration of a compound in each phase of a biphasic solvent mixture and is a physicochemical property of a particular compound in a particular solvent system. This value can be used to predict retention volume (V_{ret}) in a countercurrent separation (CCS) procedure. The *K*-values of *C*-glycosylflavones present in complex botanical fractions have been determined in a number of solvent system families (*ter*AcWat, EEtWat, ChMWat) using the shake-flask technique. Relative LC-MS quantification allowed for the determination of *K*-values of multiple compounds of interest from complex extracts and CS fractions. *K*-values of *C*-glycosylflavones were also compared to interfering compounds, such as *O*-glycosylflavones, to optimize separations to avoid co-elution of unwanted compounds. This *K*-value library was used to develop targeted centrifugal partition chromatography (CPC) and high-speed countercurrent chromatography (HSCCC) methods to purify *C*-glycosylflavones.



AN INTEGRATED PROCESS FOR THE RECOVERY OF HIGH ADDED-VAUE COMPOUNDS FROM EXTRA VIRGIN OLIVE OIL USING SOLID-SUPPORT FREE LIQUID-LIQUID EXTRACTION AND CHROMATOGRAPHY TECHNIQUES

1047 PO Session

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Keywords: Extra Virgin Olive Oil, phenols extraction, step-gradient elution, oleocanthal, hydroxytyrosol.

Extra virgin olive oil (EVOO) is recognized as the main factor responsible for the health and nutritional benefits of the Mediterranean diet. This ancient oil is an abundant source of phenolic compounds with high health-benefiting properties (1, 2). The major phenolic compounds identified in olive oil belong to three different classes: simple phenols, secoiridoids and lignans. Hydroxytyrosol, oleocanthal and oleaceine are three olive oil phenolic compounds of particular interest due to their important biological properties (3, 4). In the present study an integrated extraction and purification process for a direct and productive recovery of high added value compounds from EVOO is purposed by using liquid-liquid extraction/chromatography techniques. Two different extraction methods were studied and developed on a laboratory-scale Centrifugal Partition Extractor (FCPE300®). The semi-continues multi-dual mode method consisting of several “extraction-recovery” cycles and the co-current elution method for the continuous recovery of the phenolic fraction. Food grade n-hexane was used to dilute olive oil in proportion of 3:2 v/v (feed mobile phase) and mixture of ethanol/water 3:2 v/v was used to extract the phenolic fraction. In total, 20 L of EVOO were treated resulting in the recovery of 26 g of total phenolic fraction. The obtained phenolic mixture was then fractionated using preparative-scale Centrifugal Partition Chromatography (FCPC1000®) combined to a sequential step-gradient elution procedure. The biphasic solvent systems were composed of n-hexane, ethyl acetate, ethanol and water in different volume proportions (X/Y/2/3, v/v/v/v) producing organic mobile phases with increasing polarities. In a single run of 4 hours, 910 mg of oleocanthal, 882 mg of oleacein and 104 mg of hydroxytyrosol were successfully produced from 5 g of phenolic extract with mean purities of 85%, 92%, and 89% respectively. Additionally, 791 mg of MFOA and 421 mg of elenolic acid were directly obtained from the same fractionation also in acceptable purity (>85%). The structure elucidation of the isolated compounds was achieved by NMR analysis. The proposed combination of the CPE/CPC techniques for the production of high value bioactive compounds from olive oil is described for the first time and represents a short process with high productivity. The above methodology could be also applied for the effective treatment of numerous edible oils and the recovery of their bioactive constitutes.

References

[1] R. W.Owen, W. Mier, A. Giacosa, W. E. Hull, B. Spiegelhalder, H. Bartsch. Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, *Food Chem Toxicol*, 38 (2000), 647-659. [2] E. Tripoli, M. Giammanco, G. Tabacchi, D. Di Majo, S. Giammanco, M. La Guardia, The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. Nutrition Research Reviews,. 18 (2005), 98-112. [3] F. Visioli, A. Poli, C. Gall, Antioxidant and other biological activities of phenols from olives and olive oil. Medicinal Research Reviews,. 22 (2002): p. 65-75. [4] L. Lucas, S. Cicerale, and R. Keast, The anti-inflammatory and pharmacological actions of Oleocanthal, a phenolic contained in extra virgin olive oil. Anti-Inflammatory and Anti-Allergy Agents in Medicinal Chemistry. 10 (2012), 399-406.

Acknowledgment

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ISOLATION OF METABOLITES FROM MANGROVE PLANT *RHIZOPHORA MANGLE* BY COUNTERCURRENT CHROMATOGRAPHY

1048 PO Session

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Keywords: Rhizophora mangle, diterpenes, HSCCC, antibacterial

Mangrove plants are potential sources of biologically active compounds with numerous traditional and medicinal uses[1]. Brazil's coastline is 7408 km, from which 6786 km contain mangrove forests, covering 25.000 km^2. *Rhizophora mangle* (Rhiphoraceae), known as red mangrove, is a Brazilian native tree and occurs in all mangrove areas[2]. The plant is commonly used for the extraction of tannins (15-36% of the dry bark)[3] but phytochemical studies on the species also reported the isolation of flavonoids and triterpenes from the leaves[4].

In this work, three diterpenes – manool, jhanol and steviol – and a benzaldehyde – *p*-oxy-2-ethylhexyl benzaldehyde (**Figure 1**), were isolated from the hexane extract of aerial roots by countercurrent chromatography (Quattro HT-Prep, 98mL Vc) using a biphasic non-aqueous solvent system composed of hexane-acetonitrile-methanol 1:1:0.5 (v/v/v). The fractions and isolated compounds from *R. mangle* are being screened for their minimum inhibitory concentration (MIC) following CLSI guidelines for *Cryptococcus neoformans* T1-444, *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 6538 and *Escherichia coli*. The literature reports the presence of kaurane, labdane, pimarane and beyerane diterpenes besides several aromatic compounds in Rhizophoraceae[4]. However, as far as we know, only steviol was previously isolated from this plant family.

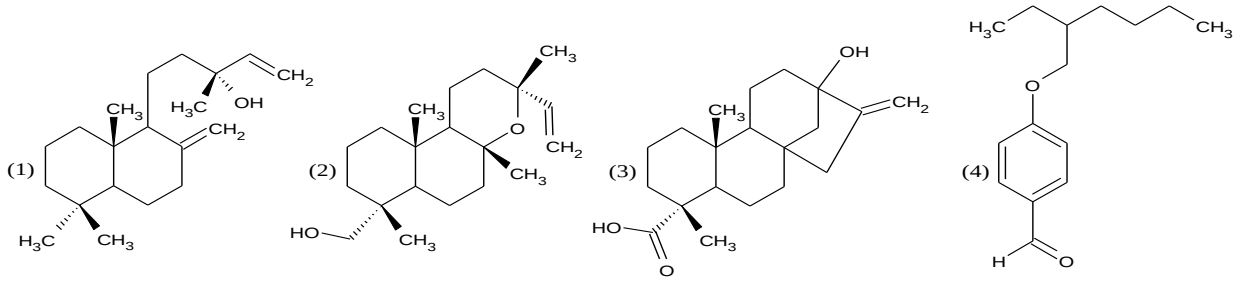

Figure 1. Isolated compounds (1) manool, (2) jhanol, (3) steviol and (4) p-oxy-2-ethylhexyl benzaldehyde

References

1. Bandaranayake, WM. Mangroves and Salt Marshes 2 (1998) 133.
2. Schaeffer-Novelli, Y. Aquatic Ecosystem Health and Management 3 (2000) 561.
3. Chapman, VJ. Mangrove phytosociology. Tropical Ecology 11 (1970) 1.
4. Nebula, M et al. Natural Products and Bioprospecting 3 (2013) 207.

MULTIPLE DUAL-MODE CPC AS AN EFFICIENT TOOL FOR THE PURIFICATION OF CAULERPENYNE FROM *CAULERPA TAXIFOLIA*

1049 PO Session

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Keywords: Caulerpa taxifolia*, caulerpenyne, centrifugal partition chromatography, isolation, purification.*

Caulerpenyne (Cyn) is a cytotoxic compound first isolated in 1978 from *Caulerpa prolifera*.(1) This molecule, constituted by a diacetoxybutadiene moiety (Fig. 1), exhibited a wide range of biological properties such as, antibacterial properties (2) and antitumor activities by inhibiting the growth of several human cancer cell lines (3).

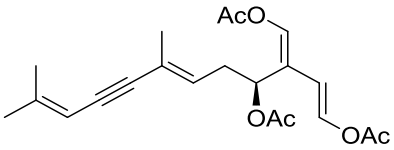

Figure 1. Caulerpenyne

Several industrial applications can be found for Cyn, so there is a need to produce and isolate it in high quantities. Since Cyn purification is time- and solvent-consuming, it is crucial to find a more green process to obtain pure Cyn with lower costs.Among the current chromatographic techniques, Centrifugal Partition Chromatography (CPC) seemed more appropriate to our objectives. Indeed, it allows low solvent consumption, can be used from analytical to preparative scale and is less time-consuming than other techniques. In the literature, CPC has been used to fractionate and/ or isolate bioactive compounds and has shown its efficiency in the purification process of natural products from diverse species.

Thus, in our study, we used CPC to separate and purify the metabolites within the crude extract of *Caulerpa taxifolia* algae. Multi dual-mode was thus chosen with the Arizona N solvent system (Hexane-Ethyl acetate--Water-Methanol 1:1:1:1) to afford Cyn with high purity (more than 98%). Further scale-up assay was made without loss of purity. Direct application of the method on the *C. taxifolia* crude extract gave caulerpenyne with 0.2% yield (*i.e.* 5 times more than with classical chromatographic techniques) and more than 98% purity. The structure of Cyn was confirmed by ^{1}H NMR and compared with previously published data (4).

Acknowledgements: E. Sfecci is the recipient of a thesis grant from the "Conseil Régional Provence Alpes Côte d'azur". M. Mehiri research is supported by the french program ENVI-Med "MEDIBIO", the ANR/Investissements d'Avenir program via the OCEANOMICs project (grant #ANR-11-BTBR-0008), and the H2020 European program via the EMBRIC project.

References

1. Amico V, Oriente G, Piattelli M, Tringali C, Fattorusso E, Magno S, Mayol L.. Caulerpenyne, an unusual sequiterpenoid from the green alga *Caulerpa prolifera*. Tetrahedron Lett. 1978; 19: 3593 – 3596.
2. Hodgson LM. Antimicrobial and antineoplastic activity in some south Florida seaweeds. Bot. Mar. 1984; 27: 387 – 390.
3. Fischel JL, Lemée R, Formento P, Caldani C, Moll JL, Pesando D, Meinesz A, Grelier P, Pietra F, Guerriero A, Milano G. Cell growth inhibitory effects of caulerpenyne, a sesquiterpenoid from the marine alga *Caulerpa taxifolia*. Anticancer Res. 1995; 15: 2155 – 2160.
4. Guerriero A, Meinesz A, D'Ambrosio M, Pietra F. Isolation of toxic and potentially toxic sesqui- and monoterpenes from the tropical green seaweed *Caulerpa taxifolia* which has invaded the region of cap martin and monaco. Helv. Chim. Acta 1992; 75: 689 – 695.

CAULERPENYNE FROM *CAULERPA TAXIFOLIA*: A COMPARATIVE STUDY BETWEEN CPC AND CLASSICAL CHROMATOGRAPHIC TECHNIQUES

1050 PO Session

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Keywords: Caulerpa taxifolia*, caulerpenyne, centrifugal partition chromatography, purification*

Caulerpenyne (Cyn) (**1**) is a cytotoxic compound first isolated in 1978 from *Caulerpa prolifera*.(1) This molecule, constituted by a diacetoxybutadiene moiety, exhibited a wide range of biological properties with mainly antibacterial properties (2) and antitumor activities by inhibiting the growth of several human cancer cell lines.(3) Several industrial applications are possible for Cyn, so there is a need to produce and isolate it in large quantities. Since Cyn purification is time- and solvent-consuming, it is crucial to find a more green process to obtain pure Cyn with lower costs.

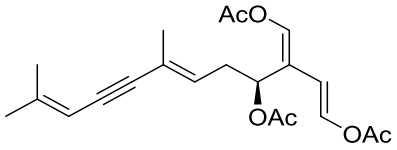

Figure 1. Caulerpenyne

Thus, in our study, Cyn has been purified from *C. taxifolia* crude extract with two different techniques: Centrifugal Partition Chromatography (CPC) and classical chromatographic techniques. CPC method involves only the CPC step with 0.2% yield (dry weight). On the other hand, other chromatographic techniques traditionally used imply at least three steps: (i) a liquid-liquid extraction, (ii) a size exclusion chromatography, and finally (iii) a diol column chromatography with a 0.04% yield (dry weight). Among the current chromatographic techniques, CPC seemed the more appropriate to our objectives for several reasons: (i) it allows low solvent consumption, (ii) it can be used from analytical to preparative scale and (iii) it is less time-consuming than other techniques. In the literature, CPC has been used to fractionate and/or isolate bioactive compounds and has shown its efficiency in the purification process of natural products from diverse species/ origin. The comparative study showed CPC to be faster at lower costs for Cyn isolation, and increased the extraction yield significantly.

Acknowledgements: E. Sfecci is the recipient of a thesis grant from the "Conseil Régional Provence Alpes Côte d'azur". M. Mehiri research is supported by the french program ENVI-Med "MEDIBIO", the ANR/Investissements d'Avenir program via the OCEANOMICs project (grant #ANR-11-BTBR-0008), and the H2020 European program via the EMBRIC project.

References

1. Amico V, Oriente G, Piattelli M, Tringali C, Fattorusso E, Magno S, Mayol L.. Caulerpenyne, an unusual sequiterpenoid from the green alga Caulerpa prolifera. Tetrahedron Lett. 1978; 19: 3593 – 3596.
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RATIONAL DEVELOPMENT OF CONICAL COLUMNS ON J-TYPE COUNTER-CURRENT CHROMATOGRAPHY FOR PROTEIN SEPARATION USING AQUEOUS-TWO PHASE SYSTEMS

1051
FP
Session IX

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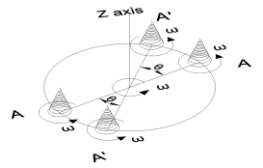
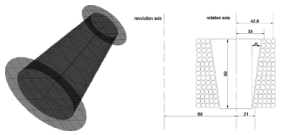
Keywords: Conical column, aqueous two-phase system, physical model, protein separation, HSCCC

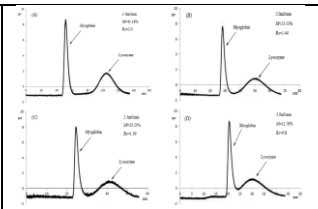
Purification and separation of large biomolecules and nanoparticles using counter-current chromatography (CCC) has been challenging yet much desired. Such usage invariably resorts to aqueous two-phase systems. For handling large molecules like proteins, it is notoriously difficult to achieve both sound stationary phase retention and phase mixing. With J-type CCC, successes have been shown for 2D spiral columns[1] and 3D toroidal columns[2]. This work considered both the pros and cons of 2D spiral columns and 3D cylindrical spiral columns, and then developed conical column geometry for protein separation and isolation (Fig. A)[3].



A: the J-type planetary motion of conical coil column. θ:elapsed rotation angle of the rotor; ω:rotation speed



B: Preparation of the the new conical bobbin (left) and winding details (right) of the column.



C: Separation of myoglobin and lysozyme using an ATPS. Phase system: 14% w/w PEG1000 and 12.5% K_3PO4, 1000 rpm rotation.

Each location is quantified by angular value φ

$$x = R\cos\theta + l\varphi\sin\alpha\cos(2\theta + \varphi x)$$
$$y = R\sin\theta + l\varphi\sin\alpha\sin(2\theta + \varphi)$$
$$z = l\varphi\cos\alpha$$

D: Mathematic description for the trajectory of arbitrary point on conical coil column on J-type CCC (R: revolution radius; *l* : positive scaling factor; φ : angular position; α : taper angle)

The conical column can be formed by lifting the center of the 2D spiral column in the vertical direction perpendicular to the 2D spiral plane. When the radius gradient over the horizontal orientation gradually reduces to complete disappearance, the conical column will then be converted to a 3D spirally wound cylindrical column. The factors influencing the stationary phase retention and phase mixing include β value, rotation speed (ω), winding pattern [Fig.B (right)], conical taper angle (α), angular position (φ). When β>0.5, stationary phase retention is favored but not for phase mixing. On the contrary, when β<0.5 (particularly β<0.25), phase mixing is favored, but not for phase retention[4]. Consequently, stationary phase retention always trades off with phase mixing for such a system. The taper angle should be between 5-15º following a compromise between stationary phase retention and phase mixing. The column winding pattern [Fig B(right)] ensures that better mixing takes place at those locations suitable for more column volume. We will show that a conical column strikes a balance for having the advantage of a spiral column for improved stationary phase retention and at the same time for having the advantage of a 3D spiral column for improved phase mixing. Based on our virtual CCC results, we then built a conical column in a real physical form (Fig. B). With the use of an ATPS, model proteins lysozyme and myoglobin have been well resolved (Fig. C).

In conclusion, this work illustrates an example for constructing and then analyzing the physical model (the conical column in this case) as a virtual column (Fig. D). Experiment vindicated the efficacy of the conical coil column for protein separation.

REFERENCES: [**1**] Y.H. Guan et al., J Chromatogr A 1151 (2007) 115. [**2**] Y.H. Guan, R. van den Heuvel, J Chromatogr A

COMBINING SEVERAL ELUTION MODES TO SEPARATE COMPOUNDS FROM COMPLEX MATRIX

1052 PO Session

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Keywords: countercurrent chromatography; elution mode; complex matrix

Counter-current chromatography (CCC) is a unique chromatographic separation technology which based on the liquid-liquid partition and extraction without the solid support stationary phase. It has been widely used for the preparative and analytical separation, isolation and purification of various products due to its advantages in separation. However, simultaneously separate multiple compounds, especially in large scope of polarity range has been an intractable problem in CCC application. Usually, it is necessary to use several different solvent systems and multiple separations to isolate many compounds from a complex matrix because of the low number of theoretical plates of CCC. This leads to the great increase of solvent consumption and extend the time of the separation.

In such a case, we consider combining several elution modes to achieve separation of compounds with a large polarity scope from complex matrix (such as extract of traditional Chinese medicine) in a single CCC separation. In this separation, a high-speed countercurrent chromatography using the gradient elution combined elution-extrusion mode to separate compounds in the extract of *Cynomorium*. It is first and important step to select a suitable two-phase solvent system in a CCC separation (1). A classical solvent system consist of *n*-hexane-ethyl acetate–methanol–water (HEMWat) was used to achieve this separation. A series of HEMWat biphasic systems with different composition were screened to optimize the solvent system for CCC separation and their partition coefficients (K values) of the compounds were measured. Two solvent systems were selected (HEMWat 5 and 6). Firstly the lower phase of HEMWat 6 was used as stationary phase and the upper phase as mobile phase. After the hydrodynamic equilibrium was established throughout the column, the sample solution was injected. Then the upper phase was continuously pumped in the column and eluted. After eluted with a column volume of upper phase of HEMWat 6, the mobile phase changed from the upper phase to HEMWat 5 and kept on eluting. Then, continued eluting a column volume with the upper phase of HEMWat 5 as mobile phase. Next began the extrusion process and the lower phase of HEMWat 5 was introduced into the column to push out the stationary phase in the column. The fraction was collected with an automatic fraction collector.

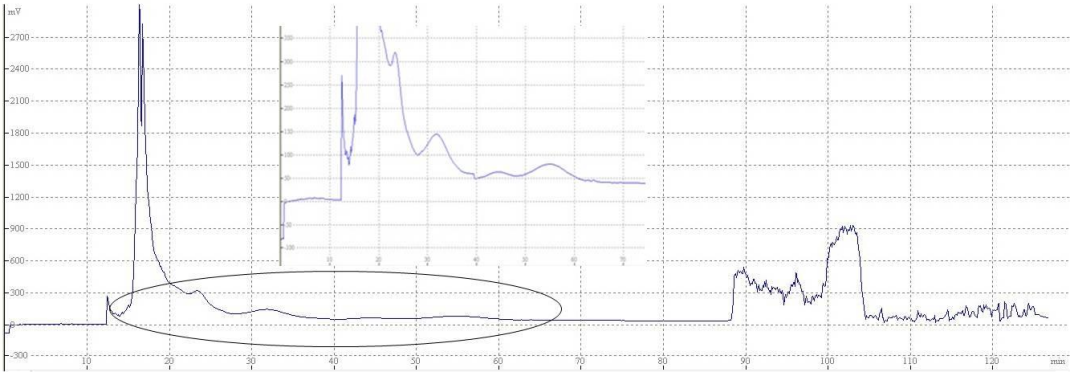

Figure 1. HSCCC chromatogram of combined elution modes separation

References

1. Y. Ito, Golden rules and pitfalls in selecting optimum conditions for high-speedcounter-current chromatography, J. Chromatogr. A 1065 (2005) 145–168.

SEPARATION AND PURIFICATION OF ACTIVE COMPONENTS FROM LYCIUM BARBARUM L. BY HSCCC USING DUAL-MODE ELUTION

1053 PO Session

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Keywords: Lycium barbarum *L., dual mode, antioxidant*

Lycium barbarum L. is a Solanaceous defoliated shrub that is widely grown in arid and semi-arid regions of Northwestern China, Southeastern Europe, and the Mediterranean areas. The fruits of *L. barbarum*, also called goji berry or wolfberry, have been used as traditional Chinese herbal medicine and functional food for more than 2500 years. *L. barbarum* has the functions of nourishing kidney and liver, improving fertility in men, and brightening eyes. In this work, a dual-mode elution was used in the separation of *L. barbarum* L. extracts by HSCCC with a two-phase solvent system composed of *n*-butanol–ethyl acetate–water (1:4:5). The separation was initiated by filling the multilayer coil column with the upper phase of the solvent system as the stationary phase. The lower phase was then pumped into the column using the head-to-tail mode for elution. After a run of a certain time, the inlet and outlet of the column were switched, and the upper phase, which was originally used as the stationary phase, was eluted in the tail-to-head direction through the column. Three compounds with a wide range of polarity were separated using this method. They are 5-hydroxymethyl furfural, rutin and quercetin. The HSCCC chromatogram is shown in Figure 1. The antioxidant activities of the compounds were evaluated by the methods of DPPH radical scavenging assay. Rutin and quercetin showed high radical scavenging activities with the EC_{50} values being 20.07 ± 0.10 and 3.11 ± 0.03 μg/mL, respectively.

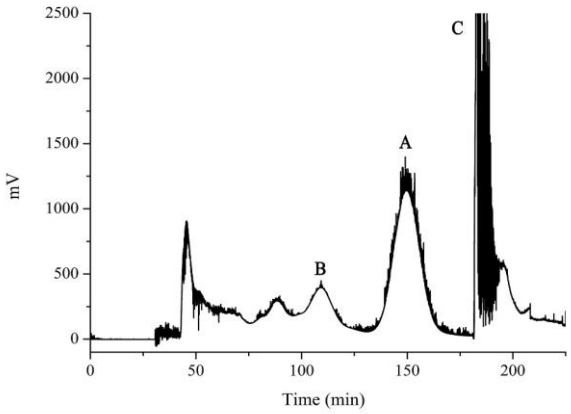

Figure 1. HSCCC chromatogram of the extract of *Lycium barbarum* L using dual-mode elution. The solvent system is *n*-butanol–ethyl acetate–water (1:4:5)

THREE SOLVENT SYSTEM CCC COMBINED THE USE OF O-CARBOXYMETHYL CHITOSAN AS AN ADDITIVE FOR SEPARATION OF CHEMICAL COMPONENTS IN *LYCIUM BARBARUM* L.

1054
FP
Session VI

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Keywords: three solvent system, O-carboxymethyl chitosan, solvent addictive, Lycium barbarum L .

Lycium barbarum (goji berry) is one of the most important traditional Chinese medicine. Their fruits are reported to have various biological activities and health-promoting properties. Therefore, the fruits and leaves have been used widely as vegetable medicines and functional tea in China, Southeast Asia, Europe, and North America (1). An increasing number of researchers have focused on the study of the active components, trying many different separation methods to obtain them. However, these methods exhibit some shortcomings such as a tedious process, time and labor consumption, solvent residue, low efficiency and so on. Recently, several applications of three-phase solvent systems in HSCCC separation have been reported. And this kind of HSCCC technique has potential application in separation of physiologically active constituents with a wide range of polarity in natural products (2,3).

A new three-phase solvent system was efficiently applied for high-speed counter-current chromatography to separate the secondary metabolites with a wide range of hydrophobicity in *L. barbarum* .The three-phase solvent system of n-hexane/methyl acetate/acetonitrile/water (4:3:4:3.5, v/v/v/v) was selected for high-speed countercurrent chromatography separation. The two phases (middle phase and lower phase) of a three-phase solvent system was as a stationary phase followed by elution with upper phase to separate the hydrophobic compounds. Then the mobile phase was switched to the middle phase to elute the moderately hydrophobic compounds, and finally the polar compounds were eluted out of the column with the lower phase. Although we get six peaks through the three-phase solvent system CCC separation, the resolution is not good enough. In order to improve the resolution, we will use the O-carboxymethyl chitosan (O-CMC) as a solvent additive. In Liu's research it was indicated that O-CMC could improve resolution not by increasing the retention of the stationary phase but by introducing intermolecular forces: hydrogen bonding interaction and electrostatic interaction (4). We will also investigated the effect of O-CMC concentration on *K* and *Rs* to optimize the CCC separation condition. Through this method, we expect to get a comprehensive separation of a wide variety of the complex sample in a one-step operation in a relative less time without any pretreatment.

References

1. S.Magiera,M.Zareba,Chromatographic Determination of Phenolic Acids and Flavonoids in *Lycium barbarum* L. and Evaluation of Antioxidant Activity,Food Analytical methods 8(2015)2665-2674.
2. A.Yanagida,Y.Yamakawa,R.Noji,A.Oda,H.Shindo,Y.Ito,Y.Shibusawa, Comprehensive separation of secondary metabolites in natural products by high-speed counter-current chromatography using a three-phase solvent system,J.Chromatography.A 1151(2007)74–81.
3. Y. Liu, X.F. Chen, J.X. Li, D.L. Di, Three-phase solvent systems for comprehensive separation of a wide variety of compounds from Dicranostigma leptopodum by high-speed counter-current chromatography, Journal of Separation Science 38(2015)2038-2045.
4. Y.J. Liu, G.H. Wang, X.Y. Huang, Y.F. Liu, D.L. Di, Use of O-carboxymethyl chitosan in high-speed counter-current chromatography: a novel additive for a biphasic solvent system,New Journal of Chemistry Science 38 (2014)1150-1157.

SEPARATION OF SAPONINS FROM *SILENE COLORATA* BY USING CENTRIFUGAL PARTITION CHROMATOGRAPHY

1055 PO Session

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Keywords: Silene colorata*, Saponins, CPC*

Saponins are a class of chemical compounds found in particular abundance in various plant species. There is a constant demand for these compounds for their amphiphilic properties that makes them natural foaming agents, especially for the cosmetic market, while they are also being promoted commercially as dietary supplements and nutraceuticals. The genus *Silene* (Caryophyllaceae) is known to contain triterpenoid saponins [1] and phytoecdysteroids [2]. *Silene colorata* is distributed over the Mediterranean basin, but little is known concerning the phytochemistry of the species. Previous work has focused on the identification of the main secondary metabolites in various *Silene* extracts, among which *S. colorata.* Especially, the content in saponins/sapogenins has been specified. There have been identified both two main sapogenin (aglycone moiety) types, triterpenes and steroids/phytoecdysteroids [3]. The co-existence of saponins and their aglycone forms in the extracts of *S. colorata*, as well as the fact that the polarity of these groups of compounds varies widely, makes it difficult to separate them and, moreover, quantify them. Several works exist focusing on analytical methods for the detection and quantification of saponins and/or sapogenins, individually or as a group of compounds [4]. Nevertheless, little work has been published regarding the separation of saponins in preparative scale. Especially from *S. colorata*, there exists no such work.

Centrifugal partition chromatography (CPC) is a preparative technology that proves to be very efficient for the separation of natural compounds. It has been successfully implied in the case of a hydroalcoholic *Silene colorata* extract that was further enriched by adsorption resin treatment, by optimizing the biphasic solvent system that effectively separates the saponins. Thus, 10 biphasic solvent systems were tested and compared between them and finally a system composed of n-Hex/EtOAc/BuOH/EtOH/H_2O at a volume ratio of 5:5:5:6:15 was found to be the optimal for the saponins separation. This was used in ascending mode, inside a 50ml rotor. A spectrophotometric method has been used for the determination of the total sapogenin content (TSC) for each extract and fraction of the CPC. The recovery of fractions that contain total saponins >95% has been achieved.

References

[1] S. Bottger, M.F. Melzig. Triterpenoid saponins of the Caryophyllaceae and Illecebraceac family. Phytochem Lett 4 (2011), pp. 59 – 68

[2] N.Z. Mamadalieva. Phytoecdysteroids from Silene plants: distribution, diversity and biological (antitumour, antibacterial and antioxidant) activities. Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas. 11 (2012), pp.474 – 497

[3] A. Termentzi, C. Croizat, K. Collin, P.Y. Morvan, R. Vallée, M. Halabalaki, L. Skaltsounis. HPLC-HRMS/MS analysis of saponins and phytoecdysteroids from a Silene colorata hydroglycerinic extract

[4] C.Y. Cheok, H. Abdel Karim Salman, R. Sulaiman. Extraction and quantification of saponins: A review. Food Res Int 59 (2014), pp. 16–40

DEVELOPMENT OF A COUNTER-CURRENT CHROMATOGRAPHY-BASED EXTRACTION METHOD FOR EMERGING CONTAMINANTS FROM RIVER WATER

1056
FP
Session VI

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Keywords: emerging contaminants, extraction, column material

Complex mixtures of emerging pollutants which include pesticides, biocides, personal care products, and pharmaceuticals are constantly present in the hydrosphere and may pose a risk to aquatic ecosystems and human health. This is becoming a major problem with ever growing density of population. The vast and ever-increasing number of chemicals, often present at very low (sub ng/L) concentrations, complicates their monitoring and subsequent regulation (1). In this context, counter-current chromatography (CCC), all liquid technique, was considered as a possible alternative to solid phase/membrane extraction as CCC has been used as a concentration method for trace level compounds before (2). The research explores the use of CCC as an extraction method for large volumes of water. In this mode, the mobile phase is the water sample potentially containing the pollutants, while the stationary phase is an appropriate organic solvent(s) with added extracting reagents to enhance the partitioning of target molecules. The model water samples contained a number of compounds, including pesticides, pharmaceuticals, and corrosion inhibitors, covering a wide polarity range with log P between 1 and 5. The preliminary results demonstrated that there is clear evidence of retaining certain types of molecules on the inner walls of a CCC column, possibly caused by adsorption. This led to further consideration of testing different types of column materials. A range of recovery efficiencies for the majority of these compounds and method development will be presented and discussed.

Acknowledgments

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References

1. W. Brack, et al (50 authors), The SOLUTIONS project: Challenges and responses for present and future emerging pollutants in land and water resources management, Science of the Total Environment 503 (2015) 22-31.
2. S.N. Ignatova, T.A. Maryutina, B.Ya. Spivakov,·V.K. Karandashev, Group separation of trace rare-earth elements by countercurrent chromatography for their determination in high-purity calcium chloride, Fresenius J Anal Chem 370 (2001) 1109–1113.

COUNTERCURRENT CHROMATOGRAPHY FRACTIONS OF PLANT EXTRACTS WITH ANTI-TUBERCULOSIS ACTIVITY

1057 PO Session

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Key Words: plant extracts, anti-tuberculosis activity

The southwestern part of the United States has a large variety of indigenous plants, many of which have not been investigated for their medicinal potential, and only very few of which have had their extracts separated into the individual compounds they may contain. Samples of numerous plant species were received from that area from Richard Spjut, who is very highly knowledgeable of the various plant species of that region, including which species might be more likely to have medicinal properties than others. All were extracted with typical solvents, giving crude residues, some of which were subjected to countercurrent chromatography (CCC) separation.

Some of the crude residues and some of the CCC fractions were tested for anti-tuberculosis activity using both MABA and/or LORA methods. Activity was found in some samples in each of the two categories. Test results will be given, including comparisons of the anti-TB activity of certain CCC fractions, with the anti-TB activity of the crude residues from which they came, respectively.

ISOLATION AND PURIFICATION OF α-MANGOSTIN FROM INDONESIAN *GARCINIA MANGOSTANA* L. RINDS USING HIGH PERFORMANCE COUNTER-CURRENT CHROMATOGRAPHY

1058
PO
Session

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Keywords: α-mangostin, Mangosteen, Garcinia mangostana

Independence of drug raw materials has been a concern by the government of Indonesia lately and exploration of natural materials is expected to be the best solution, especially the exploration of various medicinal plants that exist in Indonesia. Mangosteen rinds with a content of xanthones and derivatives including α-mangostin is one of the medicinal plants that could potentially be developed as a source of raw material for medicine because it has antioxidant activity, anti-bacterial, anti-fungal, and anti-tumor. Laboratory testing so far indicates that α-mangostin is effective against several types of cancer cells, including breast cancer, liver, and leukemia. A research on isolation and purification of α-mangostin from mangosteen rinds had been done using Mini High Performance Counter Current Chromatography (HPCCC). This study aimed to obtain α-mangostin with high purity which can be accepted not only as drug raw materials but also as reference materials. The research was begun with preparation of the extract by maceration of the rind powder in 80% ethanol, then selection of a solvent system for HPCCC by testing the solubility of α-mangostin in the upper phase and the lower phase in HEMWat solvent (hexane, ethyl acetate, methanol, water) in various proportions. Sample injection was repeated 5 times without the top up of the stationary phase. The results showed that the best solvent system for the isolation of α-mangostin by HPCCC was HEMWat (5: 5: 10: 4 v / v) with K_D = 1, upper phase was used as the stationary phase while lower phase was used as a mobile phase. Samples were dissolved in the mobile phase before injection. Running time required for each injection was 25 minutes and the α-mangostin retention time ± 20 minutes. Total α-mangostin obtained was 38.0 mg with a purity of 98.13 ± 1.11% and 98.6% recovery Identification and α-mangostin assay were done by HPLC while characterization of α-mangostin isolated was conducted by NMR. In conclusion, this HPCCC had very efficient performance because the isolation and purification was done in one step with 5 times simultaneous injections. The purity of α-mangostin isolated was > 98% which can be accepted as drug raw material or as reference material.

Key words: α-mangostin, drug raw or reference material.

References:

1. Berthod, A., Maryutina, T., Spivakov, B., Shpigun, O. and Sutherland, I.A., (2009): Countercurrent Chromatography Analytical Chemistry(IUPAC Technical Report), Pure Appl. Chem., Vol. 81, No. 2, pp. 355–387.
2. Obolskiy, D., *et al* (2009): *Garcinia mangostana* L.: a Phytochemical and Pharmacological review. Phytother Res, 23(8):1047-65
3. Cui, J., Hu, W., Cai, Z., Liu, Y., Li, S., Tao, W., Xiang H. (2010): New medicinal properties of mangostins: Analgesic activity and pharmacological characterization of active ingredients from the fruit hull of *Garcinia mangostana* L. Pharmacology, Biochemistry and Behavior 95,166–172.
4. Doshi N.B., Sheth A.H, Sen D.J. and Dave J.B.(2010):Counter Current Chromatography: A Superior and an Advanced Analytical Technique, Pharma Times - Vol 42 - No. 01 – January.
5. Jia J., et al.(2011): Preparative Separation of methyl swertianin, swerchirin and Decussatin from the Tibetan Medicinal Plant *Swertia mussotii* using High-speed Counter-current Chromatography, Phytochem Anal.,Nov 3

PREPARATIVE SEPARATION OF SUCROSE MONOESTERS, DIESTERS AND POLYESTERS MADE OF PALM OIL AND SUCROSE BY HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY

2001
FO
Session IV

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Keywords: sucrose esters, esterification degree, separation, flash countercurrent chromatography

Sucrose esters (SE) are widely employed as biodegradable, nontoxic, skin-compatible additives in cosmetics, pharmaceuticals and foods [1]. However, commercial SEs are a complicated mixture of sucrose monoesters (SME), diesters (SDE), and polyesters (SPE) prepared by transesterification methods. Application of SE in preparation of nanoparticles is widely studied [2] and have been reported for SME, SDE, or SPE [3-5]. Therefore, the preparation of SME, SDE and SPE is needed for different usages. A planetary centrifuge frame that could accommodate three columns connected in series with rotary seals and stainless steel tubes of i.d. 2.0 mm was fabricated. The total capacity of the columns was 20 L. The columns were made by winding 38 m of polytetrafluoroethylene (PTFE) tubing (i.d. 15 mm) on each holder (width 20 cm, outer diameter 20 cm). The β value of five coil layers ranged from 0.50 to 0.75. The rotation speed of the columns could be controlled from 0 to 1000 rpm. HSCCC experiments were performed by stepwise gradient mode. The upper phase of *n*-hexane–ethyl acetate–methanol-water (1:1:2:1) was used as the stationary phase of the whole separation. The first step elution employed the lower phase of *n*-hexane–ethyl acetate–methanol-water (1:1:1.2:1) as the mobile phase to harvest SME, and the second step elution applied the lower phase of *n*-hexane–ethyl acetate–methanol-water (1:1:2:1) as the mobile phase to obtain SDE. After the second step elution the apparatus was stopped, and the solution in the column was extruded out through the inlet of the mobile phase to yield the fractions containing SPE. In a preparative separation of SE-13 (200 g) with a elution flow rate of 250 mL/min, the combination of tubes 15 to 85 tube fractions resulted in 52.4 g of SME, the combination of tube fractions 90 to 190 gave 95.2 g of SDE, and the combination of tube fractions 1' to 36' from the stationary phase yielded in 45.5 g of SPE. Fig. 1 showing the HPLC analyses of the SME, SDE, and SPE obtained from the HSCCC separation demonstrates that full separation of SME, SDE and SPE was achieved in only 4 h.

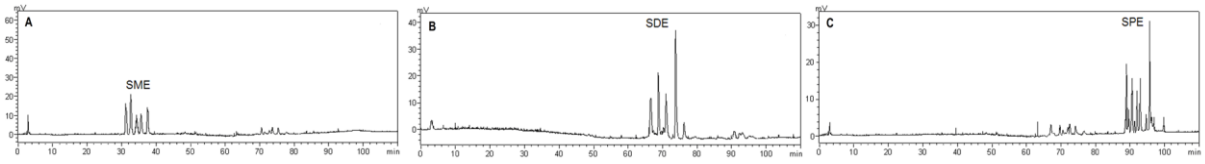

Figure 1. HPLC chromatograms of SME, SDE and SPE obtained from SE-13 by HSCCC separation.

References

1. J. Cázares-Delgadillo, A. Naik, Y.N. Kalia, Skin permeation enhancement by sucrose esters: A pH-dependent phenomenon, Int. J. Pharm. 297 (2015) 204-212
2. N. Asim, S. Radiman, M.A. Bin Yarmo, Synthesis of WO3 in nanoscale with the usage of sucrose ester microemulsion and CTAB micelle solution, Mat. Lett. 61 (2007) 2652-2657
3. S. Das, W.K. Ng, R.B.H. Tan, Sucrose ester stabilized solid lipid nanoparticles and nanostructured lipid carvers: I. Effect of formulation variables on the physicochemical properties, drug release and stability of clotrimazole-loaded nanoparticles, Nanotechnology 25 (2014) 105101 (14pp)
4. W. Li, S. Das, K.Y. Ng, P.W. Heng, Formulation, biological and pharmacokinetic studies of sucrose ester-stabilized nanosuspensions of oleanolic acid, Pharm. Res. 28 (2011) 2020-2033
5. V. Klang, N. Matsko, A.M. Zimmermann, E. Vojnikovic, C. Valenta, Enhancement of stability and skin permeation by sucrose stearate and cyclodextrins in progesterone nanoemulsions, Int. J. Pharm. 393 (2010) 152-160

ONLINE ENRICHMENT AND SEPARATION OF FIVE FLAVONOIDS COMPOUNDS FROM *MIKANIA MICRANTHA* USING MAGNETIC NANOMATERIALS COUPLED WITH HIGH SPEED COUNTERCURRENT CHROMATOGRAPHY

2002
FP
Session III

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Keywords: Flavonoids, Magnetic Nanomaterials, Countercurrent Chromatography

Mikania micrantha is an invasive weed. Flavonoids generally exist in weeds. Owing to its ability of antioxygenation, improving circulation, and cholesterol-lowering effects, the separation and enrichment of flavonoid components in weeds is important. In this study, we compared the adsorption capacity of six different magnetic nanoparticles to bind to flavonoid standards, then selected Fe_3O_4@SiO_2@DIH@EMIML magnetic nanoparticles to fill online polytetrafluoroethylene pipeline. A device of magnetic nanoparticles (MNPs) online pipeline combined with high speed countercurrent chromatography (HSCCC) through a six-way valve has been used to achieve online separation and enrichment of the five components simultaneously. Ethyl acetate-methanol-water (10:1:10, v/v) or *n*-hexane-ethyl acetate-methanol-water (1:1:1:1, v/v) was used for on-line separation of the astragalin, quercetin, luteolin, baicalein, and kaempferol. Five flavonoids targets have been enriched and separated. The method for separating flavonoids by on-line combination of magnetic nanomaterials and high speed countercurrent chromatography was established for the first time.

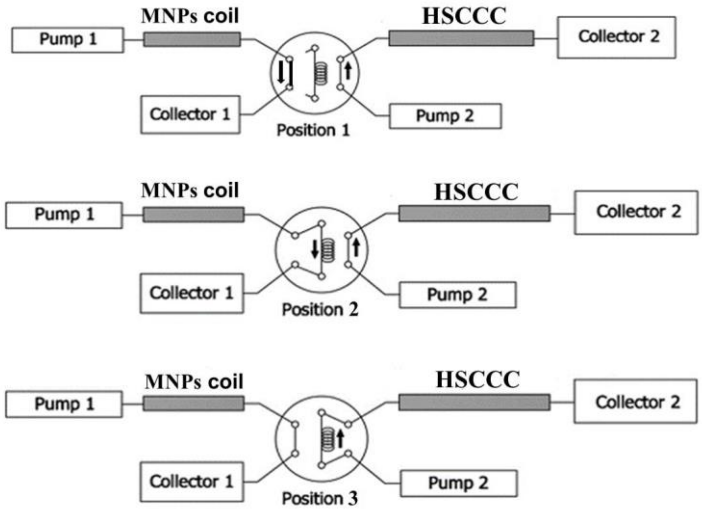

Figure 1. Scheme of the magnetic nanoparticles- high speed countercurrent chromatography system

MASS-SPECTROMETRIC SEPARATION STRATEGIES FOR THE GUIDED RECOVERY OF GENOTOXIC PYRROLIZIDINE ALKALOIDS FROM PLANT AND FOOD SOURCES USING ALL-LIQUID CHROMATOGRAPHY TECHNIQUES

2003
FO
Session I

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Keywords: pyrrolizidine alkaloids, K_D evaluation by mass-spectrometry, preparative ESI-MS/MS profile

Pyrrolizidine alkaloids (PAs) are genotoxic natural products. Almost 350 differently substituted PAs were structurally characterized with their additional occurrence as *N*-oxides. Legal regulations of PAs in food and feed are currently under discussion. So far, PA contamination has mainly been reported for honey, foods containing honey, and bee products such as pollen (1,2). PA contamination of retail ready-to-use lettuce and herbal teas is also a recurring and unsolved safety risk for the consumer. However, there is still a lack of commercially available PA reference substances for specific targeted quantification (1).

For the recovery of PA compounds, CCC and CPC techniques are highly suitable for fortification of these target compounds or final purification for NMR characterization. Hampering the preparative recovery, PAs occur in minor or trace amounts in plant sources and require sensitive LC-ESI-MS or GC-MS analysis for detection and identification. In the planning for a CCC/CPC separation, PA mixtures in rough crude plant extracts, and PA traces in food need to be evaluated to use optimised distribution values in tailored biphasic solvent systems. As consequence of weak UV chromophores in PAs, a prediction of elution and potential co-elution problems could only be achieved by quantification of selective ion traces using LC-ESI-MS. Then, calculation of respective partition ratios (K_D) using relative concentrations of respective PAs in the phase layers can be deduced. Using the specific mass-spectrometry approach, target PA compounds can normally be guided to a desired elution/extrusion volume.

In the work-flow, after performing the CCC/CPC separation, the resulting fractions need to be selected and pooled. To omit a wrong combination of already separated PAs, a so-called off-line ESI-MS injection profile is able to project the real results of a preparative experiment by displaying the selected ion traces (3,4). The injection of tube fractions in the sequence of recovery (this is not a chromatography step) clearly helps to identify co-elution effects and reveal the pure fractions to be pooled by every specific tube. LC-ESI-MS analysis of some selected specific fractions might be required to investigate the existence of isobaric stereoisomers (epimers, diastereomers) which might have been separated by the CCC/CPC approach.

The prediction of compound elution and selective monitoring will be exemplified by a preparative HPCCC experiment with the respective ESI-MS injection profile of PAs in *Chromolaena odorata*, a highly invasive plant to agricultural areas in West-Africa.

References

(**1**) EFSA Journal (2011), 9 (11), 2406. (**2**) BfR Opinion No. 018/2013 of 5 July 2013, Pyrrolizidine alkaloids in herbal teas and teas. www.bfr.bund.de/cm/349/pyrrolizidine-alkaloids-in-herbal-teas-and-teas.pdf (**3**) G. Jerz et al., Preparative mass-spectrometry profiling of bioactive metabolites in Saudi-Arabian propolis fractionated by high-speed countercurrent chromatography and off-line atmospheric pressure chemical ionization mass-spectrometry injection. *J. Chromatogr. A*, 1347 (2014) 17–29 (**4**) J. Oetken et al.. Preparative mass-spectrometry profiling of genotoxic pyrrolizidine alkaloids in *Jacobaea vulgaris* (syn. *Senecio jacobaea*) by spiral-coil countercurrent chromatography and ESI-MS/MS off-line continuous injection. Planta Med (2013) 79 - PH8, DOI: 10.1055/s-0033-1352085.

HYPHENATED HPCCC AND HPLC/DAD/ESI-TOF AS A PLATFORM FOR SEARCHING OF BIOLOGICALLY ACTIVE COUMARINS FROM APIACEAE PLANTS

2004
SO
Session I

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Keywords: psoralen derivatives, central nervous system, coumarins

Coumarins are high value secondary metabolites possessing broad spectrum of pharmacological properties and are widely distributed in the plant kingdom, mostly in representatives of Apiaceae family. Psoralen derivatives are used in treatment of psoriasis or vitiligo as PUVA therapy. Some coumarins exhibit strong antibacterial and antifungal activity. Several genera belonging to Apiaceae have been studied for their neurobiological effects on memory enhancement through cholinesterase inhibition.

As a part of our ongoing studies series of coumarins were purified and subjected to further biological test. An efficient strategy, based on bioassay-guided fractionation, LC-MS and HPCCC, was established to purify and evaluate the bioactive compounds from the dichloromethane extract of the fruits of *Heracleum mantegazzianum* Sommier & Levier. The mixture of *n*-heptane-ethyl acetate-methanol-water (6:5:6:5 v/v) was used in the reversed phase mode and pure xanthotoxin, pimpinellin, imperatorin, and phellopterin were obtained. The antimicrobial activity of extract, chromatographic fractions, and single compounds were in the range of MIC = 0.03-1 mg/mL.

Ether extract from fruits of *Mutellina purpurea* (Poir.) Reduron, Charpin & Pimenov was also subjected to similar procedure and the solvent mixture in a ratio of 3:2:3:2 (v/v) was selected for further study. Four isolates, pteryxin (2.72 mg), hyuganin C (7.94 mg), osthol (4.30 mg), and hyuganin A (3.09 mg) were obtained in a single run after injection of 300 mg of crude extract. Additionally, auraptenol (5) and hyuganin D (6) were identified using LC-ESI-(Q)TOF-MS. To the best of our knowledge, this is the first report of identification of dihydropyranocoumarins in selected plant, as well as the first report about HPCCC separation of those compounds. Series on in vitro studies provided information about strong inhibition against BChE for both extract and hyuganin C (54.05 and 87.86% at 100 µg/mL, and 68.93 and 89.37% at 200 µg/mL, respectively). Inhibition was significantly higher than the reference drug galanthamine (80.03% of inhibitory activity at the concentration of 100 µg/mL).

HPCCC technique was also extensively used for the isolation of natural coumarins for further *in vivo* biological studies on the CNS. These studies included maximal electroshock-induced seizure test, elevated plus maze test (EPM), and the passive avoidance (PA) paradigm (for naïve Swiss mice and those with scopolamine-invoked memory deficit). The results clearly demonstrated that selected coumarins may be potent anticonvulsant agents, and can be an interesting option for alleviation of depressive and anxiety disorders as well as memory impairment in animal models.

Acknowledgments: The research presented in this lecture were financially supported by Grant No. 2014/13/B/NZ4/01248 from The National Science Centre, Kraków, Poland.

WHAT IS INDUSTRIAL SCALE IN CPC?

2005
SO
Session VIII

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Keywords: process chromatography, API purification, industrial scale

Centrifugal Partition Chromatography (CPC) is one of the technical realization of liquid-liquid chromatographic, where the stationary phase is immobilized by a strong centrifugal force, caused by a circular motion. Opposed to HSCCC, CPC consists of series connected network of extraction cells, which operates as elemental extractors, and the efficiency is guaranteed by the cascade (1,2). CPC instruments vary on scale from 50 ml to 25 liter, however all advantages are realized on bigger scale use in industry. Up to date, there are no really available instruments that could be used for API purification in real situations.

By industrial size we mean process chromatography, the same scale that can be achieved by conventional HPLC DAC columns, with a diameter of 150-1200 mm and a flow rate of 1 to 20 liters per minute. Searching the CPC industry did not reveal an instrument that can provide a satisfactory solution for these flow rates with good stationary phase retention ratios. It is impossible for CPC to get a role in API purification without this ability.

This presentation will highlight the development steps that provided the pharmaceutical industry the first real industrial CPC, the RotaChrom iCPC. Starting from theoretical basics of the flow simulations, I would like to continue the journey to the realization of cells, through the troubleshooting of mechanical designs. Applications of our new technology from different examples of API purification in the pharmaceutical industry will be presented.

The first iCPC, an iCPC-S version with 2.5 L/min rated cells are under extensive testing in real separations, while the iCPC-L version with 10 L/min flow rated cells are under prototype testing right now. By the time of the conference, the first iCPCs are expected to be under operation in a cGMP environment.

References

1. Berthod, A. (2002). *Countercurrent Chromatography.* Amsterdam: Elsevier
2. Foucault, A. (1995) *Centrifugal Partition Chromatography*, New York; Marcel Dekker

COMPREHENSIVE TWO-DIMENSIONAL CCCXLC ANALYSIS FOR IMPROVED SEPARATION OF ROOIBOS POLYPHENOLS

2006
SO
Session IV

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Keywords: rooibos, two-dimensional chromatography, polyphenols

Rooibos (*Aspalathus linearis*), an herbal tea from South Africa, represents a complex mixture of polyphenols, comprising a variety of compounds naturally present in the plant, as well as degradation products formed during processing (1). Analytical separation of such a mixture represents a substantial challenge. Comprehensive two-dimensional chromatography offers a powerful approach to improve the resolution of components in such complex samples. In this regard, the comprehensive combination of countercurrent chromatography (CCC) and liquid chromatography (LC) promises to deliver a highly orthogonal separation since the two dimensions have different separation mechanisms (2).

Plant material from a single rooibos plant was sub-divided and processed to produce the conventional "fermented herbal tea", or dried immediately to represent "unfermented" (green) tea. Water extracts were prepared to represent the polyphenols present in a cup-of-tea and ethanol-insoluble matter removed to prevent formation of emulsions in the CCC (3). As first dimension separation, high-performance CCC (Spectrum model, Dynamic Extractions) using gradient elution in normal phase mode was optimized to provide an adequate spread of peaks across the gradient. The lower layer of ethyl acetate:water (1:1) was used as stationary phase, while the mobile phase was a gradient from 100% upper layer of the same solvent system to 50% upper layer of *n*-butanol:water (1:1) (total run time 60 min). In the second dimension, ultra high pressure reversed phase (RP) LC was used. The flow-rate and gradient time in the second dimension together with the 1st dimension sampling time were simultaneously optimized. This was done by measuring the column efficiency and peak width for representative peaks in the second dimension at different flow rate and gradient time combinations, measuring the peak width for aspalathin in the first dimension and calculating the practical peak capacity for the 2D analysis at different combinations of the three parameters. The best combination of the three parameters resulting in a reasonable total analysis time was a second dimension flow rate of 0.8 mL/min, a second dimension gradient time of 20 min and a first dimension sampling time of 1.25 min. These parameters resulted in 48 first dimension fractions giving a total analysis time of 16 hrs per sample. The two dimensions were coupled off-line for analysis using diode-array detection to draw up contour plots and high resolution mass spectrometry (MS) for tentative identification of peaks.

The contour plots for the off-line CCCxLC analysis showed good distribution of the peaks indicating a high orthogonality as expected. The practical peak capacity was calculated as >3000, which is higher than that achieved previously (>2000) for rooibos extracts using off-line LCxLC with hydrophilic interaction chromatography (HILIC) and RP-LC in the 1st and 2nd dimensions, respectively (4). A large number of peaks were observed in the green and fermented rooibos extracts. No major change in polyphenol profile was observed between the green and fermented sample, but peaks differed in intensity between the samples.

References

1. E. Joubert, D. De Beer, South African J. Bot. 77 (2011) 869–886.
2. T. Michel, E. Destandeau, C. Elfakir, Anal. Bioanal. Chem. 406 (2014) 957-969.
3. D. de Beer, C.J. Malherbe, T. Beelders, E.L. Willenburg, D.J. Brand, E. Joubert, J. Chromatogr. A. 1381 (2015) 29–36.
4. T. Beelders, K.M. Kalili, E. Joubert, D. de Beer, A. de Villiers, J. Sep. Sci 35 (2012) 1808-1820.

CENTRIFUGAL PARTITION CHROMATOGRAPH: A CONTINUOUS MULTIPHASE REACTOR

2007
FO
Session XII

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Keywords: multiphase reaction, biocatalysis, CPC as reactor

Multiphase reaction systems, mostly aqueous organic systems, are used in biocatalysis to convert hydrophobic substrates which are almost insoluble in aqueous media. To enhance the catalytic efficiency and long term stability as well as to enable continuous production, the biocatalysts are commonly immobilized. Unfortunately, most of the immobilization techniques reduce the initial catalytic activity of the system as steric and/or mass transport hindrances are present or the conformation of the biocatalyst changed after immobilization. Besides, multiphase reaction systems lead to different design aspects compared to monophasic aqueous systems. Efficient mixing and separation of the phases need to be realized to ensure the mass transport of substrate to the biocatalyst (reaction) and the product to the organic phase (product removal).

We are studying the Centrifugal Partition Chromatograph (CPC) as an alternative multiphase reaction device [1-2]. Commonly, the CPC is used for liquid-liquid chromatography, where one phase of a two-phase-system is immobilized in a chamber system that is arranged around a rotary axis, while the second phase is pumped through the stationary one. The dynamic partition of components between both phases enables the separation in this device. Due to the CPC set-up, the immobilization of biocatalysts in their natural environment, namely an aqueous solution, is easily feasible. The intense mixing of the two phases containing either the biocatalyst (stationary phase) or the substrate (mobile phase) and the efficient phase separation for *in situ* product removal is ensured simultaneously. Hence, the CPC combines all design aspects for an efficient multiphase reactor, namely immobilization of the biocatalyst, intense mixing, complete phase separation and *in situ* product removal in one compact device (see figure 1). As no solid phase is used to immobilize the biocatalyst structure changes due to covalent binding, loss of the hydrate shell or steric hindrances may not occur. Additionally, as no solid phase is used to immobilize the biocatalyst structure changes due to covalent binding, loss of the hydrate shell or steric hindrances may not occur.

The present study will address the process design procedure to systematically select the phase system to achieve stable and efficient reaction rates and the CPC operation conditions to enable efficient mixing and separation of the phases. The procedure is applied to several biocatalysts ranging from enzyme to whole cell systems. To evaluate the applicability of the CPC as multiphase reactor experiments using a classical stirred tank reactor are compared and discussed.

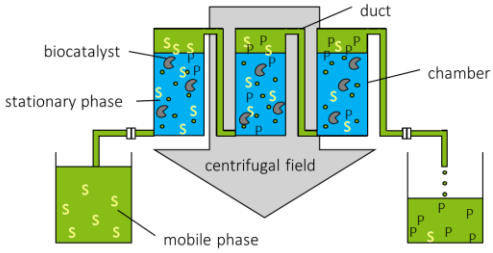

Figure 1: Scheme of the CPC as multiphase reactor

References

[1] J. Krause, T. Oeldorf, G. Schembecker, J. Merz, Enzymatic hydrolysis in an aqueous organic two-phase system using centrifugal partition chromatography J. Chrom. A 1391 (2015) 72-79
[2] C. Nioia, D. Riboula, P. Destraca, A. Martyb, L. Marchalc, J.S. Condoreta, The centrifugal partition reactor, a novel intensified continuous reactor for liquid–liquid enzymatic reactions, Biochem. Eng. J. 103 (2015) 227-233

STATIONARY PHASE RETENTION IN CCC: DIFFERENCES BETWEEN HYDROSTATIC AND HYDRODYNAMIC COLUMNS

2008
SO
Session XII

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Keywords: Sf, stationary phase retention factor, flow rate, phase density, centrifugal field, throughput.

CCC is the only chromatographic separation technique in which the stationary phase volume contained in the column is an adjustable parameter, *Sf*. Obviously, if there is no liquid stationary phase retained by a CCC column, no separation is possible. Hence, the *Sf* parameter is a critical parameter in CCC column performance measured by (1) the resolution factor, and (2) the purification throughput as CCC is mainly a preparative technique.

(1) The resolution factor is the ratio of the distance between two peaks, *ΔV,* over their average peak width (2*σ*). Using all known chromatographic retention (solute partition coefficients, *K*) and efficiency (plate number, *N*) equations, the resolution equation can be derived as:

$$Rs = \frac{\Delta V_r}{2(\sigma_1 + \sigma_2)} = Sf \frac{\sqrt{N}}{4} \frac{(K_2 - K_1)}{1 - Sf\left(1 - \frac{K_1 + K_2}{2}\right)}$$

(2) The purification throughput is the mass of compound obtained with the desired level of purity per working time and per solvent volume used.

To increase the throughput, the mobile phase flow rate must be increased, which has consequences on the stationary phase retention ratio. There are differences between the two main types of CCC columns: the hydrodynamic CCC columns based on coiled tubes and no rotary seals and working at low pressure vs. the hydrostatic CPC columns based on interconnected chambers, rotary seals and working at intermediate pressures.

For hydrodynamic CCC columns, increasing the flow rate decreased the *Sf* factor depending on the centrifugal field strength (rotor rotation) and also, specifically, on the tubing diameter used to prepare the CCC column. But the tubing diameter is related to the tubing length making *N* the column efficiency. All parameters being related, there are optimal conditions that must be sought for any new separation since they are solute dependent.

For hydrostatic CPC columns, there is also a decrease in *Sf* with increasing the flow rate. This *Sf* reduction is deleterious for resolution. It can be reduced by increasing the centrifugal field which affects the CPC chromatographic efficiency *N* and the operating pressure. All this leads to an optimization approach differing from what can be done with hydrodynamic columns.

The different optimization ways will be presented and discussed based on practical real-world purifications.

References

1. A. Berthod, K. Faure, Revisiting resolution in hydrodynamic CCC: the tubing bore effect, J. Chromatogr. A 1390 (2015) 71-77.
2. E. Bouju, A. Berthod, K. Faure, Scale-up in CPC: the free-space between peaks method, J. Chromatogr. A 1409 (2015) 70-78.

ISOLATION OF TOCOPHEROLS AND TOCOTRIENOLS AS CONSTITUENTS OF γ ORYZANOL IN BABY BANANA PEELS WITH HYPERPIGMENTATION BY MEANS OF SPIRAL COIL-LSRCCC.

2009
SO
Session XI

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Keywords: tocopherols, tocotrienols, banana, γ-oryzanol, spiral coil

The spiral-coil low-speed rotary countercurrent chromatography (spiral-coil LSRCCC) has become a powerful tool in isolation of approx. 700 mg of γ-Oryzanol, a nutraceutical edible oil identified in fraction 14 from hexane extract of baby banana peels with hyperpigmentation **(Figure 1/left)**. The APCI-HPLC MS/MS on line coupled UV contour plot spectra were analysed thoroughly to identify tocopherols in fraction 10 and 11 at the beginning of extrusion mode and sequentially tocotrienols as compounds with a strong fluorescence (*m/z* 419) at 10.7 min between fraction 12 and 15 in extrusion mode. ^{1}H- and ^{13}C-NMR spectra recorded at 600 and 150 MHz, respectively, from fraction 14 not only depicted the occurrence of one compound but also a variety of resonances with high intensity that mask low intensity chemical shift, which play an important role in the elucidation of tocopherols and tocotrienols **(Figure 1/middle)**. The polar chromanol ring of ß-tocotrienol was defined by resonances of quaternary carbons in the ^{13}C-NMR spectrum with the support of the HSQC and HMBC experiments (1,2). Additionally, elucidation of triterpene alcohol ferulates in fraction 14 was enabled due to the resonance at δ 167.88 in the ^{13}C-NMR spectrum which supported the identification of cycloartenyl *E* ferulates and 25-hydroxy-24-methylcycloartenyl ferulate as constituents of γ-Oryzanol in baby banana peels with hyperpigmentation **(Figure 1/right)** (3,4,5).

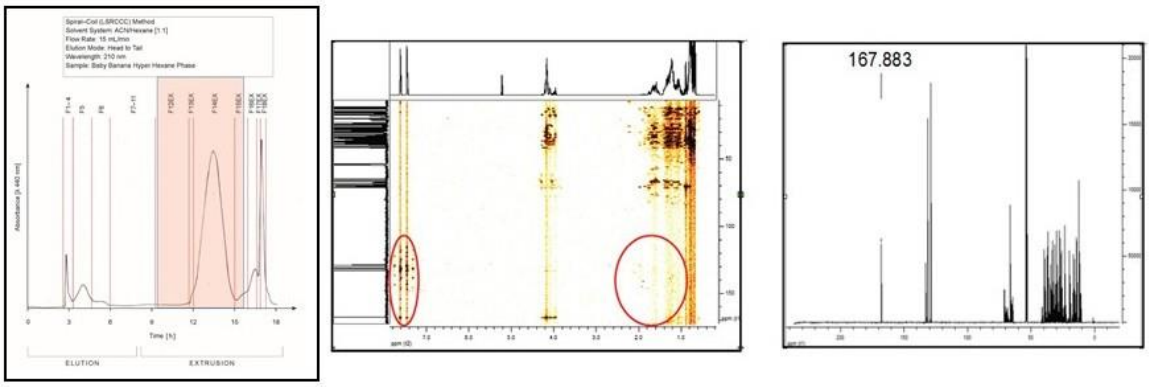

Figure 1. **Left**: Spiral-Coil LSRCCC chromatogram in elution and extrusion mode from Baby Banana peels with hyperpigmentation from hexane extract. **Middle**: HMBC spectrum of fraction 14 (150 MHz) in extrusion mode of Banana peels with hyperpigmentation. **Right**: ^{13}C-NMR spectrum (150 MHz) of fraction 14 from Spiral-Coil LSRCCC of Baby Banana peels with hyperpigmentation. The chemical shift at δ 167.88 belongs to the triterpene alcohol ferulates identified in fraction 14 as constituents of γ-oryzanol.

References

1. S. Ohnmacht, R. West, R. Simionescu, J. Atkinson, Assignment of the ^{1}H and ^{13}C NMR of tocotrienols, Magn. Reson. Chem. 46 (2008) 287-294. **2**. A.M. Lampi, T. Nurmi, V. Ollilainen, V. Piironen, Tocopherols and Tocotrienols in Wheat Genotypes in the HEALTHGRAIN Diversity Screen, J. Agric. Food Chem. 56 (2008) 9716-9721. **3**. N. Fang, S. Yu, T. M. Badger, Characterization of Triterpene Alcohol and Sterol Ferulates in Rice Bran Using LC-MS/MS, J. Agric. Food Chem. 51 (2003) 3260-3267. **4**. M. Liu, F. Yang, H. Shi, C.C. Akoh, L. Yu, Preparative separation of triterpene alcohol ferulates from rice bran oil using a high performance counter-current chromatography, Food Chem. 139 (2013) 919-924. **5**. A. Angelis, A. Urbain, M. Halabalaki, N. Aligiannis, A. L. Skaltsounis, One-step isolation of γ-oryzanol from rice bran oil by non-aqueous hydrostatic countercurrent chromatography, J. Sep. Sci. 34 (2011) 2528-2537.

MATCHING SWEET SPOTS: REFINING A TLC-BASED COUNTERCURRENT SOLVENT SYSTEM SELECTION STRATEGY

2010
SO
Session V

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Keywords: Solvent system selection, Thin-layer liquid chromatography, Rf value sweet spots

A TLC-based solvent system prediction strategy for countercurrent separation (CCS) has been proposed since 1979 [1,2] (Figure 1). The theory proposes that the *K* values that define the sweet spot of optimal CCS correspond to a similar range of R*f* values from the silica gel TLC plate developed in the organic phase of the biphasic solvent system. Despite its simplicity, there is little evidence in the literature that this method has been widely used. The present study develops a theoretical validation that an exponential correlation exists between TLC R*f* and CCS *K*, i.e., R*f* and log*K* correlate linearly (Figure 2). With the middle of the log*K* and R*f* sweet spot set a 0 and 0.5 respectively, the R*f* sweet spot range varies with the maximum and minimum values of *K* (Table 1). In order to experimentally validate the TLC prediction method, the CCS solvent systems for 37 natural products were investigated. The method correctly predicted the sweet spot in over 90% of the cases. Advanced guidelines for the TLC prediction of a solvent system can now be provided.These will equip CCS users with an enhanced practical understanding of how to apply TLC-based solvent system prediction strategies for CCS.

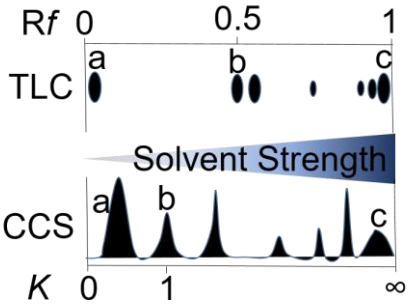

Figure 1. Model of the relative behavior of a natural mixtures in both chromatographic systems

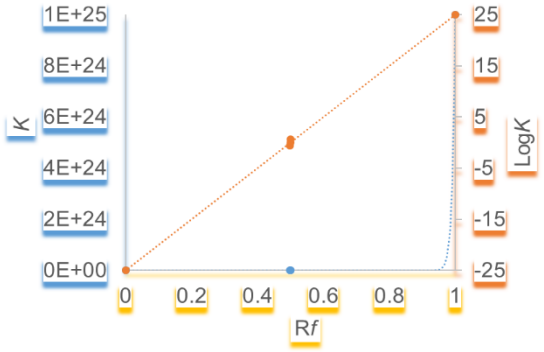

Figure 2. Proposed correlation between TLC R*f* and CCS *K* (blue), and R*f* and log*K* (red)

Table 1. Representative *K* value ranges and their corresponding R*f* value sweet spots

K	**R*f***	***K***	**R*f***	***K***	**R*f***	***K***	**R*f***	***K***	**R*f***
max	1.00	100		1000		10000		10^{25}	
4.00	Sweet[a]		0.65		0.6		0.58		0.5012
1.00	0.50								
0.25	Sweet[b]		0.35		0.4		0.43		0.4988
min	0.00	0.01		0.001		0.0001		10^{-25}	

References

1. K. Hostettmann, M. Hostettmann-Kaldas, O. Sticher, J. Chromatogr. A 186 (1979) 529.
2. J.B. Friesen, G. F. Pauli, J. Liq. Chromatogr. Relat. Technol. 28 (2005) 2777-2806.

ENANTIOSEPARATION OF AROMATIC ACIDS BY PRECOLUMN DERIVATIZATION COUNTERCURRENT CHROMATOGRAPHY

2011
FP
Session II

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Keywords: Aromatic acids; Countercurrent chromatography; Enantioseparation; Precolumn derivatization

The enantiomeric separation and analysis of chiral drugs has become essential since enantiomers exhibit different biological behaviour and may exert different pharmacodymic, pharmacokinetic, and toxicologic activities. Chromatographic enantioseparations could be divided into three categories: direct separation on a chiral stationary phase, enantioseparation by chiral mobile phase additive, and separation of diastereoisomer formed by precolumn derivatization with a chiral derivatization reagent. These methods have been substantially explored and used in traditional liquid chromatography and gas chromatography. In recent years, countercurrent chromatography has become increasingly attractive for enantioseparations due to its preparative capacity, and more than ten kinds of chiral selectors has been tested and investigated [1]. However, literature about enantioseparation by precolumn derivatization countercurrent chromatography, as far as we know, have not been published.

Enantioseparation of aromatic acids by countercurrent chromatograpy, including mandelic acid derivatives and 2-arylpropionic acid derivatives, have been mainly investigated in the recent years in our lab using different chiral selectors, such as β-cyclodextrin derivatives and L-proline derivatives [2-4]. It was found that some racemates couldn't be enantioseparated. Herein we want to report our recent study on the enantioseparation of some racemic aromatic acids by precolumn derivatization countercurrent chromatography, which was not separated in our previous work. (-)-Menthol was selected as the chiral derivatization reagent for esterification of racemic aromatic acids. A suitable biphasic solvent system was selected for separation of diastereoisomeric analytes, as shown in Figure 1.

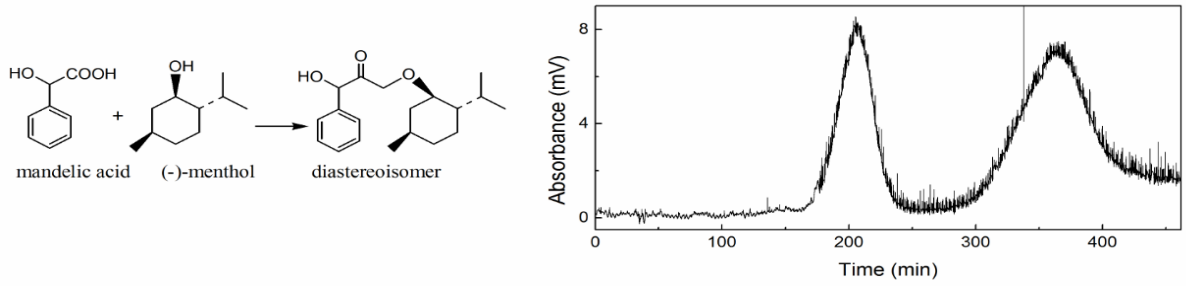

Figure 1. Esterification of mandelic acid with (-)-menthol and chromatogram of separation of diastereoisomer by countercurrent chromatography

References

1. X. Y. Huang, D. L. Di, Chiral separation by counter-current chromatography. Trends Anal. Chem. 67 (2015): 128-133.
2. S. Tong, X. Wang, M. Lu, Q. Xiong, Q. Wang, J. Yan. Enantioseparation of 2-(substituted phenyl)propanoic acids by high speed countercurrent chromatography and investigation of influence of substituents in enantiorecognition, J. Sep. Sci. 39 (2016): 1567-1573.
3. S. Tong, H. Zhang, D. Cheng. Preparative Enantioseparation of β-substituted-2-phenylpropionic acids by countercurrent chromatography with substituted β-Cyclodextrin as chiral selectors, Chirality, 27 (2015): 795-801.
4. S. Tong, M. Shen, D. Cheng, Y. Zhang, Y. Ito, J. Yan. Chiral ligand exchange high-speed countercurrent chromatography: mechanism and application in enantioseparation of aromatic α-hydroxyl acids, J. Chromatogr. A 1360 (2014): 110-118.

COMPREHENSIVE TWO-DIMENSIONAL CPC-LC: PROVIDING ANALYTICAL AND PREPARATIVE SEPARATIONS SYSTEMS FOR COMPLEX EDELWEISS EXTRACTS

2012
FO
Session II

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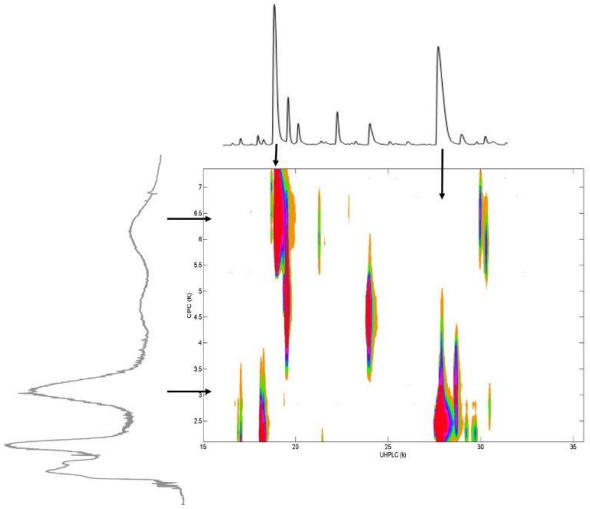
Keywords: K-driven separation, comprehensive two-dimensional chromatography, orthogonality, scale up

Driven by the need to discover key players in herbal medicine and to speed up pharmacognosy studies by providing high purity standards, we devised here the comprehensive two-dimensional CPC-LC system as an effective tool to unravel complexity. The edelweiss plant is a well-known alpine traditional medicine associated to a wide number of health benefits. Famous for its rareness in wild environment, tremendous efforts were made recently to cultivate this plant and access to its beneficial therapeutic effects. Three major antioxidants were recently identified by HPLC (1), but their closest impurities (some as isomers) refrain the use of preparative LC to provide high quality standards required for further therapeutic studies.

With a preparative purpose in mind, we developed a laboratory-scale multiple heart-cutting CPC-LC method by optimization of K-driven orthogonality on compounds of interest. Indeed, while LC solid stationary phase usually imposes high economical costs at industrial scale, liquid stationary phase used in CCC and CPC offers such a versatility that it can be tuned to provide orthogonal separation mechanisms. Once the laboratory-scale 2D chromatography was optimized (on a 30ml CPC rotor and a 4.6mm x 10cm C18 HPLC column), it was successfully transferred to industrial scale (on a 1liter CPC rotor and a 50mmx30cm C18 HPLC column).

However, the combination of CPC and LC in a comprehensive approach can also give insights to the complexity of the plant extract. By employing a smart tracking strategy for active ingredients and their closest impurities, i.e., by evaluating first dimensions CPC fractions by reconstructing them from two different second-dimension analytical HPLC methods with various chromatographic selectivity, we were able to highlight the presence of compounds that were co-eluting and hence invisible if using only one CPC or LC chromatographic dimension. The comprehensive 2D CPC-LC strategy provides deep information on plant complexity, generating K-based mapping that can be helpful indeed for preparative method development, but also for analytical insights to monitor plant variability and extraction efficiency.



1. S. Schwaiger, et al., Tetrahedron, 61 (2005) 4621-4630.

RAPID PURIFICATION AND SCALE-UP SEPARATION OF THREE MAKAMIDES FROM *LEPIDIUM MEYENII* USING HIGH-CAPACITY HIGH-SPEED COUNTER-CURRENT CHROMATOGRAPHY

2013
FP
Session I

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Keywords: Lepidium meyenii*; high-speed counter-current chromatography; makamides*

A prototype of engineering high-speed counter-current chromatograph (HSCCC) was designed containing two separation columns. Each of them is composed of three single-channel units. A rapid separation and scale-up approach has been developed by using this prototype to isolate the macamides, *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide, *N*-benzyl-9*Z*-octadecenamide, and *N*-benzyl-9*Z*- octadecenamide, from a crude extract that was obtained from *Lepidium meyenii* by Supercritical Fluid Extraction (SFE). 1g of crude macamide extract was separated and purified by HSCCC with a two-phase solvent system composed of n-hexane-ethylacetate-methanol-water (7:2:5:1, v/v/v/v) with one unit in one step for about 360 min, and the fractions were analyzed by high-performance liquid chromatography (HPLC). This large scale preparative single step run yielded 226 mg *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide with a purity of 97.0%, 313 mg *N*-benzyl-9*Z*-octadecenamide with a purity of 98.8%, and 152 mg *N*-benzyl-hexadecanamide with a purity of 95.4%. This is the first time that high-speed counter-current chromatography has been used to purify makamides in a multiple gram scale in less than 6 h and at such high purity of the final products.

PURIFICATION OF BIOACTIVE COMPOUNDS FROM *CENTAURIUM ERYTHRAEA* RAFN. BY CONVENTIONAL AND NEW GENERATION DESIGNED CENTRIFUGAL PARTITION CHROMATOGRAPHY COLUMN COUPLED WITH MS DETECTOR

2015
SO
Session IV

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Keywords: *Seco-iridoids,* Centaurium erythraea *Rafn, centrifugal partition chromatography countercurrent chromatography*

Centaurium erythraea Rafn. (Gentianaceae) is a widespread herbaceous plant used in a traditional medicine in more than twenty countries. Hepatoprotective, antibacterial or hypoglycemic are some of the activities reported in the literature [1]. *Seco*-iridoid glycosides, xanthones, phenolic derivatives and triterpenes were identified as major compounds [2]. In order to recover efficiently the polar constituents, the raw material was macerated in methanol, UHPLC-MS-UV profiles of each extract enabled to establish the yield in swertiamarin (ST), sweroside (SR) and gentiopicrin (GP), three structurally closely-related target natural products.

Centrifugal partition chromatography (CPC), a support-free liquid–liquid chromatography based on the partitioning of analytes between two non-miscible liquid phases, was used to isolate ST, SR and GP from the extract. Experimental conditions were optimized on isocratic and gradient mode using a mixture EtOAc/EtOH/H_2O on a conventional CPC 250 mL column [3]. These data were then transposed to a new generation 250 mL column coupled with an MS detector. The monitoring of the collected fractions was done with single quadrupole mass spec detector in tandem, allowing targeted *seco*-iridoid glycosides to be clearly identified and the purity determined in a single step. Furthermore, this allowed us to perform a five times faster separation compared with the conventional 250 mL column.

The structures and the purity of the recovered compounds were also unambiguously ascertained by extensive NMR analyses. This study proved the ability of new generation CPC column coupled with MS, to purify higher amount of a polar extract in a timeless duration run. The choice of an environment-friendly solvent mixture including the recycling of EtOAc was also noticeable and may contribute to upgrade CPC as a potential industrial production tool.

References

1. P. Valentao , E.Fernandes, F. Carvalho, P.B. Andrade, R.M. Seabra, M.L. Bastos ,Antioxidant Activity of Centaurium erythraea Infusion Evidenced by Its Superoxide Radical Scavenging and Xanthine Oxidase Inhibitory Activity. J. Agric. Food Chem. (2001); 49 :3476−3479
2. G. Stefkov , B. Miova, S. Dinevska-Kjovkarovska, J.P. Stanoeva,M. Stefova,G. Petrusevska,S. Kulevanova Chemical characterization of *Centaurium erythrea* L. and its effects on carbohydrate and lipid metabolism in experimental diabetes. J. Ethnopharmacol. (2014); 152:71-77
3. J. Goll, G. Audo, M. Minceva Comparison of twin-cell centrifugal partition chromatographic columns with different cell volume. J Chromatogr A (2015); 1406:129 • 135

SPECULATIONS ON THE FORMATION OF A DOUBLE PEAK DURING HIGH-SPEED COUNTER-CURRENT CHROMATOGRAPHIC SEPARATION OF AN AZO DYE

2016 SO Session XI

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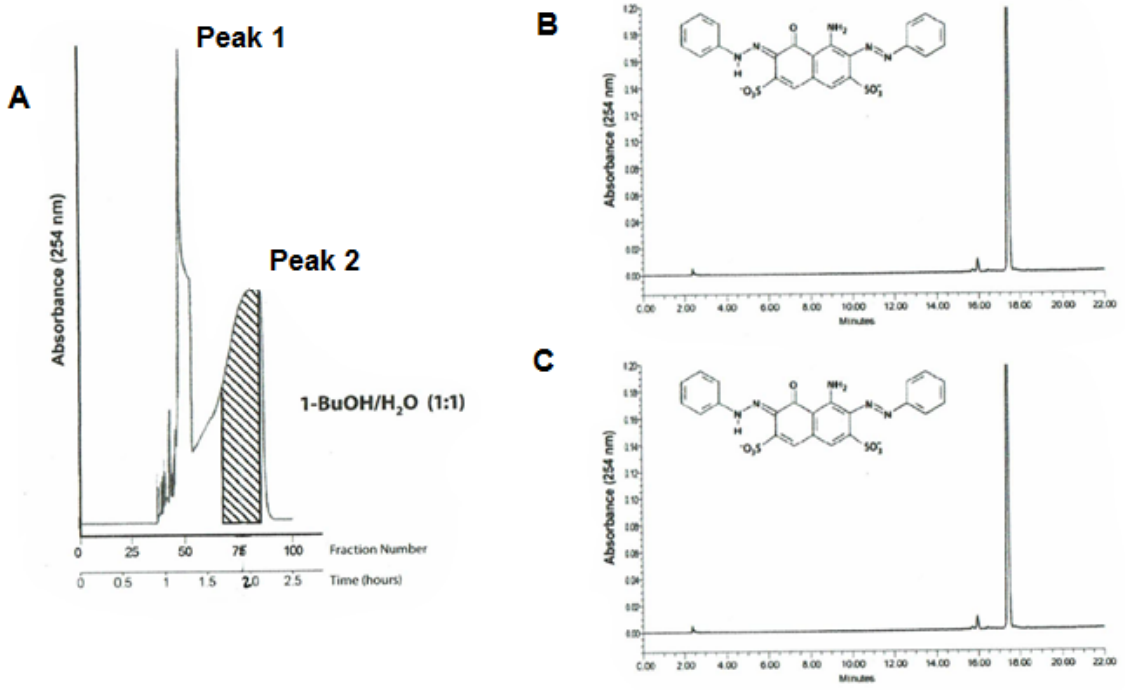
Keywords: spiral high-speed CCC; elution profile; azo dye.

High-speed counter-current chromatography (HSCCC) is a liquid-liquid partition technique in which one of the liquid phases (the stationary phase) is retained in the rotating CCC column while the other liquid phase (the mobile phase) is pumped through. HSCCC has been applied successfully to the separation of components from various synthetic dyes such as those of the xanthene, triphenylmethane, quinoline, azo, pyrene and indigo types. While many applications of HSCCC have been described, few publications have addressed the phenomena that occur in the CCC column during the process of separation.

While we were identifying several subsidiary colors of the azo color additive D&C Red No. 33 [*J. Chromatogr. A*, 1380 (2015) 120-129], we observed that the CCC elution profile obtained for one of the compounds showed two peaks (Peak 1 and Peak 2 in Figure A). HPLC analyses pointed to the same component in aliquots from Peak 1 fractions as in those from Peak 2 fractions, eluting at the same retention time and having identical UV-vis spectra (Figures B and C).The present work attempts to explain why this single compound separated in the CCC column into two peaks as if it were a mixture of two components.



(A) HSCCC elution profile obtained for the separation of a subsidiary color of D&C Red No. 33; **(B)** HPLC chromatogram of fractions from Peak 1; **(C)** HPLC chromatogram of fractions from Peak 2

SEPARATION AND IDENTIFICATION OF A NOVEL SUBSIDIARY COLOR OF THE COLOR ADDITIVE FD&C RED NO. 40 (ALLURA RED AC) USING SPIRAL HIGH-SPEED COUNTER-CURRENT CHROMATOGRAPHY

2017 PO Session

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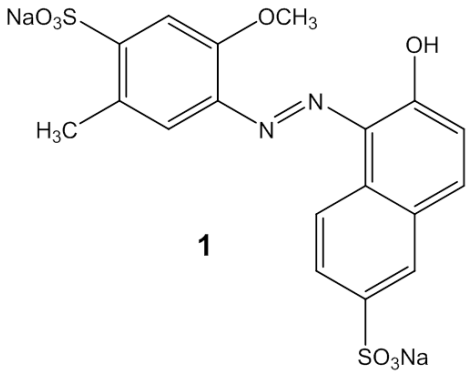
Keywords: affinity-ligand pH-zone-refining CCC; azo color additive

FD&C Red No. 40 (R40, Allura Red AC, Colour Index No. 16035) is a color additive permitted in the United States for coloring foods, drugs, and cosmetics. It consists mainly of the disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid, **1**. During its manufacture, a host of synthetic by-products are produced, and they are found in various amounts in the final product. Before it may be used as a color additive, R40 is subject to batch certification by the U.S. Food and Drug Administration (FDA) to ensure compliance with limits on levels of impurities specified in the Code of Federal Regulations (CFR).

In the current study, a method based on spiral high-speed counter-current chromatography using the affinity-ligand pH-zone-refining mode was developed for the preparative separation of a subsidiary color impurity from a batch of R40. The impurity is often observed in HPLC chromatograms of batches of R40 submitted for certification, and its identity has not heretofore been known. Very few published works describe the separation of components of R40, and none involves use of preparative methods for separating subsidiary color impurities. For identification purposes in the present study, it was necessary to isolate the impurity, which represents only about ~0.6% of the dye. The chemical structure of the isolated impurity was then determined by NMR and LC-MS/MS.



1

CCC SEPARATION STRATEGIES FOR VERY NONPOLAR LIPID COMPOUNDS

2018
FO
Session V

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Keywords: tripalmitin; stearic acid cholesteryl ester; ionic liquids; recycling CCC; bridging solvents

CCC is a well-established liquid-liquid chromatography method, the efficacy of which comes into full fruition with slightly polar analytes [1]. Limitations exist especially in the very nonpolar range. This is mainly due to the prerequisite that the analytes should preferably partition between the two phases with K values of $0.4 < K < 2.5$ [2]. In the field of very nonpolar lipid compounds, it is difficult to develop a (stable) solvent system with reasonable K values. Two very nonpolar substance classes are triacylglycerides and steryl esters, especially those without double bonds in the acyl moiety and with a given chain length. Model compounds considered to be very difficult to separate by CCC are tripalmitin (PPP, log K_{OW} ~21) and stearic acid cholesteryl ester (18:0-CE; log K_{OW} ~15).

Using the very hydrophobic solvent system of the HEMWat family, i.e. “HEMWat -7”, 18:0-CE was not detected at all in the lower phase (while free sterols showed K values of ~15). Likewise, the simple two-component mixture *n*-hexane/acetonitrile (1:1) previously used for fatty acids resulted in K values of 2000 and 20000 for PPP and 18:0-CE. The three-component solvent system suitable for carotenoids, i.e. *n*-hexane-benzotrifluoride-acetonitrile (10:3.5:6.5 [3]) provided K values of ~25 and ~30 for PPP and 18:0-CE. In either case, these K values were way too high for applications in CCC despite the notable difference in K values. Even application of CCC in the co-current mode could not provide sufficient resolution of the two compounds ($\alpha << 1.5$) [4].

Here we present two different approaches in order to further decrease the K values. One attempt was made by studying the effects of using ionic liquids (ILs), which were obtained from our partner institute in Belfast, in the solvent system. In contrast to published studies with more polar solvent systems, the share of IL had to be increased in order to obtain suitable phase separation. With the best solvent system containing IL we could achieve K values of ~2.5 and ~4 in shake flask systems. Due to the relatively high demand of IL and their limited availability, we set up CCC in recycling mode [5]. The recycling mode approach not only improves peak resolution but also supports green chemistry in that the consumption of solvent is reduced. We also noted that several solvent systems with ILs which worked well in shake-flask experiments (e.g., with regard to the settling time) were difficult to use in CCC (low stationary phase retention, S_f). In a second series of experiments, we aimed to improve the K values by adding bridging solvents. Promising results were obtained with toluene [6] as well as toluene/benzotrifluoride as bridging solvents.

References

1. Y. Ito, Golden rules and pitfalls in selecting optimum conditions for high-speed counter-current chromatography. J. Chromatogr. A 1065 (2005) 145-168.
2. J.B Friesen, G.F. Pauli, G.U.E.S.S. - A generally useful estimate of solvent systems for CCC. J. Liq. Chromatogr. Rel. Technol. 28 (2005) 2777-2806.
3. M. Englert, S. Hammann, W. Vetter, Isolation of ?-carotene, ?-carotene and lutein from carrots by countercurrent chromatography with the solvent system modifier benzotrifluoride. J. Chromatogr. A 1388 (2015) 119-125.
4. S. Hammann, M. Englert, M. Müller, W. Vetter, Accelerated separation of GC-amenable lipid classes in plant oils by countercurrent chromatography in the co-current mode. Anal. Bioanal. Chem. 407 (2015) 9010-9027.
5. Q.-Z. Du, C.-Q. Ke, Y. Ito, Recycling high-speed countercurrent chromatography for separation of taxol and cephalomannine. J. Liq. Chromatogr. Rel. Technol. 21 (1998) 157-162.
6. C. Gallistl, W. Vetter, Synthesis, liquid chromatographic fractionation and partial characterization of polybrominated dibenzofuran congeners. J. Chromatogr. A 1443 (2016) 69-79.

ADVANCEMENT OF COUNTERCURRENT CHROMATOGRAPHY INSTRUMENTATION BY TUBING MODIFICATIONS

2019
FO
Session IX

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Keywords: Tubing modifications, Separation Efficiency, Instrumentation

Countercurrent chromatography is a preparative separation technique that can be described as a combination of repetitive liquid-liquid extractions of compounds between two immiscible liquid phases and chromatography features. The separation process takes place in a long and hollow round-shaped tubing made of PTFE that is wrapped around a holder hub, resulting in so-called multilayer coils which represent the heart of a CCC system (1). CCC can currently not compete with HPLC in terms of theoretical plate number and resolution. Therefore, resolution in CCC is achieved by fine-tuning the composition of the solvent system in order to obtain enhanced selectivity between the different compounds. Compared to that, attempts to improve the CCC instrumentation and to advance the core unit of the chromatographic system, the tubing geometries, have been scarcely reported in literature. Recently, we found that modification of the round-shaped tubing, used in classic CCC, by crimpings can improve the separation efficiency by increasing the stationary phase retention. At first, a crimping tool was developed that allowed the manufacturing of different tubing modifications which differed in the arrangement of the crimpings (2). These arbitrarily selected tubing modifications were investigated in a CCC-1000 instrument one after another with self-constructed multilayer coils. The stationary phase retention in a hydrophobic, an intermediate, and a hydrophilic two-phase solvent system was tested. In the intermediate solvent system, the separation efficiency was tested with alkyl hydroxybenzoates. The most convincing tubing modification was further studied by means of a factorial design of experiments approach (3). The effect of three geometrical parameters (crimping depth, distance between the crimping and the β-value range) on the retention of the stationary phase was tested. The tubing modification showing the highest retention was also tested with the intermediate polar solvent system using alkyl hydroxybenzoates, as well as with the hydrophobic solvent system using fatty acid methyl esters. The results were evaluated based on peak widths, resolution values, and theoretical plate numbers. The optimum tubing modification featured the deepest crimping depth and the narrowest crimping distance and was superior to the conventional CCC tubing in terms of separation efficiency. Computational fluid dynamic simulations were performed to explain the effects and to establish a link between the geometrical parameters and the separation efficiency.

References

1. Y. Ito, Golden rules and pitfalls in selecting optimum conditions for high-speed countercurrent chromatography, J. Chromatogr. A 1065 (2005) 145-168.
2. M. Englert, W. Vetter, Tubing modifications for countercurrent chromatography (CCC): Stationary phase retention and separation efficiency, Anal. Chim. Acta 884 (2015) 114-123
3. M. Englert, W. Vetter, Tubing modifications for countercurrent chromatography (CCC): Investigation of geometrical parameters, J. Liq. Chrom. Relat. Technol. (2016) *in press*.

DEEP EUTECTIC SOLVENT SYSTEMS IN LIQUID-LIQUID CHROMATOGRAPHY

2021
FO
Session V

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Keywords: deep eutectic solvents, COSMO-RS, hydrophobic compounds, solvent system selection

Deep eutectic solvents (DES) are a class of solvents composed of hydrogen bond donor and hydrogen bond acceptor molecules. The mixture has a substantially lower melting point than the pure substances and remains liquid at room temperature. The number of possible combinations of DES-forming components is nearly unlimited as there is a huge pool of potential hydrogen bond donors and acceptors. DES have interesting characteristics, such as high solvation capacity and low volatility, and they are considered environmentally benign, which makes them interesting for applications in liquid-liquid chromatography. Compared to ionic liquids, DES are less expensive and their preparation is easy [1]. They can be used to replace water in biphasic systems and, therefore, increase the solubility of hydrophobic substances. To our knowledge, this is the first study showing a successful application of DES-based biphasic systems in liquid-liquid chromatography. In this work, model components from the G.U.E.S.S.-mix [2] were separated using a centrifugal partition extractor (SCPE-250-BIO from Armen Instrument, France). It could be shown that DES-based systems are in particular suitable for the separation of hydrophobic compounds with an octanol-water partition coefficient in the range of 2-12 [3].

In order to foster the selection of a DES-based biphasic system for different separation problems, the prediction quality of COSMO-RS (conductor-like screening model for realistic solvation) of important thermodynamic parameters for the design of a liquid-liquid chromatographic process has been evaluated. The results show that the accuracy of the calculated liquid-liquid equilibrium data and partition coefficients is high enough for screening and solvent system selection purposes. It could be demonstrated that DES-based biphasic systems can be applied for the separation of hydrophobic substances, and that the predictive model COSMO-RS can be employed to reduce the experimental effort in the selection process of an appropriate hydrogen bond donor and acceptor combination as well as DES-based biphasic solvent systems.

References

1. A.P. Abbott, D. Boothby, G. Capper, D.L. Davies, R.K. Rasheed, Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids, J. Am. Chem. Soc. 126 (2004) 9142–9147.
2. S. Roehrer, F. Bezold, E.M. García, M. Minceva, Deep Eutectic Solvents in Countercurrent and Centrifugal Partition Chromatography, J. Chromatogr. A 1434 (2016) 102-110.
3. J. Brent Friesen, G.F. Pauli, G.U.E.S.S.—a generally useful estimate of solventsystems for CCC, J. Liq. Chromatogr. Relat. Technol. 28 (2005) 2777–2806.

MODEL-BASED DESIGN OF A SEQUENTIAL CENTRIFUGAL PARTITION CHROMATOGRAPHY PROCESS FOR THE PREPARATIVE BATCH SEPARATION OF TERNARY MIXTURES

2022
SO
Session VIII

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Keywords: model-based design, ternary separations, sequential centrifugal partition chromatography (sCPC)

In the preparative chromatographic separation of natural products from complex mixtures, the target component often possesses an intermediate elution speed. In batch injections, peak cutting is usually required to obtain an intermediate component in highly pure form, leading to low productivity and yield. Sequential centrifugal partition chromatography (sCPC) is a liquid-liquid chromatography technique offering an alternative for the purification of intermediate target components at high column loadings. The use of sCPC for the continuous fractionation of binary mixtures at high purities was previously demonstrated [1]. In this work, a discontinuous sCPC operating mode was developed for the separation of ternary mixtures. A sCPC unit consists of two columns connected in series. For ternary mixture separation by sCPC, the feed mixture (A, B, and C) is loaded at a point between the two columns at the start of the separation process. Multiple elution cycles are then performed, each consisting of one descending and one ascending step. During this time, the neighboring components (A and B) are eluted from the column. The durations of the ascending and descending steps are chosen so as to prevent elution of the intermediate target component (B), which remains “trapped” inside the column. After completion of the elution cycles, the purified target component can be recovered. Similar methods have been reported for the enrichment and purification of intermediate components initially present at low concentrations in the feed [2,3]. In these studies, the feed stream was injected continuously or repeatedly over multiple elution cycles, rather than in one loading step. Full recovery of the intermediate components was not achieved.

The aim of this work was to develop a model-based design approach for selection of the sCPC operating parameters for the separation of a ternary mixture into three product fractions of high purity and yield. Toward this goal, the relationship between column loading, duration of the ascending and descending steps, and band broadening due to dispersion effects was investigated. First, a simple mathematical model was developed for determination of the durations of the ascending and descending steps at a certain column loading under ideal conditions (no dispersion effects). A safety factor was applied to allow for broadening of the intermediate component band during the elution cycles. Simulations based on the equilibrium cell model (4) were then performed to evaluate separation performance with the selected operating parameters under non-ideal conditions. This new design approach was validated experimentally using a model mixture of three compounds of similar molecular structure (ethyl paraben, propyl paraben, and butyl paraben). Three highly pure products were obtained at nearly 100% yield, and good agreement was found with the simulations.

References

1. E. Hopmann, M. Minceva, J. Chromatogr. A 1229 (2012) 140.
2. P. Hewitson, S. Ignatova, H. Ye, L. Chen, I. Sutherland, J. Chromatogr. A 1216 (2009) 4187.
3. N. Mekaoui, J. Chamieh, V. Dugas, C. Demesmay, A. Berthod, J. Chromatogr. A 1232 (2012) 134.
4. A. Kostanian, J. Chromatogr. A 973 (2002) 39.

THE ROLE OF COUNTER-CURRENT CHROMATOGRAPHY IN THE MODERNISATION OF TRADITIONAL CHINESE MEDICINES: 8 YEARS ON

2023
So
Session III

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Keywords: Countercurrent Chromatography, CCC, Traditional Chinese Medicines, TCM, Chinese Herbal Medicines

In 2009, the authors published their first review on the role of counter-current chromatography in traditional Chinese medicines (1). This publication analyzed 198 papers covering 108 different plant species from 56 plant families, involved collaboratively 109 different organizations, and described the isolation of 354 different molecules across a wide range of polarities. Details of the range of compounds isolated, their numerous medical indications, the different organizations involved, the range and frequency of the journals they publish in, the different phase systems employed, and the CCC centrifuges used were presented at CCC2008 in Brazil. The inspiration for this review came from Roger Giese of the Journal of Chromatography following the signing of the Beijing Declaration on November 29th, 2007 which stated:

"The combination of traditional Chinese medicine and other schools of medicine may lead to a novel healthcare model for humans, and will effectively lower healthcare costs for both individuals and institutions. Innovation and diffusion of traditional Chinese medicine needs the support of modern science. Newly emerged disciplines, such as genomics, and the steady growth of basic knowledge, in particular, bioinformatics, has provided both the means and way forward for interpreting the basic principles of traditional Chinese medicine, and is leading to associated innovation. It is necessary to promote innovations of traditional Chinese medicine through enhanced international cooperation, in an attempt to further enrich its theoretical knowledgebase, improve people's understanding of traditional Chinese medicine, raise the level of safety, effectiveness, and quality of traditional Chinese medicine, and accelerate the modernization and internationalization of the traditional Chinese medicine industry."

Now, 8 years on, we are in process of writing a follow-up review. Whereas in 2008, after a huge surge of publications between 2003-2005, the publication rate appeared to plateau at about 40-50 papers a year, we now find that 847 papers have been published in the last 8 years, which compares to 175 in the previous 8-year period, with the rate rising from 85 in 2008 to 120 in 2015. This growth in activity represents a maintained annual increase of 22% per annum which is quite amazing.

The presentation will highlight different approaches to the modernization of Chinese herbal medicines, highlight the valuable role CCC is playing and review the different technological approaches being adopted.

References

1 I. A. Sutherland and D. Fisher, Role of counter-current chromatography in the modernisation of Chinese herbal medicines, J. Chromatogr. A 1216 (2009) 740-753.

PURIFICATION OF MONOSACCHARIDES FROM CRUDE HYDROLYSED SUGAR BEET PULP FOR THE PRODUCTION OF SUSTAINABLE CHEMICAL FEEDSTOCKS

2024
SO
Session II

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Keywords: monosaccharaides, sugar beet pulp, biorefinery

Over 8 million tonnes of sugar beet are grown annually in the UK, and sugar beet pulp (SBP) is a significant waste product from processing. Currently, the SBP is pressed, dried, and pelleted in an energy intensive process before being sold as low value animal feed. SBP is a rich source of carbohydrates with great potential for further utilisation. It consists mainly of cellulose (a polymer of D-glucose) and pectin (a copolymer of various hexose and pentose sugars, primarily L-arabinose and D-galacturonic acid). The development of SBP as a sustainable feedstock for chemical and pharmaceutical intermediates is an increasingly important aspect when considering a whole crop biorefinery.

The present work demonstrates the single step separation of crude hydrolysed material following pretreatment. Pre-treatment of the SBP by steam explosion removes the cellulosic fraction while subsequent acid hydrolysis converts the remaining pectic sugars into component monosaccharides. CPC separation was performed on a Kromaton FCPC-A with a 200 mL column using an ethanol: aqueous ammonium sulphate (300 g/L) (0.8:1.8 v:v) phase system in the ascending mode. Impurities were removed in the separation as well as the isolation of three main product fractions: L-rhamnose, L-arabinose combined with D-galactose and a high purity D-galacturonic acid fraction. Throughput maximisation and linear scale up allowed a 10 mL injection on a 200 mL column to be scaled up to a 152 mL injection on a 1000 mL column in a 100-minute separation. The isolated arabinose and galacturonic acid fractions are useful precursors to a number of high value chemical intermediates via chemical and enzymatic synthesis routes.

Acknowledgments

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COUNTERCURRENT SEPARATION OF NATURAL PRODUCTS: VERBENONE FROM ROSEMARY ESSENTIAL OIL

2025
FP
Session VI

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Keywords: Rosemary essential oil, Rosmarinus officinalis*, verbenone*

Countercurrent chromatography has become increasingly popular in the separation of natural products [1] due to its low solute decomposition, no irreversible solute adsorption (total recovery of injected sample) and high loading capability. Besides this, its relatively simple retention mechanism (liquid-liquid partitioning) allows working with many sample types. All this turns CCC into a robust technique for the preparative isolation of target compounds from plant extracts. Nevertheless, there are relatively few publications describing its use for the isolation of compounds of interest from essential oils.

Verbenone is an unsaturated bicyclic terpene ketone that possesses an odor reminiscent of camphor, menthol and celery, and has been actively pursued as a potential candidate for the protection of individual trees and forest stands because it decreases the response to pheromone in many bark beetle species. It can be naturally found in rosemary (*Rosmarinus officinalis* L.), Spanish verbena (*Verbena triphylla*), Spanish *Eucalyptus globulus* essential oils and other plants [2].

The aim of this work was to use countercurrent chromatography for the isolation of verbenone from rosemary essential oil. The essential oil was obtained from fresh leaves of rosemary, upon hydrodistillation in a Clevenger-type apparatus. Analyzes of the essential oil were performed by GC-MS and GC-FID systems. The identification of the components of the essential oil was carried out by comparison of mass spectral data with NIST 14 and Willey 275 libraries and also, by the calculated and experimental linear retention indices comparison. The major compounds identified were camphor (28.4 %), 1,8-cineole (15.0 %) and α-pinene (12.5 %). Verbenone (5.5 %) was a minor compound.

To make possible the separation of verbenone, it is important to choose an adequate solvent system. Due to the chemical composition of the essential oil, rich in hydrocarbon and oxygenated terpenes, five different solvent systems with low to medium polarities were chosen for test tube partitioning test selection: **A.** hexane-ACN 1:1, **B**. hexane-ACN-MeOH 1:1:0.1, **C**. hexane-ACN-EtOAc 1:1:0.1, **D**.hexane-EtOH-H_2O 4:3:1 and **E**. hexane-EtOH-H_2O 4:2:2 . Solvent systems **B**, **C** and **D** gave very similar results and hexane:EtOH:H_2O 4:3:1 was chosen for the purification of 113.7 mg of rosemary essential oil, as it doesn't contain ACN and so is considered more eco-friendly and less toxic solvent system. The purification was done using the 26 mL coil of an HTPrep apparatus, with a flow rate of 1 mL/min (upper phase as mobile), rotation speed of 860 rpm. The retention of stationary phase was 71 %. A total of 25 fractions (2 mL) were collected and verbenone was isolated from fractions 10-13. These fractions were analyzed by GC-FID and GC-MS and verbenone was identified, with a relative area of 100 %. So, CCC showed to be a capable tool to isolate verbenone, one of the minor constituents of rosemary essential oil.

References

1. G.G., Leitão et al. 2012. Strategies of solvent system selection for the isolation of natural products by countercurrent chromatography. Chapter 21 *In*: Medicinal Plants: Diversity and Drugs.1srt ed. ENFIELD : SCIENCE PUBLISHERS, v.1, p. 641-668.
2. U. Ravid.2008. Enantiomeric distribution of odorous oxygenated monoterpenes in aromatic plants, in: R. IKAN, Selected topics in the chemistry of natural products, World Scientific Publishing Co., Singapore, pp. 166.

ALTERNATING ISOCRATIC AND GRADIENT ELUTION CCC FOR THE ISOLATION OF MINOR PHENOLICS FROM *ORMOCARPUM KIRKII* BARKS

2026
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Session IV

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Keywords: Ormocarpum kirkii*, biflavonoids, biscoumarins, gradient elution CCC*

Ormocarpum kirkii S. Moore (Fabaceae), an African medicinal plant, is a shrub or small tree that can grow up to 9 m. It is mainly distributed in Eastern and Southern areas of Africa. In Western and Central Africa, this plant is found in the North of Cameroon, Niger, and Nigeria where it is known as "Tsa" in Haussa [1]. It is a traditional medicine for the treatment of oedema, rheumatism, stomach pains, epilepsy, hernia, diarrhea, headache, fever and malaria [2]. Previous phytochemical investigations revealed the presence of biflavanoids, flavonoids, isoflavonoids, and bisdihydrocoumarins [3, 4, 5]. (I-3,II-3)-Biflavonoids were amongst those compounds responsible for the antiplasmodial activity [4].

The ethanol extract of the bark of the plant was first fractionated with the generic solvent system hexane-ethanol-water 4:2:2 (reversed phase mode) affording 5 fractions (**A-E**). This first CCC procedure aimed the fractionation of the crude extract into fractions of low, medium and high poilarity, avoiding the tedius liquid-liquid partitioning on a separatory funnel. The major part of compounds was contained in the first polar fractions. Fraction **A** was further fractionated with the solvent system ethyl acetate-water 1:1, giving 4 sub-fractions (**A1**-**A4**) Two of them (**A1** and **A2**) contained the major compound ormocarpin, a glycosylated biflavoinoid previously isolated by Nyandat *et al*. [3] from the same plant by countercurrent distribution with the Craig and Posta apparatus [3]. **A3** contained ormocarpin and its diastereomer in a 1:1 ratio whereas **A4** contained two main non glycosylated biflavonoids and other minor compounds. Fraction **A1** was purified using EtOAc–n-BuOH–H_2O (9.5:0.5:10), using the upper phase as mobile phase, affording 7 sub-fractions (**A1a**-**A1g**). In this procedure ormocarpin was obtained directly as fraction **A1b.** Fraction **A1g** (410 mg) was further purified using a preparative gradient elution consisting of EtOAc–n-BuOH–H_2O (X:Y:10), (X:Y = 9:1 (I); 8:2 (II); 7:3 (III); 6:4 (IV); 5:5 (V); 4:6 (VI) 3:7 (VII) in seven steps. Two new flavonoids, isovitexin-4'-O-glucopyranosyl-(1-5)-apiofuranoside and apigenin-6-C-apiofuranosyl-(1-2)-glucopyranoside were isolated as minor compounds, together with an unknown one, which structure is under investigation. The less polar Fraction **A4** was also purified using gradient elution mode but with the HEMWat solvent system 1:X:1:1, X=1,2,2.5,3, affording three biscoumarins and three bisflavonoids (two aglycones and one monoglucoside). A total of 15 componds were isolated from the bark extract of *O. kirkii* by alternating isocratic and gradient elution CCC, where several families of solvent systems were used according to the polarity of compounds being purified in each fraction**.**

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References

1. DH Fulton *et al.,* 1976. East African Literature Bureau, Kampala, Nairobi, Dar es Salaam
2. S. M. Maregesi *et al.,* 2007 Ethnopharmacological survey of the Bunda district, Tanzania: Plants used to treat infectious diseases. *J. Ethnopharmacol*. 113, 457–470.
3. E. Nyandat *et al*., 1990. The 7,7″- β-diglucoside of (2S,3R)-chamaejasmin from *Ormocarpum kirkii*. *Phytochemistry* 29, 2361-2364.
4. L. Dooghe *et al*., 2010. Antiplasmodial activity of (I-3,II-3)-biflavonoids and other constituents from Ormocarpum kirkii.*Phytochemistry* 71, 785–791.
5. Y.J. Xu *et al*., 2012. Rapid isolation and identification of minor natural products by LC–MS, LC–SPE–NMR and ECD: Isoflavanones, biflavanones and bisdihydrocoumarins from *Ormocarpum kirkii*.*Phytochemistry* 79, 121–128

NOVEL APPROACH TO SAMPLE INJECTION IN COUNTER-CURRENT CHROMATOGRAPHY: CASE STUDY OF HONOKIOL PURIFICATION

2027
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Session IX

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Keywords: sample injection, honokiol, magnolol, sample loading, throughput

Counter-current chromatography (CCC) has been widely used as a preparative separation method to purify natural products from plant extracts and fermentation broths. Traditionally, throughput optimization in CCC has focused on sample concentration and sample volume. In this study, for the first time, the sample injection was considered as consisting of three variables: injection flow rate, post-injection flow rate, and sample solvent. The effects of these parameters were studied using a honokiol purification from a *Magnolia officinalis* bark extract as a case study aiming to achieve the highest throughput/yield ratio for greater than 99% purity of this potential anti-cancer drug obtained for submission to the Chinese FDA.

It has been demonstrated before [1] that CCC can purify honokiol with a hexane-ethyl acetate-methanol-water (5:2:5:2) two-phase system at preparative and pilot scales. However, in this study, to reduce the toxicity of residual solvents, methanol was replaced with ethanol, which consequently changed the physico-chemical properties of both liquid phases including the polarity difference between them. Therefore, in the present work, a complete stationary phase retention study and the effect of different CCC operational parameters on throughput, purity, and yield was systematically studied. Various sample injection scenarios were investigated, aiming for increased sample loading on a 1 L lab scale CCC instrument.

An injection method was established that increased the throughput of honokiol by 43.7% (from 3.11 g/h to 4.47 g/h), and decreased the solvent consumption of mobile phase and stationary phase per gram of honokiol by 43.5% (from 0.63 L/g to 0.37 L/g) and 48.0% (from 0.57 L/g to 0.30 L/g) respectively. These results show the importance of understanding the whole injection process when optimizing a given CCC separation.

Acknowledgments

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References

1. L. Chen, Q. Zhang, G. Yang, I. Garrard, S. Ignatova, D. Fisher, I. A. Sutherland., Rapid purification and scale-up of honokiol and magnolol using high-capacity high-speed counter-current chromatography, J. Chromatogr. A 1142 (2007) 115.
2. A. Peng, P. Hewitson, H. Ye, L. Zu, I. Garrard, I. Sutherland, L. Chen, S. Ignatova, Novel approach to sample injection in counter-current chromatography: case study of Honokiol purification. J. Chromatogr. A, submitted (2016)

THE EFFECT OF COLUMN ASPECT RATIO ON SEPARATION IN COUNTER-CURRENT CHROMATOGRAPHY

2028
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Session XI

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Keywords: column, aspect ratio, resolution, stationary phase retention

Traditionally, Counter-counter Chromatography (CCC) columns are made from round shape tubing simply because it is readily available on a market at a reasonable cost. One of the first attempts to look into different column shape was done by Degenhardt et al. (1), who demonstrated that use of rectangular tubing, with an aspect ratio of 1.33 (2x1.5 mm I.D. tubing) led to a slight improvement in the resolution, though with reduced stationary phase retention.

In the present study, the retention and resolution of a model mix in higher aspect ratio rectangular columns, with an aspect ratio of 3.1 & 0.32 (2.4 x 0.8 mm I.D. tubing) have been evaluated against that of standard round column with an identical cross-sectional area. These columns have improved packing profiles, allowing larger columns on the same sized instrument.

CCC experiments were done in a two phase system with heptane, ethyl acetate, methanol, and water, separating three pharmaceutical molecules (caffeine, aspirin, and naproxen) at the concentration of 2 mg/mL each.

Stationary phase retention in these rectangular columns with a volume about 20 ml each is shown to be highly dependent on the orientation of the rectangular tubing. When aligned horizontally to the *g*-field, the column provides a higher resolution over standard round tubing (Rs 1.86 cf. 1.46, respectively) despite a slightly reduced retention (80% cf. 85%, respectively). Results will be discussed at a range of flow rates from 1 to 4 mL/min.

Methodology for column production of these rectangular tubing bobbins will be shown. The further analysis of the flow in these different shaped columns using computational fluid dynamics (CFD) is ongoing and initial results will also be discussed.

References

1. Degenhardt, A., Schwarz, M., Winterhalter, P., & Ito, Y. (2001). Evaluation of different tubing geometries for high-speed counter-current chromatography. *Journal of Chromatography A*, *922*, 355-358.

PROCESS INTENSIFICATION AND SCALE-UP IN PH-ZONE REFINING CENTRIFUGAL PARTITION CHROMATOGRAPHY: STUDY CASE OF THE PURIFICATION OF ALKALOIDS FROM *CATHARANTHUS ROSEUS*

2029
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Session VII

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Keywords: alkaloids, modelling, process intensification, scale up.

Centrifugal Partition Chromatography (CPC) is a support free chromatographic technique involving the distribution and transfer of solutes between at least two immiscible liquid phases according to their partition coefficient. It avoids irreversible adsorption of the analytes due to the absence of solid support, allows a total recovery of injected samples, and enables separations of a variety of structures within a large polarity range. The introduction of the so-called displacement mode dedicated to the purification of ionizable compounds, as well as the development of modeling approaches[1] taking into account of both hydrodynamic and mass transfer aspects have attracted increasing interest to the area. Today, the technology starts to affirm its benefits as a useful addition to the palette of techniques available for preparative chromatography.

This communication describes recent work investigating the use of CPC whilst performing the pH-zone refining mode for the purification of indolomonoterpenic alkaloids. In particular, the purification of both vindoline and catharanthine from an industrial extract of *Catharanthus roseus* will be presented. These two alkaloids are used as starting material in the semi-synthesis of a two anti-cancer drugs: Navelbine® and Javlor®. First, the different experimental parameters were optimized on a 25 mL CPC column using a Design of Experiment approach to reach the best compromise between purity, recovery, and productivity. Then, the scale up of the separation on various pilot-scale columns with different column design was achieved thanks to a recently developed model allowing the prediction of chromatograms. Productivities up to 2.5 kg/day/L of column were obtained, highlighting the potential of CPC for the industrial purification of high added value compounds.

References

1. Kotland A, Chollet S, Diard C, Autret JM, Marchal L, Renault JH Modeling pH-zone refining countercurrent chromatography: a dynamic approach, J. Chromatogr. A, 2015, 1391:80-87.

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SPIRAL COUNTERCURRENT CHROMATOGRAPHY IN ITS MANY FORMS

2030
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Session X

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Keywords: spiral tubing support, mixer-settler, natural products, carbon nanotubes

We have continued along in developing the spiral design CCC columns originally invented by Yoichiro Ito in 2000 [1]. Our first version of the spiral tubing support (STS) column or rotor made by laser lithography in 2007 has been further modified to increase the volume. The number of spiral loops in the tubing layers have been increased to improve the efficiency of separation. We made a comparison of the first STS-4 rotor with the STS-12 rotor that has 12 radial channels, forming the tubing in 12 spirals per layer, fo the separation of flavonoids from a butanol extract of *Sutherlandia frutescens,* a South African plant. This is related to our previous work with other natural products isolated from this extract [2]. Next, the strategy of designing a STS rotor for analytical application will be described as well as some results of separating proteins. This rotor fits into the PC Inc. type planet centrifuge. Most of the rotors in the table are built for this size planet centrifuge. Presently, we are building a larger scale chromatograph to use the last two rotors in the list. The prep-scale STS-4 will have a volume of over 400 ml.

Table 1. Spiral CCC Rotors Fabricated

Type	OD (cm)	Description
Spiral Tubing Support (STS-4) v 2	17.5	Radial channels to make 4 spirals/level, filled with 1.6 mm ID tubing
Spiral Tubing Support (STS-4) v 2	12	PharmaTech Res. inst. 1.6 mm ID tubing
Spiral Tubing Support (STS-12)	17.5	12 spirals/level 1.6 mm ID tubing
Analytical STS (STS-12)	17.5	12 spirals/level 0.95 mm ID tubing
Mixer-Settler Spiral Disk	17.5	5 X 4-spiral disks (glass beads sections)
Mixer-Settler Spiral Disk v 2	17.5	6 X 4-spiral disks (glass beads sections)
Prep-scale STS-4	22.5	Filled with 2.6 mm ID tubing
Prep-scale Mixer-Settler	19.3	8 X 4-spiral disks (glass beads sections)

There has been high interest in applying spiral CCC to separate single wall carbon nanotubes, which are potent semiconductors. Previously, in studying the rotors available, the best results were obtained with the mixer-settler spiral disk [3] over the STS-4. Presently, we are investigating newer materials, 3D printing related methods, and the metal hardware design to improve the mixer-settler rotors. The fabrication issues to optimize the rotors and instrument for separation of single wall carbon nanotubes are challenging. We are separating carbon nanotubes from various sources and will present results using ATPSs. The spiral design rotors are successful because they are proven useful for separating the large molecules, such as proteins and now a new class of large molecules, carbon nanotubes. These purification methods will prove useful for complex molecules to be developed for therapeutics and diagnostics.

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References

1. Exclusive License from NIH # 144-2007 to CC Biotech LLC
2. Brownstein et al. *J. Liq. Chrom. and Rel. Technol*. (2015) 38: 423-429, doi:10.1080/10826076.2014.913518
3. M. Zheng et al. Anal. Chem. (2014) 86: 3980-3986, doi.org:/10.1021//ac50031891

ON-DEMAND PURIFICATION OF NATURAL PRODUCTS BY COUNTER-CURRENT CHROMATOGRAPHY

2031
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Session III

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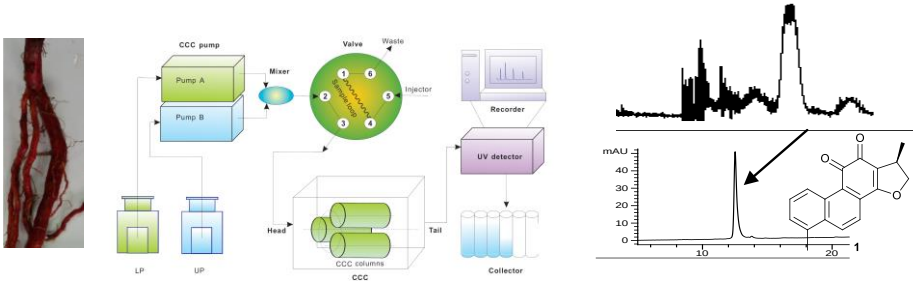
Keywords: On-demand production; Natural products isolation.

Natural products have been recognized as important resources of drug discovery and pharmaceutical agents for their diversity in structure and bioactivities. However, natural products resources such as plant and microorganism extracts are very complex and usually contain large numbers of hydrophobic and hydrophilic components. Despite of substantial developments of extraction and separation techniques, it is still a challenging task to get multiple pure targets from complex biological materials including plants, marine organisms, or microorganisms. CCC is a unique liquid-liquid partition chromatography without support matrix, which relies on the continuous partition of one sample between two immiscible solvents to achieve separation. Due to lack of solid support matrix, CCC eliminates several complications resulting from the solid support matrix, such as irreversible adsorption and denaturation of samples. Thus CCC has gained in popularity in natural products separation. Generally speaking, CCC is a simple and easy to learn process. A common CCC separation process involves several steps such as selection of solvent systems, instrumentation and separation theory mode. The selection of appropriate solvent system for target compound(s) is the first and the most important step in CCC separation which may be estimated as 90% of the entire work. Once the solvent system is selected, the whole CCC process may be pre-estimated [1,2] and can be run on schedule. Therefore, current efforts largely focus on the developed smart CCC. There are numbers of CCC devices and methods developed for the isolation of natural products [3-5]. This presentation will cover the current advancements on the CCC purification on-demand of natural products.



Smart CCC extraction and separation of targeted components

References

1. A. Berthod, J.B. Friesen, T. Inui, G.F. Pauli, Elution-extrusion countercurrent chromatography: Theory and concepts in metabolic analysis. Analytical Chemistry 79 (2007) 3371-3382.
2. S. Wu, D. Wu, J. Liang, A. Berthod, Modeling gradient elution in countercurrent chromatography: Efficient separation of tanshinones from *Salvia miltiorrhiza* Bunge. Journal of Separation Science 35 (2012) 964-976.
3. J. Liang, J. Meng, D. Wu, M. Guo, S. Wu, A novel 9 x 9 map-based solvent selection strategy for targeted counter-current chromatography isolation of natural products. Journal of Chromatography A 1400 (2015) 27-39.
4. J. Meng, Z. Yang, J. Liang, H. Zhou, S. Wu, Comprehensive multi-channel multi-dimensional counter-current chromatography for separation of tanshinones from *Salvia miltiorrhiza* Bunge. Journal of Chromatography A 1323 (2014) 73-81.
5. J. Meng, Z. Yang, J. Liang, M. Guo, S. Wu, Multi-channel recycling counter-current chromatography for natural product isolation: Tanshinones as examples. Journal of Chromatography A 1327 (2014) 27-38.

SEPARATION AND RESEARCH OF ANTI-TUMOR ACTIVE COMPONENTS IN *ZANTHOXYLUM AILANTHOIDES* SIEB. & ZUCC.

2032
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Session III

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Keywords: Zanthoxylum ailanthoides*, anti-tumor activity, G-quadruplex ligands, isoquinoline alkaloids, HSCC*

Zanthoxylum ailanthoides Sieb.& Zucc. is a traditional Chinese medicine, authentic as “Hai-Tong-Pi” in the Beijing Area. *Z. ailanthoides* distributed in Taiwan has been well investigated. However, the chemical constituents and modern pharmacological effects of Z. ailanthoides originating from mainland China are still lacking research. This *study* investigated the anti-tumor activities on several human cancer cells in vitro and the stabilization on G-quadruplex of the crude extract of Z. ailanthoides. Moreover, the silica gel column chromatography, counter-current chromatography (CCC), pH-zone refinement CCC, and preparative HPLC were employed to separate active compounds from the petroleum ether and *n*-butanol extracts. Eleven pure compounds were isolated and identified as four alkaloids, four lignans, two coumarins, and one phenylpropanoid glycoside respectively by ESI-MS, ^{1}H and ^{13}C-NMR. One is new compound and four are firstly isolated from this plant. Three isoquinoline alkaloids were found displaying remarkable stabilization effect on G-quadruplex, thus being the most promissing antitumor constituents in *Z. ailanthoides*.

1. MTT assay combined with G-quadruplex stability test temperature-dependent circular dichroism (CD) were employed for the investigation of anti-tumor activities of the crude extract and its sub-fractions. The results indicated that the 75% ethanol extract, its ethyl acetate, *n*-butanol, and water fractions displayed good inhibitory activity on hepatocellular carcinoma (Bel-7402), colon cancer (HCT-8), and lung cancer cells (A-549), but not so the petroleum ether extract. The 75% ethanol extract, and its petroleum ether, ethyl acetate, and *n*-butanol fractions displayed almost the same stabilization effects on G-quadruplex by increasing G-quadruplex's Tm by approximately 5 ºC.

2. Silica gel column chromatography combined with counter-current chromatography (CCC) was used for the separation of minor constituents from petroleum ether fraction. Two coumarins and two lignans including luvangetin (1), hinokinin (2), xanthyletin (3), and asarinin (4) were isolated and identified by MS, ^{1}H and ^{13}C NMR. Among them, asarinin has not previously been isolated from this species to our knowledge.

3. pH-zone refinement CCC *was* employed for the separation of less polar alokaloids, and CCC combined with preparative HPLC were used for the separation of polar alkaloids and related constituents from the butanol fraction. Four isoquinoline alkaloids including tetrahydro-thalifendine (5), berbine,10,11-dimethoxy-2,3-(methylene dioxy)-14α-hyroxyl radical-7-methyl(6), *N*-methylhydroxysinactine(7), and magnoflorine (9), the lignans (+)-lyoniresiol-3α-O-β-D-glucopyranoside(10) and (-)-lyoniresinol-3α-*O*-β-D-glucopyranoside, and the phenylpropanoid glycoside, Syringin (8), were isolated and identified. Among them, compound 6 is a new compounds, while 5, 7, and 8 were not isolated previously from *Z. ailanthoides*.

4. The CD test indicated that the eleven isolates presented different stabilization effects on G-quadruplex. Among them, three isoquinoline alkaloids displayed remarkable stabilization effect on G-quadruplex by increases G-quadruplex's Tm by approximately 10 ºC, making them the most potent antitumor constituents identified from *Z. ailanthoides*.

References

[1] Su-Tze Chou, Hsiu-Hui Chan, Hsin-Yi Peng, Meei-Jen Liou. Isolation of substances with antiproliferative and apoptosis-inducing activities against leukemia cells from the leaves of Zanthoxylum ailanthoides Sieb. & Zucc [J]. Phytomedicine, 2011, 18: 344–348.
[2] Zhou Q J, Li L, Xiang J F, et al. Fast screening and structural elucidation of G-quadruplex ligands from a mixture \via G-quadruplex recognition and NMR methods [J]. Biochimie, 2009, 91 (2): 304-308.

PILOT SCALE PURIFICATION OF XANTHOPHYLLS FOR D-FACTORY USING CCC

2033
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Session VII

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Keywords: pilot scale, automation, method development, biorefinery

Industrial scale application of countercurrent chromatography has progressed in the past decade. The potential advantages for CCC at pilot and production scale are significant, especially when considering non-classical chromatography operational modes such as pH-zone-refining, displacement, and ion-exchange. Sutherland, et al. reported the results of a three year joint program to evaluate the application of hydrodynamic CCC to real world pharmaceutical industry purification problems (1). Large scale, kilogram and beyond, applications of CCC remain few however (2,3). Pilot and production scale application of CCC requires robust instrumentation designed for automated continuous operation with minimal downtime. In addition, method development must be sufficiently robust to ensure repeatability and reproducibility.

Countercurrent chromatography is a key component of the EU FP7 KBBE (D-Factory) algal biorefinery project (4). The objective of D-Factory is to demonstrate a commercial scale biorefinery for sustainable *Dunaliella* biomass processing. CCC is one of three separation/extraction techniques to be utilized and will be integrated with scCO2 extraction and membrane methods to produce various high value compound products. Potential high value products obtainable from *Dunaliella* extracts include carotenes and xanthophylls. *Dunaliella* extracts are an excellent source of beta-carotene. Lutein and zeaxanthin are the dominant xanthophylls produced.

Xanthophyll purification using CCC has been reported by several labs, most using HEMWat or modified HEMWat solvent systems. These reports targeted small amounts of pure compounds. We have examined the potential throughput of CCC methods for large scale production of xanthophylls. Scaleup from test tube distribution studies, analytical scale CCC, and finally to pilot scale CCC will be presented. Throughput and factors influencing reproducible and automated pilot and production scale CCC will be discussed.

References

1. I. Sutherland, C. Thickitt, N. Douiellet, K. Freebairn, D. Johns, C. Mountain, P. Wood, N. Edwards, D. Rooke, G. Harris, D. Keay, B. Mathews, R. Brown, I. Garrard, P. Hewitson, S. Ignatova, *J. Chromatog. A*, 1282 (2013), 84.
2. C. DeAmicis, N. A. Edwards, M.B.Giles, G.H.Harris, P. Hewitson, L.Janaway, S. Ignatova, *J. Chromatog. A*, 1218 (2011) 6122.
3. A.Toribo, J.M.Nuzillard, L.Boudesocque, M.Lafosse, F. De La Poype, J.H.Renault, *J. Sep. Sci.* 32 (2009) 1801.
4. http://www.d-factoryalgae.eu/2-eng-the-project.html

TWO-PHASE MOTION IN HYDRODYNAMIC COUNTER-CURRENT CHROMATOGRAPHY: WHICH PHASE TRAVELS TOWARD THE HEAD OF THE COIL IN HYDRODYNAMIC COUNTER-CURRENT CHROMATOGRAPHY?

2034
FO
Session XII

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Keywords: *Two-phase motion; Archimedean screw; type-I planetary motion; type-J planetary motion; ß value.*

Motion of the two mutually immiscible phases in hydrodynamic countercurrent chromatographic systems is explained based on the observation of two-phase behavior enclosed in a rotating coiled tube in unit gravity (1, 2). The experiment revealed four stages. In the low rotation speed below 10 rpm (stage I), the two phases evenly occupy the head end. With an increased speed at 60-100 rpm, the lower phase completely occupies the head end (stage II). With further increase of the rotation speed, however, more upper phase occupies the head end (stage III). At high rotation speeds of over 200 rpm, motion of the two phases by the Archimedean screw effect will cease and two phases are distributed in such a way that the lower phase occupies the outer portion and the upper phase the inner portion throughout the entire length of the coil (stage IV). These two-phase behaviors are comprehensively explained on the bases of interplay between the unit gravity and centrifugal force generated by rotation of the coil. This theory is successfully extended to explain the two-phase behavior in a coil undergoing the type-I and type-J planetary motions. In the type-I coil planet centrifuge which produces the centrifugal force distribution similar to the rotating coil in unit gravity at a low speed (stage I), both upper and lower phases competitively move toward the head of the coil regardless of the revolution speed or location of the coil on the holder. In contrast, in the type-J coil planet centrifuge which displays various patterns of centrifugal force distribution according to the location of the coil, the two phase motion varies dependent on the ß values. At ß = 0.25. which generates centrifugal force distribution similar to that of the coil rotation at a moderate speed of 60-100 rpm (stage II), the lower phase moves toward the head of the coil leaving the upper phase behind. At ß = 0.5 -0.75 which produces centrifugal force distribution similar to rotating coil in the unit gravity at 125 rpm (stage III), the upper phase moves toward the head of the coil (stage III). Finally, at the higher ß values, a strong centrifugal force suppresses the Archimedean Screw effect, where two phases are distributed in such a way that the upper phase occupies the inner portion and the lower phase in the outer portion throughout the coil resulting in no mixing of the two phases (stage IV). This clearly demonstrates the importance of the proper choice of ß values in high-speed countercurrent chromatography utilizing the type-J planetary motion.

References

1. Y. Ito, Speculation on the mechanism of unilateral distribution of two immiscible solvent phases in the rotating coil, J. Liq. Chrom. 12 (1992) 2639-2675.
2. Y. Ito, Studies on hydrodynamic distribution of two immiscible solvent phases in rotating coils, J. Liq. Chromatogr. 11 (1988) 1-19.

SCHINUS TEREBINTHIFOLIUS SCALE-UP: CCC METHOD TRANSFER TO CPE AND CPC

2035
SO
Session VII

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Keywords: Schinus terebinthifolius*, 3β-masticadienonic acid, masticadienolic acid, method transference, scale-up*

All-liquid partition chromatography, both CCC and CPC, uses liquid stationary phases retained in the separation column without any solid support. When working with these techniques, a common approach to choose a suitable solvent system for the separation is based on literature search for systems that have been used in the purification of similar compounds [1]. It is not rare to find a single method/solvent system for CPC while working with CCC, or vice versa. The aim of this study was to demonstrate how a method developed on an analytical scale CCC can be transferred to CPE and CPC instruments applying scale-up theory as the first step.

An optimized method for the isolation of tirucallane triterpenes, 3β-masticadienonic, and masticadienolic acids, from the CH_2Cl_2 extract of *Schinus terebinthifolius* berries, previously developed on an analytical scale Mini-DE (17 mL Vc) [2], was transferred to two models of centrifugal partition chromatography devices, CPE FCPE300 (303 mL Vc) and CPC ASCPC250 (250 mL Vc), using the volumetric scale-up approach, where parameters are increased proportionally to the ratio of volume. HEMWat 1:6:1:6 was used as solvent system.

CPE machines are designed robust for industrial applications and for high productivity. Nevertheless, some adjustments to the operating parameters, obtained directly from analytical CCC, were required. The flow-rate was increased to reduce diffusion effects and enhance partition kinetics [3]. Loading studies were done in a range of sample mass until loss of resolution and flooding occurred. As final conditions, for the CPE experiment the flow-rate was three times faster (9 to 27 mL/min) to obtain a good resolution; whereas sample concentration could be doubled (100 to 200 mg/mL) with an increase in number of collected fractions during the elution step (1 to 2 Vc).

The same optimization procedure of operating parameters was repeated for the CPC device. Experiments were done in order to concurrently enhance throughput and improve separation by increasing flow-rate and sample loading. A better separation was obtained when increasing flow-rate three times (7 to 21 mL/min) but sample loading could not be increased without losing resolution.

Based on the fact that a better resolution is frequently observed when using larger columns [4], a method developed on an analytical scale CCC can be beneficial prior its transfer to CPE and/or CPC columns with some additional improvements, what means that analytical CCC can be used as a *scouting* technique to find a good separation system and then go directly to CPC with slight parameter modifications. This study continues with method transfer from CCC to CPE and CPC with equivalent volume columns.

Acknowledgments

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References

1. Journal Chromatogr A 1065 (2005) 145
2. Journal Chromatogr A 1389 (2015) 39
3. Bioresource Technol 132 (2013) 406
4. Journal Chromatogr A 1409 (2015) 70

PREPARATIVE SEPARATION OF MARINE BIOACTIVE COMPOUNDS BY CENTRIFUGAL PARTITION CHROMATOGRAPHY

2036
SO
Session II

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Keywords: Soliera chordalis*, bioactive molecules, centrifugal partition chromatography, fractionation*

In response to stresses like herbivory, epiphytism, fouling, or predator-prey, seaweeds are known to possess different chemical defense mechanisms by secondary metabolites production. The aim of this study is to present the capability of Centrifugal Partition Chromatography (CPC) for the fractionation of bioactive molecules isolated from the red seaweed, *Solieria chordalis* (Gigartinales, Solieriaceae). CPC is known for the low solvent consumption compared to traditional chromatographic methods.

CPC was first applied for the separation of crude samples prepared in various solvents. The determination of the mobile phase was based on the partition of metabolites between two polar and apolar solvent systems which belong to the Arizona solvent system (1). Anti-UVB capacity and anti-aging activity were studied and used as a bioassay-guided fractionation approach. The crude extracts were prepared with yields ranging from 1.0% (ethyl acetate) to 19.6 % (methanol). Thin layer chromatography analysis led to the determination of the Arizona system composition (heptane, ethyl Acetate, methanol and water). The mode of elution (ascendant or descendant) and the mobile phase composition influenced the nature and the number of collected fractions. So far, this technique has allowed separating concentrated fractions characterized by their UV-spectrum and LC-MS profile. Among the different compounds, the UV-absorbing Mycosporine-like Amino Acid (MAA) palythenic acid was detected (2). The preliminary anti-UVB test has shown a positive photoprotective activity by an important increase of the half-life $t_{1/2}$ of chlorophyll. Moreover, additional anti-elastase activity was shown for different fractions (3).

In conclusion, preliminary results highlighted the hypothesis that the anti-UVB activity of the extract is correlated to the presence of the MAAs. Complementary tests are in progress in order to evaluate the activities of purified extracts obtained by the CPC method with the aim to characterize the bio compounds fraction by biochemical composition and by their bioactivities level. Based on these results, new solvent systems are studied in order to develop an eco-friendly preparative separation of marine bioactive compounds.

Acknowledgements: Financial support: The Region Bretagne and The Department of Morbihan.

References

1. A. Berthod, M. Hassoun, Using the Liquid Nature of the Stationary Phase: The Elution-Extrusion Method, J. Liq. Chromatogr. Relat. Technol., vol. 28, no. 12–13, pp. 1851–1866, 2005.
2. G. Bedoux, K. Hardouin, Chemical characterization and photoprotective activity measurement of extracts from the red macroalga Solieria chordalis, Bot. Mar., vol. 57, no. 4, pp. 291–301, 2014.
3. G. Bedoux, K. Hardouin, Bioactive components from seaweeds: Cosmetic applications and future development, vol. 71. Elsevier, 2014.

Connect fundamentals to applications for Counter-Current chromatographY: new rules, third force, virtual column and scaling-up for protein separation

2037
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Session X

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Keywords: Type-J counter-current chromatography; Flow mode of counter-current chromatography; Multiplayer coil planet centrifuge; 2D spiral column; Stationary phase retention.

Introduction: Compared to column chromatography (e.g. HPLC), advantages for counter-current chromatography (CCC) lie mostly in its preparative-scale potential. Great efforts have been made in scaling up the equipment for increasing small molecule processing capacity[1], but challenges remain for large biomolecules (e.g. proteins and their complexes)[2]. Based on more recent results, we will illustrate new modes for CCC operation using aqueous two-phase systems (ATPS), demonstrate how we now understand the presence of a third force for multi-layer columns, how much info can be conveyed using a virtual CCC platform, and how columns for protein separation can be designed rationally.

New rules: While CCC rules-of-thumb work well for aqueous-organic two phase systems, they fail to apply to ATPS. E.g., for 2D-spiral columns (Fig.1A), it has been suggested to (a) pump the lower mobile phase from "head" (centre) to "tail" (periphery), or (b) pump the upper mobile phase from "tail" (periphery) to "head" (centre)[3]. However, experiments show that it is better to pump the lower mobile phase from centre to periphery (regardless of "tail" or "head" location), or pump the upper mobile phase from "head" (periphery) to "tail" (centre) [do not pump the upper mobile phase from "tail" (periphery) to "head" (centre)].

The third force: For nearly three decades, the most widely used multi-layer columns (Fig.1C and 1D) have been understood using a "ring" model as if such columns are 2D systems only (Fig.1B)[4]. However, recent research showed the existence of a third force that acts almost in the axial direction of the winding bobbin. This force alternates between two orientations and is largely beneficial to phase mixing[5].

The virtual CCC: In order to enhance our understanding on the CCC working principles, we have developed different physical models for CCC columns on J-type CCC. This approach has now turned into building virtual CCC columns where thorough assessments can be made without even having to construct the real CCC columns in conjunction with a CCC machinery. Examples on studied column geometries (Fig.1) will be discussed.

Scale-up of column bore size for large biomolecules and nanoparticles: Biopharmaceutical industry has kept growing at an annual rate of ca.15% globally and there have been pressing needs for downstream processing protein-based products. Both CC Biotech and Tauto Biotech have developed 2D spiral columns for separating proteins. We have recently scaled up toroidal columns for J-type CCC from 1.6 to 5 mm bore size and are able to process model proteins at 20 ml/min mobile phase flow rate. Comparison between toroidal and 2D spiral columns[6] will be presented .

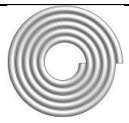				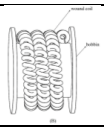	
A: 2D spiral coil on J-type CCC[4]	B: Simplify 3D cylindrical spiral coil into 2D ring on J-type CCC[5]	C: Single layer 3D cylindrical spiral coil on J-type CCC[6]	D: Multi-layer 3D cylindrical spiral coil on J-type CCC[6]	E: Toroidal coil on J-type CCC[1] (Zhou et al., presented in CCC2016)	F: Conical coil on J-type CCC (Ding et al., presented in CCC2016)

Fig.1: Column geometries investigated for protein separation and building of virtual columns.

References: 1. I. Sutherland et al., J. Chromatogr. A 1216 (2009) 4201. **2.** Y.H. Guan et al., J. Chromatogr. A 1424 (2015) 102. **3.** Y.H. Guan et al., J. Chromatogr. A 1239 (2012) 10. **4.** Y. Ito, J. Chromatogr. 188 (1980) 33-42. **5.** Y.H. Guan, R. van den Heuvel, J Chromatogr A 1218 (2011) 5108. **6.** Y.H. Guan et al., J Chromatogr A 1151 (2007) 115.

THE WORKING MECHANISM OF TOROIDAL COLUMNS ON J-TYPE COUNTER-CURRENT CHROMATOGRAPHS

2038
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Session X

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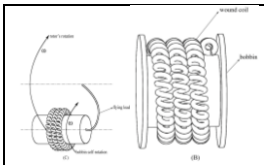
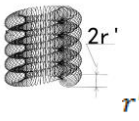
Keywords: Counter-current chromatography, toroidal column, mathematical model, protein separation and purification, optimization of toroidal column design

INTRODUCTION: To address the ever increasing challenges in biopharmaceutical industry, toroidal columns mounted on a J-type counter-current chromatograph (CCC) have applications potential[1,2]. Presently, the largest toroidal column bore size is 5mm and the largest processing capacity allows for 20 ml/min as mobile phase flow rate[1]. Compared to the most popular 3D cylindrical columns, how do we understand the reported encouraging protein separation outcomes and what can be done further to rationally design and improve this type of column?

PHYSICAL MODEL:

 **R:** rotor rotation radius **r:** bobbin self-rotation radius	 r': pre-tightly wound chain radius. An increase of 2π for φ value leads to one rotation yet moves the coil forward.	The toroidal coil augular location φ (an independent variable) is expressed by Cartesian coordinate system as, $x = R\cos\theta + \beta R\cos(2\theta + n\varphi) + r'\cos\varphi\cos(2\theta + n\varphi)$ $y = R\sin\theta + \beta R\sin(2\theta + n\varphi) + r'\cos\varphi\sin(2\theta + n\varphi)$ $z = \frac{r'n}{\pi}\varphi + r'\sin\varphi$ where *n* is the number of toroidal chains (with radius r') over a bobbin turn, and is determined by the r' value, and θ is the elapsed rotation angle of the rotor.
A: real appearance of the column in the CCC.	B: modelled appearance	C: Mathematic description for each location on a toroidal column on J-type CCC.

RESULTS & DISCUSSION: The J-type CCC planetary rotation creates a unique pattern of centrifugal force in terms of the rotary roration (quantified by the angle θ) and the column position (quantified by the locational angle φ). Two coordinate systems were used to study various physical interactions between the two liquid phases and the column internal wall surface. For the first coordinate system, we separated this CCC centrifugal forces into three perpendicular forces, namely the tangential (a_t), the normal (a_n), and the binormal force (a_b). For the second coordinate system, we separated the CCC centrifugal forces into two perpendicular forces: the tangential direction of the coil (a_t) and the plane orthogonal to the tangential direction. On the latter plane, the orthagonal force ($a_{\vec{N}+\vec{B}}$) is specified by its magnitude ($|a_{\vec{N}+\vec{B}}|$) and direction (by angle α).

Using the second coordinate system, it could now be observed that the combined normal force ($a_{\vec{N}+\vec{B}}$) rotates once for every 2π increment of the φ value for the toroidal column (i.e., for each pre-wound toroidal chain). In contrast, this pattern of the combined normal force ($a_{\vec{N}+\vec{B}}$) rotation takes place for each turn of column on the bobbin for the most popular 3D cylindrical spiral column[3,4]. In view of the fact that the toroidal column studied has ca. 20 toroidal chains for each turn on the bobbin, this means that for each bobbin turn of the toroidal column, the liquid inside has gone through ca. 20 times of pulsified mixing. With the column length being constant, it is observed that when the combined normal force ($a_{\vec{N}+\vec{B}}$) rotates once for the 3D cylindrical spiral column, this force has rotated ca. three times for the toroidal column. Such frequent change in direction of this normal force undermines establishment of the friction forces between the two liquid phases and also between the liquid phases and the column internal wall, and hence may well significantly reduces forces directly related to stationary phase retention. These results provide insights into both the mixing nature and the stationary phase retention mechanism, and formed a basis for the subsequent improvement of toroidal columns.

References: 1. Y.H. Guan et al., J. Chromatogr. A 1424 (2015) 102. **2.** Y.H. Guan et al., J. Chromatogr. A 1217 (2010) 3525. **3.** Y.H. Guan, R. van den Heuvel, J. Chromatogr. A 1218 (2011) 5108. **4.** Y. Ito, J. Chromatogr. 188 (1980) 33.

MELAMINE MODIFIED COUNTER-CURRENT CHROMATOGRAPHY COLUMN AND ITS SEPARATING MECHANISM

2039
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Session IX

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Keywords: Poly-dopamine, Auxiliary stationary phase, Adsorption, Melamine, Mechanism.

Counter-current chromatography (CCC), which is based on the liquid–liquid partition mechanism, is a unique separation and purification technique. It has become one of the most potential research fields of CCC for the separation and purification of natural products over the past two decades for its advantages such as no irreversible absorption and degeneration of the sample on the support. However, because of its relatively low theoretical plates and single separation mechanism, it is very difficult to obtain satisfying results of separating two analytes with similar structures by CCC even under the optimal chromatography conditions, which has largely hampered the further practical application of CCC in the separation field. To solve this problem, a novel solid-liquid two-stationary phases CCC (ASP-CCC) column was prepared employing graphene oxide (GO) conjugated poly-dopamine (PD) coating (GO/PD) as auxiliary stationary phase (ASP) in our preliminary work (1,2). The result show that the modified surfaces can improve resolution by introducing intermolecular forces and by enhancing the stationary phase retention ratio. Thus, the separation mode becomes a combination of partition chromatography and adsorption chromatography. Encouraged by the aforementioned work, here, we adopted melamine as the functionalization agent to in situ prepare a novel CCC with large amounts of amidogen. Steviol glycosides (mainly containing stevioside, rebaudioside A, and rebaudioside C) were selected as model molecules. In order to study the effect of melamine on the separation mechanism and chromatography behavior of the model molecules, dose-effect relationships and molecular simulation have be adopted as main means of study. The result suggests that the melamine-modified CCC column can effectively separate the model molecules with a satisfactory resolution. It will be a new way with great potential to improve the current instruments.

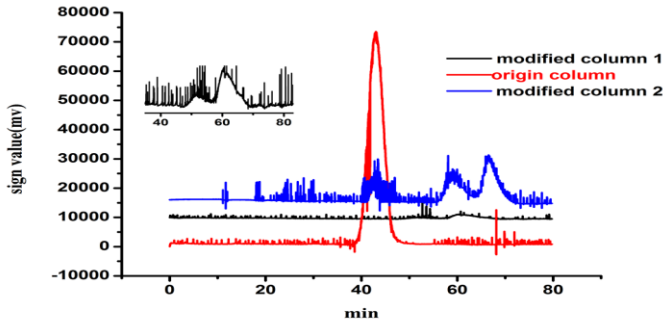

Figure 1. Comparison of the separation behavior of model analytes in TBE-300B between original and novel column

(Modified column 1 and 2 for one or two of three columns was modified with melamine, respectively)

References

1. K.J. Quan, X.Y. Huang, X.T. Li, G.H. Wang, D.L. Di, Surface modification of PTFE column for two-stationary phase separations by counter-current chromatography. J. Chromatogr. A 1422 (2015) 156-162.
2. W.P. Zhang, J. Zhang, T. Bao, W. Zhou, J. Meng, Z.L. Chen, Universal multilayer assemblies of grapheme in chemically resistant micro[tubes for micro-extraction, Anal. Chem. 85 (2013) 6846–6854.

HIGH-THROUGHPUT FUNCTIONAL ANNOTATION OF NATURAL PRODUCTS

3002
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Session VI

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Keywords: metabolomics, biological characterization, synergy, botanical

Determination of the mechanism of action to botanicals and natural products (mixtures and pure compounds) is often rate limiting for the safe and efficacious use as supplements and therapeutics. Botanicals and dietary supplements are particularly difficult, as these are often complex mixtures that can change in the constituents and concentration of individual compounds. The rapidly growing botanical dietary supplement industry faces a number of challenges regarding the quality, safety and benefit of these products. In order to address these issues, rigorous and sustained efforts are needed to chemically and biologically characterize these products. The Center for High Throughput Functional Annotation of Natural Products (HIFAN) is developing and utilizing complementary biological platforms to study the mechanism of action of botanicals and natural product libraries and integrate the use of metabolomics and other analytical methods to fully understand the contributions of individual compounds of complex mixtures to a given biological activity.

The two biological platforms that we have developed include: 1) Cytological profiling (CP) is a powerful method for quantifying and comparing the phenotypic effects of small molecules on cells. Combining automated fluorescence microscopy with computer-aided image processing, CP provides an information-rich phenotypic profile for each tested sample. Rather than focusing on a single narrowly defined phenotype, CP uses multiple cytological probes to generate hundreds of quantifiable cytological features, giving rise to a phenotypic fingerprint of each compound. 2) Functional Signature of Ontology (FUSION) is takes advantage of gene expression signatures in mammalian cell lines to generate mechanism of action hypotheses. By probing the expression signatures of small molecules and genetic perturbations (siRNA, miRNA) we utilize pattern-matching tools that produce verifiable mode-of-action. We carry out this analysis using a minimal mRNA reporter cohort and technologies for high-throughput quantitative multi-analyte detection. The endogenous reporter gene signatures resulting from each perturbation were assembled into a similarity matrix using Euclidean and Mahalanobis distance distributions. In this way we produce FUSION maps that link bioactive agents to the molecular entities and/or biological processes they engage in cells.
In this presentation we will describe the biological platforms and describe the analytical chemistry and chromatography challenges associated with our efforts to carry out comprehensive biological characterization and the need to generate well defined samples to study synergistic interactions.

Index of Authors

Index of Keywords