4th World Congress on

CHROMATOGRAPHY

August 07-09, 2017 | Rome, Italy

Posters

Aleš Štrancar et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Platform chromatographic purification of AAV gene therapy vectors

Aleš Štrancar and **R Sekirnik**
BIA Separations, Slovenia

Adeno-associated virus (AAV) vectors of various serotypes are considered to have high potential for gene therapy applications. Currently, manufacturing of AAV vectors faces the challenge of co-production of incompletely formed particles lacking a recombinant viral genome. Empty capsids increase the dose of total AAV administered for efficient transduction and are thought to cause unwanted immunological reactions against the virus. Removal of empty capsids during manufacturing, as well as analysis of empty/full AAV particle content is therefore a critical requirement for any AAV production process. Poster demonstrates how CIMmultus™ QA monolithic columns can be used to remove empty AAV capsids from the product chromatographically in a single step.

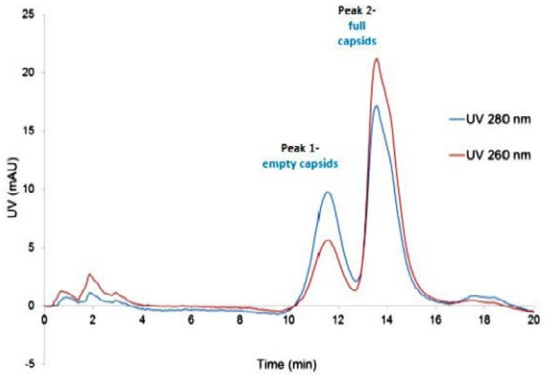


Image 1: CIMmultusTM QA column strong ion exchange chromatographic separation of empty and full capsids.

Biography

Aleš Štrancar is the CBDO of the BIA Separations and one of the main inventors of the CIM Convective Interaction Media®. He is author and co-author of more than 60 scientific papers dealing with separation and purification technologies, a co-author of five granted USA patents and their foreign equivalents in the field of biomolecule separations and purification and a co-author of several book chapters dealing with novel chromatography technologies for biomolecule separation. He co-developed several industrial scale purification processes. He was the President of Technology Council of Ministry of Economy of the Republic of Slovenia and Member of Science and Technology Council of the Republic of Slovenia.

ales.strancar@biaseparations.com

Notes:

Aleš Štrancar et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

PATfixTM - At-line monitoring of impurities and critical quality attributes in biopharmaceutical up- and downstream processes using HPLC fingerprinting

Aleš Štrancar[1], Sebastijan Peljhan[1], Tomas Kostelec[1], Romina Žabar[1], Blaž Goričar[1], Vid Skvarča[1], Vignesh Rajamanickam[2], Valentin Steinwandter[3] and **Patrick Sagmeister[3]**

[1]BIA Separations, Slovenia
[2]Vienna University of Technology, Austria
[3]Exputec GmbH, Austria

Production of high value biological therapeutics usually involves complex manufacturing processes with high process variability. Additionally, development of robust and reliable bioprocesses can be challenging. PAT aims to enhance bioprocess understanding and implies a holistic approach to ensure that quality is built into products by design. Efficient PAT therefore calls for fast and robust analytical techniques which enable to assess high quality information about critical quality attributes and key performance indicators as parallel as possible to the manufacturing process. PATfix™ is unique HPLC system for routine gradient separations that enables every analytical task. Equipped with bio-inert ceramic pump heads is deliberately tailored to meet the demands of analytical applications covering wide range of biomolecules. Highly sensitive and fast multi-wavelength detector enables to detect component peaks even in very fast gradients.

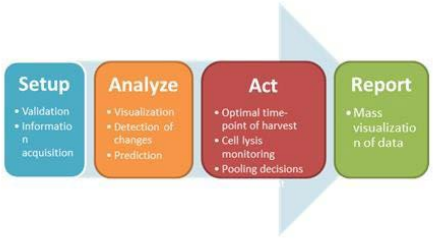


Figure 1: Exputec inCyght® Chromatography Data Science Software offers a user-friendly and powerful toolbox for the analysis of chromatographic data sets.

Biography

Aleš Štrancar is the CBDO of the BIA Separations and one of the main inventors of the CIM Convective Interaction Media®. He is author and co-author of more than 60 scientific papers dealing with separation and purification technologies, a co-author of five granted USA patents and their foreign equivalents in the field of biomolecule separations and purification and a co-author of several book chapters dealing with novel chromatography technologies for biomolecule separation. He co-developed several industrial scale purification processes. He was the President of Technology Council of Ministry of Economy of the Republic of Slovenia and Member of Science and Technology Council of the Republic of Slovenia.

ales.strancar@biaseparations.com

Notes:

Aymen K Al-Suwailem et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

HPLC-fluorescence method for the enantioselective analysis of propranolol in rat serum using immobilized polysaccharide-based chiral stationary phase

Aymen K Al-Suwailem[1], Amer M Alanazi[1], Mohamed M Hefnawy[1], Abdulrahman A Al-Majed[1], Mohamed G Kassem[1], Gamal A Mostafa[1], Sabry M Attia[1] and **Mohammed M Khedr[2]**

[1]King Saud University, Saudi Arabia

[2]Cardiff University, UK

A stereoselective high-performance liquid chromatographic (HPLC) method was developed and validated to determine S-(-)- and R-(+)-propranolol in rat serum. Enantiomeric resolution was achieved on cellulose tris(3, 5-dimethylphenylcarbamate) immobilized onto spherical porous silica chiral stationary phase (CSP) known as chiralpak IB. A simple analytical method was validated using a mobile phase consisted of n-hexane-ethanol-triethylamine (95:5:0.4%, v/v/v) at a flow rate of 0.6 mL min-1 and fluorescence detection set at excitation/emission wavelengths 290/375 nm. The calibration curves were linear over the range of 10–400 ng mL^{-1} (R=0.999) for each enantiomer with a detection limit of 3 ng mL^{-1}. The proposed method was validated in compliance with ICH guidelines in terms of linearity, accuracy, precision, limits of detection and quantitation, and other aspects of analytical validation. Actual quantification could be made for propranolol isomers in serum obtained from rats that had been intraperitoneally (i.p.) administered a single dose of the drug. The proposed method established in this study is simple and sensitive enough to be adopted in the fields of clinical and forensic toxicology. Molecular modeling studies including energy minimization and docking studies were first performed to illustrate the mechanism by which the active enantiomer binds to the β-adrenergic receptor and second to find a suitable interpretation of how both enantiomers are interacting with cellulose tris(3,5-dimethylphenylcarbamate) CSP during the process of resolution. The latter interaction was demonstrated by calculating the binding affinities and interaction distances between propranolol enantiomers and chiral selector.

Biography

Aymen K Al-Suwailem is working as an Assistant of Program Director at Prince Sultan Cardiac Center, KSA. He has completed PhD and Master's degree in Analytical Pharmaceutical Chemistry. He has also completed his Master's degree in Clinical Pharmacology and Hospital Administration.

aymen_4120@hotmail.com

Notes:

Elizaveta Fedorova et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

High performance and ultra-high performance liquid chromatography for determination of organic acids - intermediates of branched-chain amino acids biosynthesis in *Escherichia coli* strains

Elizaveta Fedorova, D O Varlamova, A D Kivero, K D Guk and **L R Ptitsyn**
Ajinomoto-Genetika Research Institute, Moscow, Russia

Recent advances in the development of branched-chain amino acids (BCAA) production strains is mainly based on using of system metabolic engineering strategies. Such approach needs thorough understanding of BCAA biosynthetic pathways and its regulations. In particular, the qualitative and quantitative evaluation of organic acids (OA), which are intermediates of BCAA biosynthesis, may help to clarify the key points of this process. Currently, different analytical methods are used for OA determination, including gas chromatography (GC), HPLC and mass spectrometry (MS). From other hand, the UHPLC approach is able to provide the analysis of numeral culture fluid (CF) samples with high sensitivity and high speed. The assessment of possibility to apply UHPLC and HPLC approaches for OA determination in CF of *E. coli* strains is the goal of this work. The following OA were analyzed: ketoisovalerate (KIV), 2-isopropylmalate (2-IPM), 3-isopropylmalate (3-IPM), ketoisocaproate (KIC), α-keto-β-methylvalerate (KMV), ketoisobutyrate (KIB), ketoglutarate (KG). For each method, the capacity factor (K), number of theoretical plates (N), asymmetry of the peak (Fasy), relative standard deviation by area (RSDPA), relative standard deviation of migration retention time (RSDRT) were calculated. It was shown that method UHPLC has lower qualitative detection limit, lower Fasy, RSDPA, and RSDRT. The time for analysis of one sample was much lower in case of UHPLC (5 min) in comparison with those for HPLC (10 min). Quantitative analysis of OA was performed in CF samples of *E. coli* strains. Two intermediates: KIV (1.53 mg/l from UHPLC and 1.87 mg/l from HPLC) and 2-IPM (2.07 mg/l from UHPLC and 2.37 mg/l from HPLC) were detected in analyzed samples. It was shown that the method UHPLC for determination of OA in CF of *E. coli* strains has a number of advantages, higher sensitivity and less analysis time, compared with HPLC.

Biography

Elizaveta Fedorova completed her Graduation from Russian State University named after A. N. Kosygin in 2006. She has been employed as an Analytical Chemist/ Engineer at Ajinomoto-Genetika Research Institute in 2007 and as Junior Research Associate since 2013. Her research interests include the development of UHPLC methods for analysis of organic acids in cultural fluids. She is the author of original research papers published in international journals.

lorensdar@yandex.ru

Notes:

Danijela Ašperger et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Chromatographic monitoring of febantel after biodegradation and advanced oxidation processes

Danijela Ašperger[1], Josipa Papac[1], Mirta Čizmić[1], Sandra Babić[1], Tibela Landeka Dragičević[1], Davor Ljubas[1], Lidija Ćurković[1] and **Sanja Radman[2]**
[1]University of Zagreb, Croatia
[2]Pliva d.o.o., Croatia

Classical methods of wastewater treatment fail to remove small molecules of contaminants such as pharmaceuticals. For many years pharmaceuticals have been used for medical therapeutic purposes and therefore have been uncontrollably released in the environment, through excretion, inappropriate disposal or because of inadequate waste water treatment. Recently, they have been recognized as potentially harmful pollutants, so accordingly the term emerging contaminants has been formed. One of commonly used pharmaceuticals is the anthelmintics whose representative is febantel. Febantel is used in human medicine and in veterinary practice very often. In spite of its frequent usage, about febantel behavior during and after wastewater treatment, there are still no sufficient data to predict its behavior and possible impact on the environment. The goal of this paper was monitoring the degradation products of febantel after biodegradation with activated sludge and compares them with the degradation products obtained after advanced oxidation processes (AOPs), so that, it can be used in system for waste water treatment. The effectiveness of febantel degradation and identification of degradation products formed during the processes was monitored by analytical and bioanalytical methods. Degradation products have been detected and identified by high and ultra-high performance liquid chromatography coupled to mass spectrometers. Assessment of toxicity of degradation process was done by measuring the bioluminescence inhibition of *Vibrio fischeri* bioassay. Finally, the results indicated that degradation products obtained during biodegradation are different from degradation products obtained by AOPs. Also degradation products after AOPs proved to be more toxic.

Biography

Danijela Ašperger has her expertise in Analytical Chemistry, Environmental Chemistry and, Toxicology. In 2013, she was appointed as an Associate Professor at Faculty of Chemical Engineering and Technology, University of Zagreb. She is currently working as a participant on the project "Fate of pharmaceuticals in the environment and during advanced wastewater treatment" funded by Croatian Science Foundation. She teaches instrumental analytical chemistry, characterization of materials, nondestructive methods of chemical analysis in art and archeology, and quality management.

diva@fkit.hr

Notes:

Felix Anyakudo et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Analysis of chlorinated compounds, phenolic compounds, styrenes, indene, dicyclopentadiene, dihydrocyclopentadiene, cumene, benzene, toluene, ethylbenzene and xylenes in fuel oil by headspace GC-MS

Felix Anyakudo[a, b], **Erwin Adams**[a] and **Ann Van Schepdael**[a]
[a]Farmaceutische Analyse, Belgium
[b]Inspectorate Antwerp NV, Belgium

Fuel oils are mostly used in marine applications and in power plants. They are known to contain hazardous volatile organic compounds (VOCs) that are of health and environmental importance. Chlorinated compounds, phenolic compounds, styrenes, indene, dicyclopentadiene, dihydrocyclopentadiene, cumene, benzene, toluene, ethylbenzene and xylenes are some of the hazardous compounds that have found their way into fuel oil through various streams. A static headspace GC-MS method was developed for the analysis of these compounds in fuel oil. Styrene D8 was used as internal standard for quantitation. Linear calibration curves were achieved for all components with determination coefficients R2 >0.99. Repeatability, limit of detection, limit of quantitation and recovery were reported. Matrix effect caused by fuel oil was minimized by 1:1 dilution with mineral oil. This method was sucessfully applied to the analysis of commercial samples.

Biography

Felix Anyakudo is a PHD student in the department of pharmaceutical analysis katholiek universiteit Leuven. He is currently the technical development and chromatography manager Inspectorate Antwerp NV. Felix received his bachelor in chemistry from university of Nigeria Nsukka and his master degree in chemistry from katholieke universiteit Leuven, Belgium. He also received additional masters in pharmaceutical science from katholieke universiteit Leuven in 2002. Felix joined Inspectorate Antwerp NV in 2004 and has been actively involved in projects relating to expansion of laboratory capabilities mostly in the field of chromatography. His current research interest lies in the area of method development for the analysis of environmental and pharmaceutical samples using various kinds of chromatographic techniques.

Felix.anyakudo@Kuleuven.be

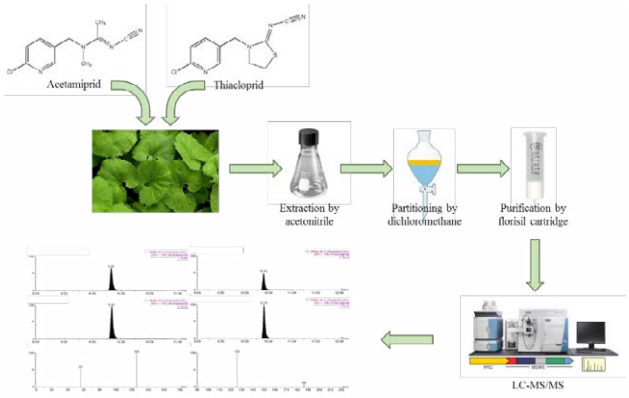
Notes:

Han Sol Lee et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Quantification of acetamiprid and thiacloprid residues in field-incurred butterbur samples using liquid chromatography-tandem mass spectrometry

Han Sol Lee, Hyung Suk Chung, Md Humayun Kabir, Md Musfiqur Rahman and **Jae-Han Shim**
Chonnam National University, South Korea

An analytical method was developed to quantify the residual levels of the neonicotinoid insecticides acetamiprid and thiacloprid in field-incurred butterbur samples using liquid chromatography-tandem mass spectrometry. Samples were extracted with acetonitrile and partitioned with dichloromethane. After partitioning, purification was conducted using a Florisil® cartridge. Linearity of the two compounds over a concentration range of 0.004–0.4 µg/mL was excellent, with determination coefficients (R2)≥0.9998. The limits of detection (LOD) and quantitation (LOQ) for both acetamiprid and thiacloprid were 0.0006 and 0.002 mg/kg, respectively. The average recoveries for acetamiprid and thiacloprid at two spiking levels (0.02 and 0.1 mg/kg, i.e., 10×LOQ and 50×LOQ) were between 78.23 and 82.15%, with relative standard deviations≤7.22%. The method was successfully applied to field-incurred samples treated with a commercial pesticide product, either once (zero or 7 days before harvest) or twice (0 and 7, 7 and 14, or 14 and 21 days before harvest). The highest and lowest residues were obtained for the 0 and 7 days' treatment and the 14 and 21 days' treatment, respectively. The developed method is simple and accurate and can be extrapolated to other leafy vegetables.



Biography

Han Sol Lee is pursuing her Master degree at Chonnam National University, South Korea. She is interested in agricultural food safety from harmful materials such as pesticides and veterinary antibiotics. She participated in many projects that developed the analytical method for determining pesticides and veterinary antibiotics in several environmental factors such as crops, water, and monitoring.

fjqngksthf@naver.com

Notes:

Hyung Suk Chung et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Determination of kinetics and pre-harvest residue limit of pyriofenone in oriental melon (*Cucumis melo* var. *makuwa*) grown under regulated climatic conditions

Hyung Suk Chung, Md Humayun Kabir, Han Sol Lee, Md Musfiqur Rahman and **Jae-Han Shim**
Chonnam National University, South Korea

A high-performance liquid chromatography–ultraviolet detection was used to estimate the disappearance rates as well as the pre-harvest residue limits (PHRLs) of pyriofenone in oriental melon (*Cucumis melo* var. *makuwa*) grown under greenhouse conditions at two different locations (A and B) in Seongju, Republic of Korea. The identity of the compound in standard solution and representative field incurred samples was confirmed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method was validated in terms of linearity, limits of detection and quantification, accuracy (expressed as recovery %), and precision (expressed as relative standard deviation %) for accurate and precise quantitation. Notably, the residual levels of field incurred samples collected over 0 day through 10 days post-application were below the maximum residue level (MRL=0.2 mg/kg) established by the Korean Ministry of Food and Drug Safety. Site A showed lower residue levels and a higher decline rate than site B, which might be attributed to seasonal variation (high temperature) and increased metabolic and enzyme profiling in the mature fruits. The half-lives were similar, 4.9 and 4.3 days, at sites and B, respectively. Using the pre-harvest residue limit, we predicted the residue amounts at ten and five days before harvest, which resulted in concentrations lower than the provisional MRL at harvest time.

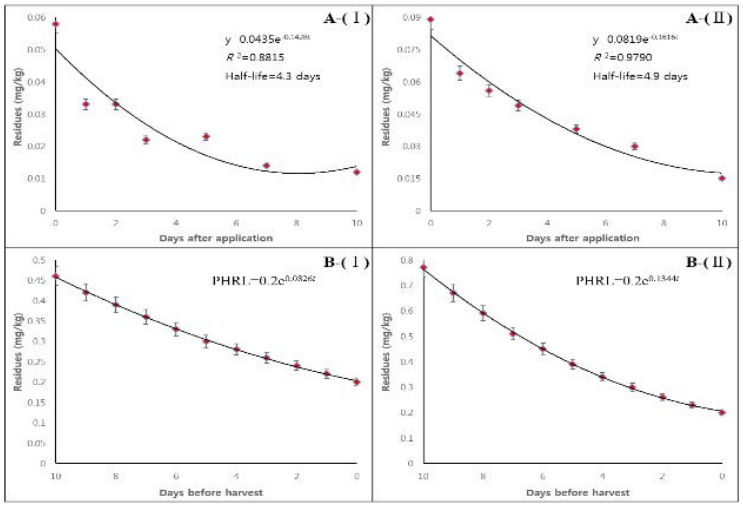


Figure 1: Dissipation pattern and pre-harvest residue limit curves of pyriofenone in oriental melon.

Biography

Hyung Suk Chung is pursuing her Master degree at Chonnam National University, Republic of Korea. He is interested in Food Safety from harmful materials such as pesticides and veterinary antibiotics. He participated in many projects that developed the analytical method for determining pesticides and veterinary antibiotics in several environmental factors such as crops, water, and monitoring.

jhsgudtjr@naver.com

Marco Ruijken, J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Product control using differential GC/MS and comprehensive GCxGC/MS

Marco Ruijken
MsMetrix, Netherlands

During product control or trouble shooting, the investigator is often confronted with two samples; one sample relates to the good quality product, while the other sample can be an out-of-spec batch, or a sample with bad product characteristics, e.g. having a different smell or taste. Typically for the above examples is the limited time available to solve these problems. GC-Analyzer is a software product developed to detect differences between two samples at very low levels. All fragmentation ions are searched for being different between both samples. De-convolution and subsequent identification (NIST) is implemented to quickly identify possible components of interest. Similar algorithms have been developed to handles comprehensive GCxGC-MS data. Certainly, GCxGC/MS is a technique having superior separation capabilities compared to 1-dimensional GC/MS, but co-elution or near co-elution still might occur, especially in complicated matrices. Whereas most software tools for GCxGC/MS use processing of "TIC" data only, our new methods apply data analysis using the "all ions" approach. The implemented method allows for the detection and de-convolution of differential components that are not or badly separated, even in two dimensions. It will be demonstrated that processing using the "all ion" approach will substantially detect more (differential) components, compared to the analysis using TIC data only. Technical details of the algorithms will be explained and examples will be given from applications like food analysis, product control in flavor & fragrance industry and from base chemistry industry.

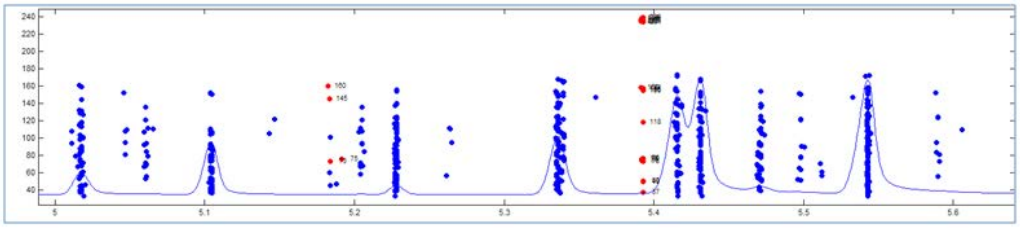


Figure 1: Differential analysis Dot Plot, showing differential peaks in red and similar peaks in blue.

Biography

Marco Ruijken is the Owner/ Head of Research of MsMetrix, Maarssen the Netherlands. MsMetrix develops informatics solutions for LC/MS and GC/MS Data Analysis in the area of: Metabolite Profiling, Metabolomics, Proteomics, BioMarker Discovery, and Impurity / Degradation Profiling. Our mission is to be the premier provider of fast, affordable, user-friendly and reliable software in the above application fields. His educational background is in Chemometrics/Statistics and Processing of complex data. Current research topics are advanced deconvolution in GC/MS and GCxGC/MS with the focus on Differential Analysis. Furthermore, we are specialized in implementing ideas or requirements from universities or companies into our existing software tools.

mr@msmetrix.com

Notes:

Maria de Fátima Simão Jucá Cruz et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Application of combined chromatographic techniques in the screening and separation of complex triterpenoid saponins from *Pithecellobium saman*

Maria de Fátima Simão Jucá Cruz, Gabriela M Pereira, Bernadete P da Silva and **José P Parente**
Institute of Research on Natural Products Walter Mors, Brazil

Introduction & Aim: *Pithecellobium saman* (Leguminosae) is native to tropical America, nowadays widely cultivated and naturalized through tropical regions with ornamental purposes. The leaves are livestock forage supplement and this species is used in traditional medicine as a remedy for the treatment of different diseases. Nonetheless, no chemical investigations or biological evaluations were carried out on the constituents of this species. According to the literature, complex triterpenoid saponins are shown to possess several physiological properties depending on their amphipathic chemical structures, such as the capacity for alteration of membrane permeability. Additionally, these compounds have been reported to possess therapeutic potential for immune system modulation through different mechanisms. As part of our ongoing efforts in discovering potentially bioactive compounds from natural sources, the present study is concerned with the combination of different chromatographic methods to improve the isolation of individual substances from a complex mixture of compounds with similar chemical structure.

Methodology: The root barks of *Pithecellobium saman* were extracted with MeOH. After concentration, the resulting residue was partitioned between water and n-BuOH. The organic phase was concentrated, submitted to molecular exclusion chromatography and monitored by thin layer chromatography, affording four fractions containing a complex mixture of saponins. The fractions were submitted to reversed phase high performance liquid chromatographic analysis, using an ultraviolet detector, with MeOH:H2O (65:35) as solvent system, a flow rate of 0.5 mL and a chromatographic run time of 45 minutes.

Findings: By a combination of chromatographic methods and spectroscopic techniques, it was demonstrated that the fractions were composed of a multicomponent mixture of complex triterpenoid saponins in different proportions.

Conclusion: A combination of different chromatographic techniques was required to perform the isolation of the substances from a complex mixture and to establish a preliminary structural characterization of its constituents.

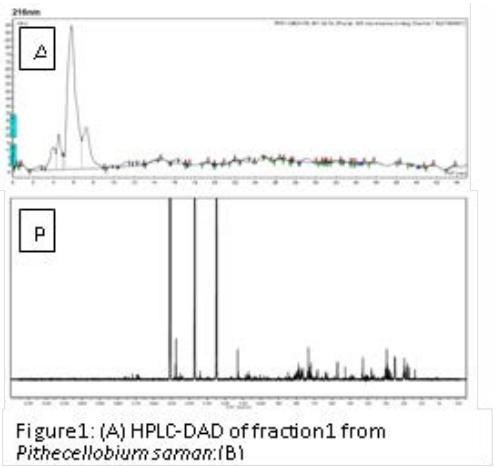


Figure1: (A) HPLC-DAD of fraction1 from *Pithecellobium saman*;(B)

Biography

Maria de Fátima Simão Jucá Cruz completed her Graduation in Chemistry and Master's Degree at Federal Rural University of Rio de Janeiro (UFRRJ). She is pursuing her Doctorate degree in Natural Products Chemistry at UFRJ in Brazil.

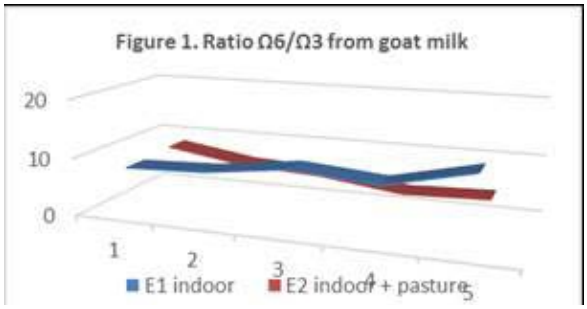
fatima_ufrrj@hotmail.com

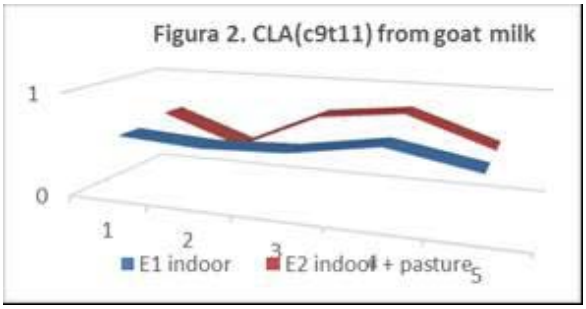
Mariana Ropota et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Goat milk polyunsaturated fatty acids determination by gas chromatography

Mariana Ropota[1], **Margareta Olteanu**[1], **Ilie Voicu**[1] and **Angela Sava**[1,2]
[1]INCDBNA-IBNA, Romania
[2]USAMV-Bucharest, Romania

The differences in the fatty acids content of the goat milk were determined by gas chromatography in two groups of Carpatina×Saanen (F3) goats (n=30), in the same physiological state (second lactation), fed from April to August according to the intensive system (E1 indoor) or extensive system (E2 indoor + pasture). Group 1 received briquettes made of hays and concentrates, alfalfa hay and barley straws. Group 2 received a supplement of alfalfa hay plus concentrates (corn and barley). Ten milk samples from each group were collected monthly. The method involved the chemical processing of the samples by transformation in fatty acids methyl esters (FAME), followed by separation of the components in the chromatographic column, fatty acids identification by comparison with standard chromatograms and quantitative determination of FAME (g FAME of % g Total FAME). Reference materials (CRM) were: Standard solution of methyl fatty acids SUPELCO 37 Component FAME Mix; 10mg/mL and; infant/adult nutritional formula- standard reference material 1849. The method was validated "in house", determining the following parameters: accuracy, fidelity, reproducibility, sensitivity, detection limit, quantification limit and tracing in agreement with SR EN ISO/CEI 17025:2005. The experimental determinations showed that Ω6/Ω3 ratio ranged from 7.93 to 12.44 in E1, and from 3.62 to 8.32 in E2. CLA (conjugated linoleic acid) level increased in the middle period of sampling (months 2, 3 and 4), from 0.52% to 0.68% in group 1, and from 0.35% to 0.80% in group 2. In the first and last months, CLA level decreased from 0.57% to 0.53% in group 1, and from 0.63% to 0.52% in group 2, but the average CLA value for the whole experimental period was 6.02% in group 2, compared to just 5.7% in group 1.





Biography

Mariana Ropota completed her PhD at Bucharest University. She is coordinating the compartment of gas chromatographic analyses within the laboratory of chemistry and nutrition physiology of the National Research-Development Institute for Animal Biology and Nutrition-IBNA-Balotesti. She is a Researcher in Analytical Chemistry. She has published more than 20 papers in national and international scientific journals, rated by ISI or by other databases.

m.ropota@yahoo.com

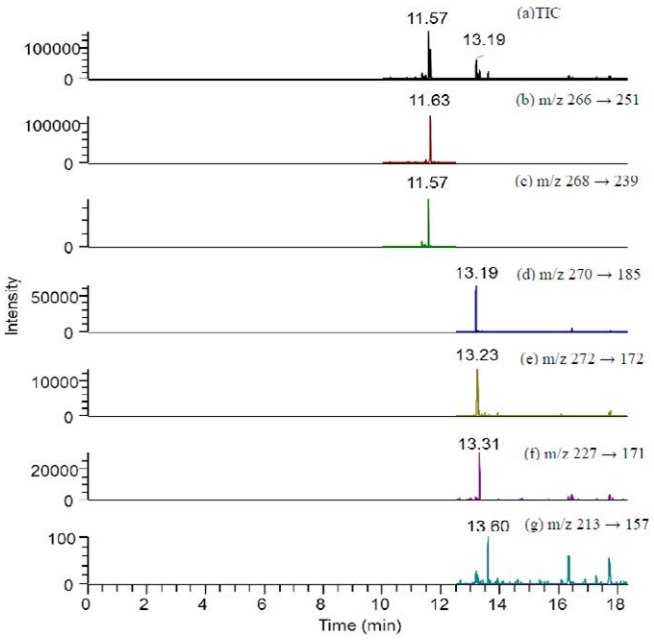
Notes:

Maw-Rong Lee et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Liquid phase micro-extraction *in-situ* derivatization for determination of estrogens in water by gas chromatography-tandem mass spectrometry

Maw-Rong Lee, Yi-Yu Chen and **Chung-Yu Chen**
National Chung Hsing University, Taiwan

Endocrine disrupting chemicals (EDCs) in environmental aqueous system have gained global attention because they may interfere with central regulatory functions by antagonizing or mimicking the effects of endogenous hormones, even at extremely low concentrations. In human life, the use of estrogens, the group of steroidal hormones of EDCs, in animal feeds, contraceptives, and hormone replacement therapy drugs is increasing dramatically. Estrogens enter the human living environment may cause many diseases such as breast and prostate cancers. Hence, to develop efficient analytical method for determination of estrogens in aquatic samples is important. This study develops liquid phase micro-extraction (LPME) *in situ* derivatization combined with gas chromatography-tandem mass spectrometry (GC-MS/MS) for analyzing of trace estrogens in aquatic samples. The targeted estrogens include mestranol (MES), dienstrol (DIE), diethylstilbestrol (DES), estrone (E1), 17α-estradiol (17α-E2), and 17α-ethinylestradiol (17α-EE). 11.25 mL pH adjusted (pH 6) water sample containing 5% sodium chloride was mixed with 3.75 mL dansyl chloride derivatization agent. After derivatization, the derivatives were extracted by LPME with octanol as extraction solvent and the extractant was detected by GC-MSMS. The linearity of the proposed method ranged between 0.05 to 50 ng/mL. The detection limits (LODs) of targeted estrogens were between 0.3 to 1.1 ng/mL. The feasibility of applying the proposed method to analyze the six estrogens in aqueous samples was also examined. The results showed the method proposed is suitable for determination of trace estrogens in environmental water samples.



Biography

Maw-Rong Lee has his expertise in developing new analytical techniques using modern instrumentation and sample preparation methods, and the applications of these techniques in areas such as environmental, clinical, and forensic chemistry. His special research interests include Mass Spectrometry, Tandem Mass Spectrometry and Integrated Separation/Identification Techniques. He is also interested in using tandem mass spectrometry to study ion molecule reaction, proton affinity in gas phase. The sample preparation methods used are LPME, SPME, SPE, microwave and SFE.

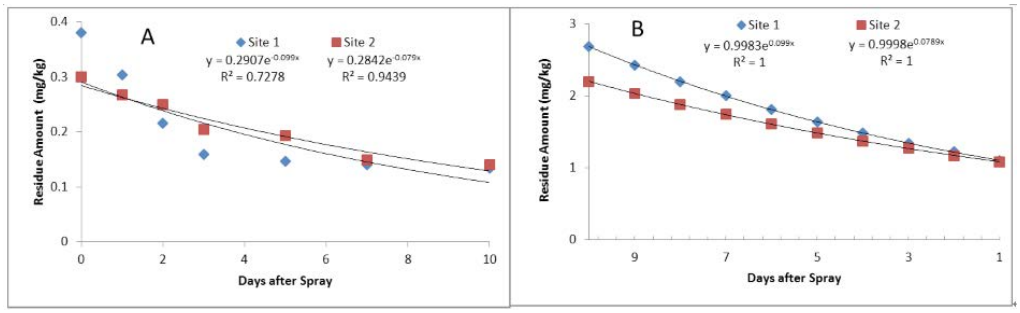
mrlee@dragon.nchu.edu.tw

Md Humayun Kabir et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Residue analysis of amisulbrom in oriental melon (*Cucumis melo* L. var. *makuwa*) cultivated in plastic house conditions: Dissipation kinetics, pre-harvest residue limits, and risk quotients assessment

Md Humayun Kabir[1], **Md Musfiqur Rahman**[1], **A M Abd El-Aty**[2], **Hyung Suk Chung**[1], **Han Sol Lee**[1] and **Jae-Han Shim**[1]
[1]Chonnam National University, South Korea
[2]Konkuk University, South Korea

Amisulbrom residue levels in oriental melon were determined using liquid chromatography–ultraviolet detector (LC-UVD). Real sample mass confirmation was performed with liquid chromatography-mass spectrometry (LC-MS/MS). Samples were prepared using liquid-liquid extraction (LLE) and cleaned up with solid phase extraction (SPE) florisil (1 g, 6 cc). The standard showed good instrument response linearity. Its determination coefficient R2=0.9999 and recovery ranged from 87.5% to 97.3%. The dissipation patterns of this pesticide were determined using samples from two different sites. Half-lives were assessed at 7.0 d and 8.8 d for site 1 and site 2, respectively. A pre-harvest residue limit graph was also constructed from the data. It indicated that if the residue levels were less than 0.55-0.59 mg/kg 3 d before harvest or less than 0.61-0.74 mg/kg 7 d before harvest, then they would be lower than the maximum residue levels. Risk assessments showed that the risk quotient was 4.39-3.47% at 0 d and declined to 1.53-1.63% at 10 d. Therefore, the experimental data indicate that the amisulbrom dosage recommended for oriental melon is unlikely to induce adverse health effects in Korean consumers.



Biography

Md Humayun Kabir completed his BSc (Hons) and MS degree in Chemistry from University of Dhaka, and working as Scientific Officer at Bangladesh Council of Scientific and Industrial Research, Bangladesh. Currently, he is pursuing PhD under the supervision of Professor Shim Jae Han at Chonnam National University, Republic of Korea. He is interested in food safety from harmful materials such as pesticides and antibiotics. He participated in many projects to develop the analytical method for determining pesticides and antibiotics in several matrices such as vegetables and fruits.

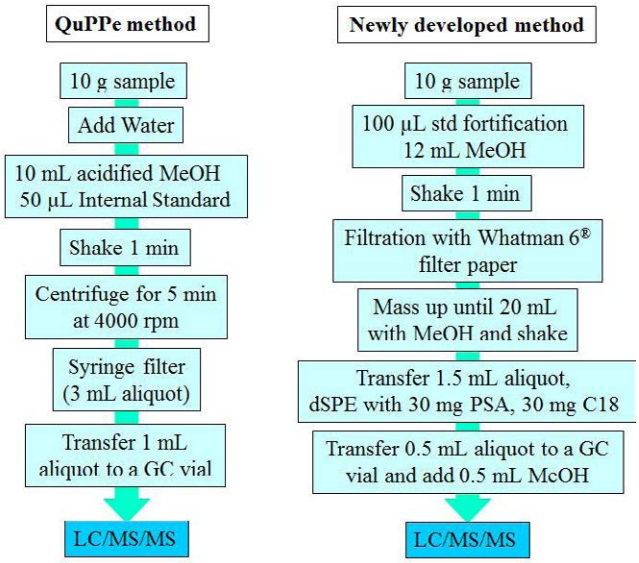
h.kabir79yahoo.com

Md Musfiqur Rahman et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

An effective methodology for analyzing dinotefuran and its metabolites in plum using liquid chromatography-tandem mass spectrometry

Md Musfiqur Rahman[1], **Jae-Han Shim**[1] and **A M Abd El-Aty**[2]
[1]Chonnam National University, South Korea
[2]Konkuk University, South Korea

A simple and effective method was developed for analyzing dinotefuran and its three metabolites, MNG, UF, and DN in plum using liquid chromatography-tandem mass spectrometry. Due to polarity and high water miscibility, dinotefuran and some of its metabolites were difficult to extract using acetonitrile and salt following QuEChERS sample preparation methodology. Therefore, sample was extracted with methanol, and purified with dispersive solid phase extraction procedure (d-SPE) using primary secondary amine (PSA) and C18 sorbent after filtration, and marked up. Due to the suppression effect from plum matrix, a matrix matched calibration curve was constructed for all of the analytes which provided good linearity with determination co-efficient R2≥0.998. Method was validated after fortification of two different standard concentrations (10 X LOQ and 50 X LOQ) into control plum matrices with three replicate for each of the concentration. Acceptable recovery was observed for each of the fortified analytes and was ranging between 83.01 and 110.18% with relative standard deviation (RSD)≤8.91. The method was successfully applied to field-incurred plum samples and dinotefuran and all of its metabolites were found as residues. The method can be extended to those polar compounds which have solvent and partitioning problems in any of the versions of QuEChERS.



Biography

Md Musfiqur Rahman is a Postdoctoral Research Fellow at Chonnam National University, South Korea. He is an author/co-author of 60 articles published in prestigious international journals with good impact factors. He is working on method development of pesticide/antibiotics and their metabolites residues in fruits, vegetable, and livestock product. His aim is to make methodology more simple and effective utilizing latest chromatographic technique.

musfiq_707@yahoo.com

Raluca Paula Turcu et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Use of an RP-HPLC method to determine the lysine content in seven varieties of peas

Raluca Paula Turcu[1, 2], **Georgeta Ciurescu**[1] and **Margareta Olteanu**[1]
[1]INCDBNA-IBNA, Balotesti, Romania
[2]University of Agronomic Sciences and Veterinary Medicine, Romania

Peas (*Pisum sativum* L) are a plant with excellent potential as a source of energy and protein for monogastric animals. The compound feeds formulations that include peas are characterized by: High protein digestibility, excellent balance between the component amino acids, and high content of lysine, favorable to meat production. Lysine is a limiting amino acid and also irreplaceable in pig and poultry feed. The purpose of this study was to determine, by reversed phase high performance liquid chromatography (RP-HPLC), the lysine content of seven varieties of peas (Biathlon, Nicoleta, Specter, Windham, Vedea, Rodil and Aurora), existing in the seed bank of National Agricultural Research and Development Institute (Fundulea, Romania), likely to be used in farm animal diets. Determination of lysine in peas, as essential amino acid, is a key tool for animal nutrition specialists. Lysine was determined by reversed phase chromatography, using high performance liquid chromatograph, Finnigan Surveyor Plus HPLC (Thermo-Electron Corporation, Waltham, USA), equipped with a BDS Hypersil C18 column with silica gel, the mobile phase consisting of two solvents, solvent A (phosphate buffer) and solvent B (water: acetonitrile: methanol 20:20:60 v/v/v). The column temperature is 45oC, and the flow rate of 1.7 mL/min for 40 min. Lysine concentration in the analysed varieties of peas ranged from 1.489 g/100 g (Nicoleta variety) to 1.940 g/100 g (Vedea variety). The series of analytical values determined in the seven varieties of peas has a coefficient of variation of 0.085. The use of liquid chromatography is a relatively simple and accurate method to assess the lysine content of feeds. Using RP-HPLC method is a tool for nutritionists for the formulation of diets, particularly to decide whether to use, or not to use synthetic lysine in these diet formulations.

Biography

Raluca Paula Turcu is a first year PhD student from University of Agronomic Science and Veterinary Medicine of Bucharest. She holds an Engineering degree and Master's degree in "Security management environment and food safety". She works as Scientific Research Assistant in the laboratory of chemistry and nutrition physiology of the National Research Development Institute for Animal Biology and Nutrition, IBNA-Balotesti. She published two papers in national scientific journal, indexed in international databases.

raluca.socoliuc@yahoo.com

Notes:

Sebastian Witek et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

UHPLC-MS/MS analysis of thyreostats in serum

Sebastian Witek, Barbara Wozniak, Iwona Matraszek-Zuchowska and **Andrzej Posyniak**
National Veterinary Research Institute, Poland

Thyreostats are thioamide antithyroid drugs. Activity of these compounds consists in inhibiting the synthesis of thyroid hormones triiodothyronine (T3) and thyroxine (T4), which favors the processes of animal fattening. Increase in weight of animals is mainly due to the water retention in the tissues and the gastrointestinal tract. The consequence is not only the production of lower meat quality, but also the risk of drug residues to human health. According to the International Agency for Research on Cancer, some compounds of this group possess carcinogenic and teratogenic properties. In accordance with the Council Directive 96/23/EC thyreostats belong to the group A2 - compounds with anabolic properties, which must be controlled on slaughter animals. To extend the range of methods used in the national control plan, a fast and efficient UHPLC-MS/MS method was developed and validated to detect five thyreostatic compounds: tapazole, thiouracil, methylthiouracil, propylthiouracil and phenylthiouracil in bovine serum. Previously published method of thyreostats in urine was used as a starting point for the development of our method. Thyreostats were extracted from serum samples with diethyl ether after derivatization with 3-iodobenzylbromide in basic medium (pH 8.0) and analyzed by gradient elution within 7.5 min on a SB-C18 column (50 x 2.1 mm; 1.8 µm, Agilent) using a mobile phase consisting of acetonitrile/0.1% acetic acid. The analysis was performed on UHPLC Shimadzu NEXERA X2 with triple quadrupole MS 8050 instrument operating in positive electrospray ionization mode. The procedure was validated according to the Commission Decision 2002/657/EC requirements. The recovery and repeatability satisfy the performance criteria specified in this document for banned compounds. The recovery ranged from 97.5% to 113.5% for all examined compounds, and the repeatability did not exceed of 14.1%. The decision limits (CCα) did not exceed 3.95 µg L^{-1}, and also detection capabilities (CCβ) did not exceed 6.73 µg L^{-1}. The developed procedure is sensitive and robust, and therefore, useful for quantification and confirmation of thyreostats in residue control programme.

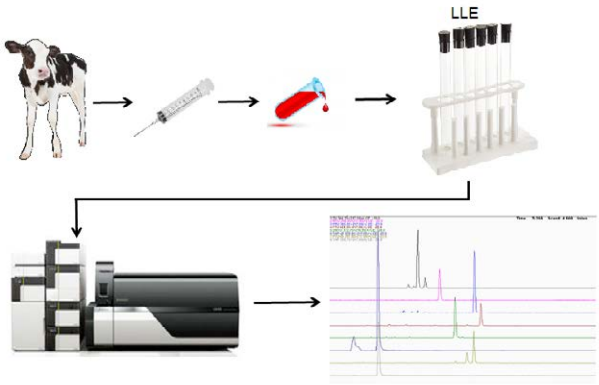


Fig.1: Graphical scheme of determination thyreostats in serum.

Biography

Sebastian Witek works at National Veterinary Research Institute, Poland since 2009. He deals with residues of hormones and thyreostats in biological samples of animal origin. He has a lot of experience in LC-MS/MS and GC-MS.

sebastian.witek@piwet.pulawy.pl

Taeik Hwang et al., J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Development of LC-Q-TOF-MS and LC-MS/MS method for the determination of grayanotoxins in foodstuffs and its application to food science study

Taeik Hwang, Dongwoo Shin, Eunyoung Noh, Ji Hye Jeong, Jun Hyoung Kim, Hyoung Joon Park, Seok Heo, Jeonghwa Jo, Nam Sook Kim, Ji Hyun Lee, Sung Kwan Park, Seong soo Park and **Sun Young Baek**
Ministry of Food and Drug Safety, Republic of Korea

A sensitive and specific high-performance liquid chromatography–Quadrupole-time of flight mass spectrometry (LC-Q-TOF-MS) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) method was developed for the determination of grayanotoxin I (GTX I) and grayanotoxin III (GTX III) in foodstuffs. Grayanotoxins (GTXs) were extracted from foodstuffs via solid-phase extraction using HLB solid-phase extraction cartridges. LC-Q-TOF-MS was used to determine the fragmentation patterns of GTXs and to characterize their fragmentation pathways. LC-MS/MS method was developed and validated for quantitative determination of GTX I and GTX III in foodstuffs. Separation of LC-Q-TOF-MS was made using a Waters ACQUITY BEH C18 column (100 × 2.1 mm, 1.7 μm) and separation of LC-MS/MS was made using a Capcell pack MG II C18 column (2.0 mm × 100 mm, 3 μm). The mobile phase consisted of distilled water (v/v, A) and methanol (v/v, B) containing 0.1% formic acid. Electrospray ionization mass spectrometry was operated in the positive ion mode. The calibration curves of LC-MS/MS obtained were linear over the concentration range of 10–100 ng/mL (GTX I), 20-400 ng/mL (GTX III) with a lower limit of quantification of 7.5 ng/mL (GTX I) and 15 ng/mL (GTX III), respectively. The relative standard deviation of intra-day and inter-day precision was below 10.35% and accuracy ranged from 83.7 to 112.0%. The analytes were stable in the stability studies. An investigation of 51 foodstuffs from the online and offline market reveals the presence of GTXs. A quantitative estimation indicated total toxin concentrations of 1.84-101 ng/mL (GTX I) and 1.53-37.4 ng/mL (GTX III) in foodstuffs. The potential of this approach is especially demonstrated by the fact that at least two of these toxins have not been previously described in the literature. The validated method was successfully applied to the quantification study of GTXs in foodstuffs for the first time.

Biography

Taeik Hwang is working as Scientific Officer at Food and Drug Safety evaluation in Ministry of Food and Drug Safety. He completed his Masters from Soonchunhyang University in Korea.

taelee93@korea.kr

Notes:

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Zhenli Liu, J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

4th World Congress on

CHROMATOGRAPHY

August 07-09, 2017 | Rome, Italy

Application of a strategy based on metabolomics guided promoting blood circulation bioactivity compounds screening of vinegar

Zhenli Liu
Institution of Basic Theory-China Academy of Chinese Medical Sciences, China

Statement of the Problem: Rice vinegar (RV) and white vinegar (WV) as daily flavoring, have also used as accessory in traditional Chinese medicine processing. As we know, the promoting blood circulation efficiency could be enhanced when herbs processed by vinegar. Number of reports focused on health benefits derived by consumption of vinegar. However, few concerned the blood circulation bioactivity.

Methodology & Theoretical Orientation: In this paper, a metabolomics guided strategy was proposed to elaborate on the chemical constituents' variation of two kinds of vinegar. GC-MS coupled with multivariate statistical analysis were conducted to analyze the chemical components in RV and WV and to discriminate them. The anti-platelet activities *in vitro* were investigated by whole blood aggregometry platelet test. And the anticoagulant activities were monitored by the whole blood viscosity, plasma viscosity, packed cell volume, prothrombin time, and four coagulation tests (PT, TT, APTT, FIB) *in vivo*.

Findings: Constituents of RV and WV were globally characterized and 33 potential biomarkers were identified. The contents of four potential alkaloid biomarkers increased with aging time prolonged in RV. RV and its alkaloids metabolites exhibited some anti-platelet effects *in vitro* and anticoagulant activities *in vivo*. WV failed to exhibit promoting effects.

Conclusion & Significance: Alkaloid metabolites were demonstrated to be the principal compounds contributing to discrimination and it increased with aging time prolonged in RV. RV exhibited the blood circulation bioactivity. The alkaloids of RV contributed to the blood circulation bioactivity.

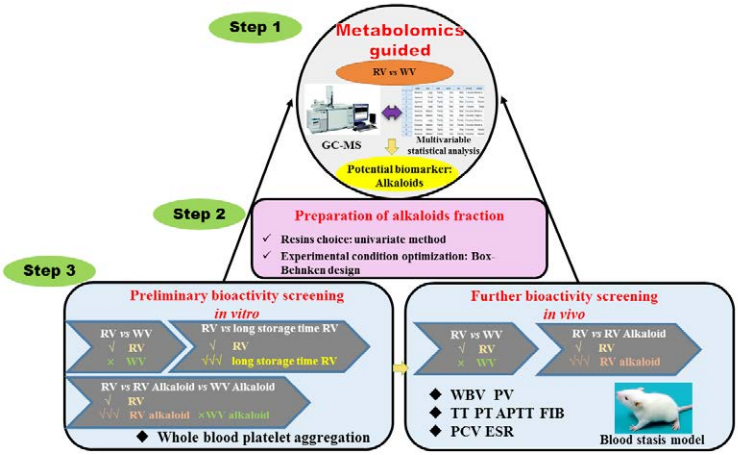

Figure 1: Description of the strategy

Biography

Zhenli Liu has her expertise in "The quality control of Chinese herbal medicine using analytical methods and in separating active ingredients from herbal medicine using chemical separation methods". She has published more than 100 journal articles in both Chinese and English.

zhenli_liu@sina.com

4th World Congress on

CHROMATOGRAPHY

August 07-09, 2017 | Rome, Italy

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Kahtan J Hasson, J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Complexation of diclofenac sodium with hydroxypropyl beta-cyclodextrins improves its solubility and stability in ampoule solution which is determined by HPLC

Kahtan J Hasson
Al-Rasheed University College, Iraq

Diclofenac sodium is widely used in medicine as anti-inflammatory and antirheumatic agent. The therapeutic dose of this drug is 75 mg by parenteral administration, however, diclofenac sodium is slightly soluble in water, and therefore, it is prepared as 3 ml ampoule contains 25 mg/ml. The available commercial products of diclofenac sodium ampoules have used different types of solubilizing agents as benzyl alcohol which is an irritant in a concentration more than 3% while the other manufacturers used propylene glycol which has toxic impurities. In this work, I tried to prepare diclofenac sodium injection by using hydroxy propyl beta cyclodextrins, a natural and safe excipient in formulation of ampoule solution which formed an inculcation complex compounds with diclofenac sodium, render it very soluble and more stable. The finished product of ampoules were subjected to the stability study by storing the samples at 40°C and 75% RH for six months and the physico-chemical properties of the samples were tested at different periods. The results showed no change in appearance of the ampoules solution along the study time. In addition, a reversed –phase high pressure liquid chromatographic method was developed and applied in studying the behavior and resistance of diclofenac sodium in its solution to the high temperature challenger. The developed HPLC method was proved to be accurate and able to detect the degradation products of diclofenac sodium in solution.

Biography

Kahtan J Hasson is a Pharmacist since 1970 with Master degree from Herriot-Watt University, UK. He is a Lecturer at Al-Rasheed University and an R&D Consultant at Al-Safa Company for pharmaceutical industries, Baghdad.

kjhassoun@yahoo.com

Notes:

María Ramos Payan, J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Advantages of using a microfluidic-chip as sample treatment miniaturization for a subsequent analysis by HPLC

María Ramos Payan
Instituto de Microelectrónica de Barcelona IMB-CNM (CSIC), Spain

Statement of the Problem: The most critical stage of the analytical process is the preparation of the sample requiring different stages prior to analysis, long extraction times, large volumes of reagents, etc., with the objective of obtaining a good clean-up for the analysis by instrumental techniques (as HPLC). Nowadays, one of the dominant trends in sample preparation is miniaturization and automation. In this paper, we present the advantages presented by the use of microfluidic systems in this field. These devices allow working in different configurations depending on the nature of the analyte to be extracted: either by liquid phase micro-extraction or by electro membranes.

Methodology: Our group has demonstrated the possibility of on-line and off-line analysis by HPLC. Two pumps are used to introduce the sample and the acceptor phase into the microfluidic device. The microfluidic device is fabricated using two patterned plates of poly (methyl methacrylate), which are symmetrical. The channels are separated by a polypropylene membrane. For off-line analysis, the acceptor outlet (extract) is collected and injected directly into a HPLC. For on-line analysis, the acceptor outlet is connected to the HPLC.

Findings: This type of device provides high selectivity, clean-up, reduces sample volume and low consumption of reagents, significantly reduces time of analysis and has demonstrated its ability to online coupling with HPLC. Additionally, the microchip-devices are reusable (allow membrane exchange) and each membrane is stable during more than 10 consecutive micro extractions.

Conclusion & Significance: The miniaturization and automatization of sample treatment procedures (on-chip) offer multiple advantages compared with existing traditional techniques. It also, offers excellent clean-up either with biological or environmental samples and significantly reduces the time of analysis from the sample collection till data obtaining.

Biography

María Ramos Payan has expertise in "Improving sample preparation techniques focused on microfluidic-chip devices as miniaturization". The novelty of her microfluidic devices offer more advantages than the existing methodologies. The devices work either using biological and environmental samples and can be coupled on-line to HPLC or mass spectrometry. She has also demonstrated the applicability of microchip devices for diagnostic diseases as diabetes. She has worked at different institutions (University of Seville, University of Huelva, University of Lund, University of Copenhagen and University of North Carolina, USA). Currently, she works at Microelectronic National Center of Barcelona and Universitat Autonoma of Barcelona with the aim of implementing optical detection into microfluidic devices for multiple different applications.

ramospayan@us.es

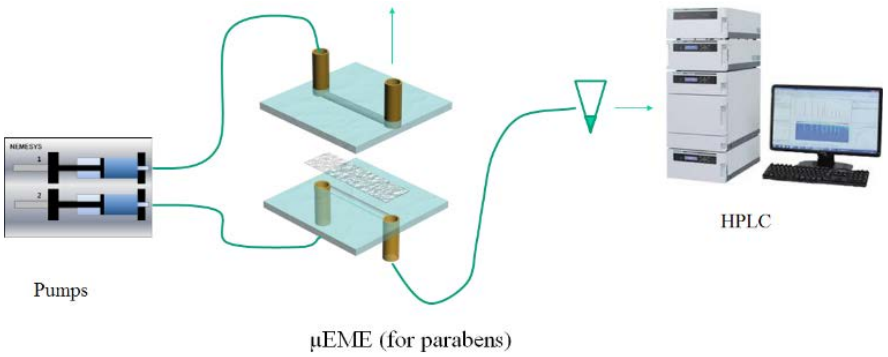
Notes:

María Ramos Payan, J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Double-flow or stopped-flow conditions as different operating working modes for microfluidic devices in sample preparation depending on the application field

María Ramos Payan
IMB CNM, Spain

This experimental work reports the study based on two working modes that can be used when liquid phase micro extraction is integrated into a microfluidic-chip device. The study compares enrichment factors, extraction efficiencies and detection and quantification limits. The microfluidic device has been tested with acid drugs as model analytes. Transport phenomena are faster on the micro-scale, and therefore extractions can be carried out quickly. This comes from the fact that the transport in LPME is governed by diffusion and that the time required for mixing scales with the square of the length, and then, the diffusion path is much shorter in microfluidic devices on chip. The study was carried out using a microfluidic device consisting on two patterned plates of poly (methyl methacrylate), which are symmetrical. In the front side, a channel was used as donor (sample) solution whereas in the back side, the channel was used as acceptor phase. Five non-steroidal anti-inflammatories were extracted from acid sample solution (pH 1.5), through the SLM, and into alkaline solution functioning as acceptor phase (pH 12). Two different working modes were tested: double-flow mode and stopped-flow mode. For double-flow working mode, a donor flow rate within the range of 1-30 µL min-1 was tested, keeping the acceptor flow rate at 1 µL min-1 (table 1). However, the acceptor flow rate was stopped during the extraction (stagnant conditions). As seen in table 1, when the acceptor flow is turned off during the extraction time, high enrichment factor significantly increases with the extraction time for all compounds. As an example, the IBU is enriched by a factor of 75 after 25 minutes extraction consuming only 500 µL of sample. On the other hand, a double-flow working mode is preferred when very high extraction efficiencies are desired.



Table

	Double-flow conditions[a,c]		Stopped-flow conditions[b]	
	EE*	EF	EE*	EF
SAC	35 (2)	3	9 (2)	29
KTP	38 (1)	4	8 (2)	25
NAX	42 (2)	4	10 (1)	34
DIC	42 (2)	4	13 (1)	45
IBU	80 (1)	7	22 (2)	75

Table 1: Results: *% Extraction efficiency (%RSD, n=4)
[a] Extraction time: 5 min
[b] Extraction time: 25 min
[c] Acceptor flow rate of 1 µL min-1 and donor flow rate of 9 µL min-1

Biography

Maria Ramos Payan has expertise in improving sample preparation techniques focused on microfluidic-chip devices as miniaturization. The novelty of her microfluidic devices offer more advantages than the existing methodologies. The devices work either using biological and environmental samples and can be coupled on-line to HPLC or Mass Spectrometry. She has also demonstrated the applicability of microchip devices for diagnostic diseases as diabetes. She has worked at different institutions (University of Seville, University of Huelva, University of Lund, University of Copenhagen and University of North Carolina, USA). Currently, she works at Microelectronic National Center of Barcelona and Universitat Autonoma of Barcelona with the aim of implementing optical detection into microfluidic devices for multiple applications.

ramospayan@us.es

conferenceseries.com

María Ramos Payan, J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

An effective microfluidic device for the extraction of fluoroquinolones using liquid phase microextraction and its analysis by HPLC

María Ramos Payan
IMB CNM, Spain

This experimental work reports the first microfluidic-chip based system for liquid-phase microextraction (LPME-chip) for the determination of fluoroquinolones in water samples. In 2011, a HPLC DAD–FLD method combined with prior traditional hollow fiber-liquid phase microextraction was developed for the sensitive determination of eight widely used fluoroquinolones. However, high sample volumes and longer extraction times were needed. In the recent years, miniaturization of analytical procedures has been a tendency with the aim of reducing costs, extraction times and improving extraction efficiencies. We present a poly (methyl methacrylate) microfluidic chip based on a double-flow working mode for the extraction of six fluoroquinolones in water samples. 1-octanol was used as support liquid membrane. Extraction parameters were fixed at pH 3 (donor phase), pH 12 (acceptor phase) and 1 µL/min for both acceptor and sample flow rate; resulting in extraction efficiencies over 40%. This technique offer faster extractions in only 5 minutes and minimum sample volume (less than 10 µL).

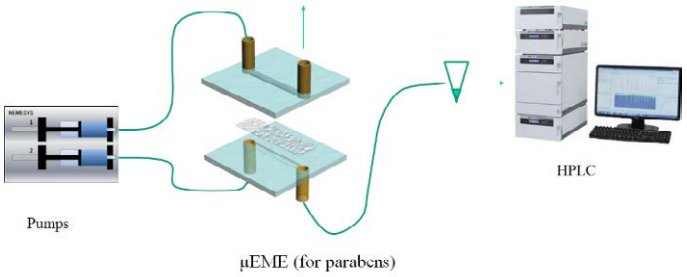


Figure1: Schematic of microfluidic device. The extract collected is analyzed by HPLC.

Biography

Maria Ramos Payan has expertise in improving sample preparation techniques focused on microfluidic-chip devices as miniaturization. The novelty of her microfluidic devices offer more advantages than the existing methodologies. The devices work either using biological and environmental samples and can be coupled on-line to HPLC or Mass Spectrometry. She has also demonstrated the applicability of microchip devices for diagnostic diseases as diabetes. She has worked at different institutions (University of Seville, University of Huelva, University of Lund, University of Copenhagen and University of North Carolina, USA). Currently, she works at Microelectronic National Center of Barcelona and Universitat Autonoma of Barcelona with the aim of implementing optical detection into microfluidic devices for multiple applications.

ramospayan@us.es

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Accepted Abstracts

J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Chromatography at high viscosity

A Schultze-Jena[1, 2], M A Boon[1], P J Th Bussmann[1], A E M Janssen[2] and **A Van Der Padt[2, 3]**
[1]TNO, Netherlands
[2]Wageningen University, Netherlands
[3]FrieslandCampina, Netherlands

Costs associated to chromatographic separation hinder implementation in purification processes in a wide range of industries. On one hand, large food process streams containing valuable complex molecules in low concentration are not fully utilized; processing and auxiliary costs could be reduced if process stream size is reduced. On the other hand, concentrated viscous streams are often diluted prior to chromatographic processing. The added water needs to be removed later on in energy intensive processes. Both situations have sparked interest of researchers and industry in high viscosity chromatography. Minimizing process streams is advantageous for the size of equipment, but leads to higher viscosities which will decrease mass transfer and increase pressure drop. The influence of feed viscosity on separation performance has largely been ignored in literature and practice. The objective of this research was to investigate separation performance as a function of viscosity for food type streams. Benefits due to decreased stream volume and disadvantages due to increased viscosity were evaluated, aiming to find maximum productivity (gproduct/m^3resin hour). Separation performance was evaluated for a range of tracers in a preparative lab scale system using a size exclusion resin for different viscosities. Viscosity was increased using sucrose. For comparison either linear velocity or pressure drop over the column bed were kept constant. Mass transfer models were applied to account for observed effects on column efficiency and peak shape due to viscosity. These models were used to predict the influence of viscosity on productivity. The results are especially relevant for industries other than pharmaceuticals, where main driver for processes development is cost reduction.

anton.schultze-jena@wur.nl

Determination of ochratoxin A in food samples by liquid chromatography/electrospray ionization triple quadrupole-MS-MS spectrometry

Deming Song
Food and Drug Administration Northeast Region Laboratory, USA

Ochratoxin A (OTA) is the most important compound in a group of structurally related mycotoxins. It is a potent carcinogen in rats and possibly associated with human kidney diseases in certain countries. OTA is a moderately stable molecule that will survive to some extent most food processing (e.g. boiling, baking, roasting or fermentation) and may thus occur in consumer products. Therefore, food chemistry branch, Northeast Regional Laboratory of FDA is monitoring OTA in a number of food products. We have established a method by HPLC-ESI-MS-MS for the analysis of OTA. By engaging SRM (selective reaction monitor) mode mass spectrometry for four OTA most distinguish and strong ions m/z 404, 386, 358 and 239, not only has the specific identification power against the method by HPLC-fluorescence detector, but also this method can reach the sensitivity of 1 parts per billion concentration (ppb) with injection volume 8 µl equivalent to 8 pg on column for quantification analysis of OTA.

deming.song@fda.hhs.gov

J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Novel technology for protein capture: Mixed-mode expanded-bed adsorption

Dong-Qiang Lin and **Shan-Jing Yao**
Zhejiang University, China

For biopharmaceuticals downstream processes, it always requires highly productive and robust technologies to improve the process efficiency. Expanded-bed adsorption (EBA) is an innovative technology that allows capturing proteins directly from un-clarified feedstock, such as cell culture broth and homogenization. EBA technology combines solid-liquid separation with an adsorption step in a single-unit operation, aiming at increased overall yield, reduced operational time, and less requirements for capital investment and consumables. Mixed-mode chromatography (MMC) is a novel technology for bio-product separation, which combines multiple binding modes like hydrophobic and electrostatic interactions, hydrogen bonding, etc. High capacity, salt-tolerance, good selectivity and relatively low cost are the major advantages of MMC for direct capture process. In the present work two chromatographic techniques, EBA and MMC, were integrated to develop new separation technology, mixed-mode EBA, improving the protein capture efficiency and reducing the pretreatments on the feedstock, such as clarification, dilution and salt-adjustment. Several MMC ligands were coupled onto typical matrices (densified agarose beads) for EBA. The static adsorption, adsorption kinetics and dynamic binding were investigated, and the effects of pH and salt addition were evaluated. New technology was challenged with two typical biopharmaceutical processes, monoclonal antibody (mAb) capture from CHO cell culture broth and recombinant human albumin serum (rHSA) isolation from Pichia pastoris fermentation broth. After the optimization of operation conditions, high separation efficiency (purity, recovery, productivity) was obtained. The results demonstrated that mixed-mode EBA, combining the advantages of EBA and MMC, would be a promising new platform for protein capture with reduced feedstock pretreatments, high efficiency and relative low cost. New technology developed in the present work could also be expanded to other bio-product processes.

lindq@zju.edu.cn

Adsorptive removal of Triton X-100 from human plasma and its derivatives

Gargi Redkar and **Sandeep Kale**
[1]DBT-ICT-Centre for Energy Biosciences, India
[2]Institute of Chemical Technology, Maharashtra, India

Viral transmission during the use of human plasma and its derivatives to treat various medical conditions can be fatal. Solvent/detergent treatment using non-ionic detergents like Triton X-100 inactivate the lipid enveloped viruses. However, the detergent interferes with downstream processing and analysis. Also, WHO permits a residual level of <25 ppm of Triton X-100 and thus it needs to be removed from post viral inactivation. Removal of Triton X-100 poses a challenge due to its low CMC and non-ionic character. Selective removal of Triton X-100 was studied using various hydrophobic resins screened on the basis of adsorption capacity, uptake kinetics and effect of plasma proteins on these parameters. Resins showing higher adsorption capacity and uptake rate with lower protein binding were selected for column studies. Breakthrough capacity of the shortlisted resins was determined at different flow rates and concentrations along with the effect of proteins. A simple and sensitive HPLC method was developed to detect Triton X-100 in the treated samples at ppm level. This research work asserted the impact of various resin characteristics and plasma proteins on selective detergent removal and thus the mechanism of adsorption of Triton X-100 onto these resins.

sb.kale@ictmumbai.edu.in

J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Gas chromatography: A novel and new technique for on-line cure monitoring studies of carbon-phenolic composite structures

K Veera Brahmam and **V Venkateswara Rao**
Advanced Systems Laboratory, Hyderabad, India

We report the use of gas chromatography technique for on-line cure monitoring of the carbon-phenolic(C-P) composites to identify the gelation region for pressure application to enhance the thermal performance. Experimental trials are made on the laminates prepared by hand lay-up process in autoclave. During curing process methylol-phenol (M-phenol) and water are evolved as reaction by-products and the concentrations of the evolved byproducts are monitored by on-line gas chromatograph connected to autoclave facility. Experimental parameters like sample injection method, sample volume and time of injection have been optimized and m-phenol and water evolution concentrations are monitored as a function of component temperature. M-phenol evolution is more consistent compared to water evolution and therefore based on the falling trend of the methylol phenol concentration a broad region of gelation for pressure application is identified. The region thus identified is further narrowed down based on the diminishing trend of the M-phenol concentration and the experimental analysis of the laminates focusing on resin content and void content. Finally the on-line pressure application criterion was established based on the resin content and void content data. Based on the resin content and the low void content the performance of the C-P structure is evaluated.

kveerabrahmam@rediffmail.com

Efficient HPLC enantiomer separation using novel homo chiral metal-organic frameworks as chiral stationary phases

Koichi Tanaka
Kansai University, Japan

Porous metal-organic frameworks (MOFs) have attracted much attention in the last decade because they can be used for various potential applications such as gas storage, separation, sensing, and catalysis. Recently, MOFs have been considered as separation materials for chromatography owing to their high surface area, uniform structural cavities, outstanding thermal and chemical stability, and selective adsorption phenomena. To date, a number of chiral MOFs have been developed and synthesized. Chiral MOFs have great potential as chiral stationary phases (CSPs) for HPLC enantioseparation of racemic compounds. However, there are only a few attempts of chiral MOFs being used in liquid chromatographic enantioseparation; most of which exhibited a relatively narrow range of chiral enantioselectivity. Herein, we report novel homochiral pillared MOFs (1, 2) with excellent selectivity for HPLC enantiomeric separation of various racemates such as sulfoxides, sec-alcohols and flavanones. The racemates of sec-alcohols were well separated on both the MOF-1 and MOF-2 columns. The separation factors ( ) were also larger for p-substituted derivatives than for their o- and m-substituted counterparts, which is most likely because of steric constraints. Interestingly, the introduction of electron-withdrawing substituents on the aromatic ring gave rise to a prominent base-line separation of enantiomers as shown in Figure 1. The π-π interactions between the N-donor linker of the framework and electron-deficient aromatic group of compound may be one of reasons.

ktanaka@kansai-u.ac.jp

J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Chromatographic detection of inborn error of metabolism in Egyptian pediatrics, two years of experiences

Shimaa Abdelsatar, Manar Obada and **Hala Elsaid**
National Liver Institute, Egypt

Background: Inborn errors of metabolism (IEM) represent a special challenge in pediatric practice. Newborn screening approaches by tandem mass spectrometry (MS/MS) coupled to gas chromatography (GC) early in infancy help in rapid and well-timed therapeutic interference to prevent overwhelming neurological outcomes.

Aim: Aim of this study is to estimate the effectiveness of the metabolic alterations as a rapid non-invasive metabolomics screening technique for diagnosis of different type of IEMs to establish diagnostic clue to IEM in high risk Egyptian pediatric for proper treatment and better outcome.

Methods: During 2015-2016, samples of 480 patients were analyzed. Quantitative measurements of amino acids and acylcarnitine profiles using MS/MS and of organic acids using GC/MS were done.

Results: 39 (39/480, 8.1%) of the patients were diagnosed to have IEM. The following disorders were identified; organic acidopathies: 25 (64.1%), amino acidopathies: 9 (23.1%), fatty acid disorders (FAO): 3 (7.7%) and lactic academia (LA): 2 (5.1%).

Conclusion: Newborn screening program should be established in Egypt as the overall incidence of IEM was found to be high due to raised ratio of consanguinity; this approach could prevent, or at least, reduce serious neurological and developmental sequelae, improve survival and reduce mortality and morbidity of patients.

manarobada@yahoo.com

Chemical composition of medicinal plant of *Atractylis* genus

Mohamed Ibrahim Badaoui, Mohamed Benkhaled and **Hamada Haba**
Batna-1 University, Algeria

The research of active principles extracted from the aromatic and medicinal plants is of a key importance, because it allows the development of drugs to maintain the health of the human being. It is for this reason that we are interested in this type of investigation in our research laboratory. Besides, our laboratory has recently started a research program intended for the systematic study of medicinal plants belonging to the genus Atractylis (Asteraceae family) in view of their valorization. The present study focuses mainly on the species *Atractylis cancellata*. The Asteraceae family is characterized by its wealth in secondary metabolites of biological interest such as the triterpenes, the phenolic compounds of type flavonoids, and sesquiterpenes. The plants of the genus Atractylis are deemed in traditional medicine for treating many diseases. The main purpose of our work is to isolate and identify secondary metabolites that may have a biological activity from the different organic extracts of the species *A. cancellata*. The hydro-alcoholic extraction: alcohol/water, separation and purification chromatographic: VLC, CC and TLC of extracts petroleum ether and ethyl acetate acquired from the whole plant *A. cancellata*, allowed us to isolate and identify secondary metabolites of different types flavonoids, sterols and triterpenes. The determination of their molecular structures is performed by the spectroscopic analysis methods such as the NMR 1D (1H, 13C J-modulated and DEPT) and the NMR 2D (COSY, HSQC, HMBC, and NOESY) and the mass spectrometry ESI-MS. It is mainly of lupeol, oleanolic acid, β-sitosterol and apigenin.

badaoui.ibrahime@univ-batna.dz

J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Variations in GC–MS response between analytes and deuterated analogs

Muhammed Alzweiri
University of Jordan, Jordan

Isotopic analogue is commonly used as an appropriate internal standard. It was reported that analytes have usually higher mass responses than their equimolar deuterated analogues (DAs) leading to quantification discrepancy. Standard addition method on dimethyl azelate (DMA) and d6- dimethyl azelate (d6-DMA) was adopted to examine possible reasons for the problem. Cross contribution of mass responses, intermolecular deuterium-hydrogen exchange during chromatographic separation, and deviation in mass ionization response of C-H against C-D bonds were studied as possible reasons for this discrepancy. GC-MS analysis revealed that neither cross contribution of ions nor H2/H exchange were possible reasons behind the difference in responses between DMA and d6-DMA relying on linearity and trans-esterification studies respectively. On the other hand, a study of carbon nucleus relaxation conducted by C13-NMR depicted that energy dissipation through C-D bond is faster than that through the C-H bond; relaxation rate of carbonyl carbon in d6-DMA and DMA were 9 and 3 sec-1 respectively. Accordingly, the energy transfer through the carbon skeleton of analytes and its mass ionization degree are more efficient than those in their DA counterparts. Conclusively, GC-MS analysis of analyte, relying on the assumption of equal response with its DA, generates overestimated analytical results of analytes.

m.alzweiri@ju.edu.jo

Simultaneous determination of sofosbuvir, paracetamol and methionine in rat plasma using thin-layer chromatography and its application to pharmacokinetic study

Nada Abdelwahab
Beni-Suef University, Egypt

Sofosbuvir (SOF) is a widely used drug for treatment of chronic hepatitis C while paracetamol (PAR) is the recommended analgesic for patients with hepatitis C because of its effectiveness and safety. Combination of PAR with methionine (MET) is preferred to reduce the severity of liver damage that may be produced from PAR overdose. A sensitive and highly selective TLC-densitometric method was developed for the first time for simultaneous analysis of SOF and the accompanied medications PAR and MET in the presence of the internal standard, naphazoline HCl (NAP) in rat plasma. Complete separation between the studied components peaks and plasma peak was obtained where Rf value of MET=0.18, NAP=0.39, PAR=0.59 and SOF=0.82. FDA recommendations for bio-analytical method validation were obeyed. The linearity of the method was assessed over the concentrations range 160-3000 ng mL-1 for both SOF and PAR and 300-3000 ng mL-1 for MET. Moreover, the accuracy, intra-and inter-day precision of the quality control samples at low, medium and high concentration levels exhibited relative standard deviations (RSD)<10%. Freezing-thawing stability was also tested; additionally pharmacokinetic and pharmacodynamics co-relation of the studied drugs in animal model has been done. The developed method can be easily used during accurate monitoring of the studied drugs.

nadasayed2003@yahoo.com

J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Chromatographic analysis of ledipasvir and sofosbuvir: New treatment for chronic hepatitis C infection with application to human plasma

Nehal Farid
Beni-Suef University, Egypt

A RP-HPLC/DAD method was developed and validated for the first time for the analysis of the newly formulated anti-HCV combination, in pure form, pharmaceutical formulation and in human plasma. In the developed method separation was carried out on Zorbax® Eclipse C18 column using a gradient mixture of acetonitrile: water as a mobile phase and scanning was performed at 260 nm (for SOF) and 330 nm (for (LED). The two drugs were completely separated from each other and from plasma, where plasma peak appeared at 2.76±0.05 min, SOF at 4.25±0.05 and LED at 7.35±0.05. The developed method showed high sensitivity, the drugs showed linearity in the range of 1-45 µg/mL for both pure form and spiked human plasma. Three freeze-thaw cycles were carried out separately at two different temperatures, -8oC and -20oC. No significant loss of the studied drugs was observed during repeated thawing and freezing. Validation parameters such as accuracy, precision, robustness and ruggedness were tested in compliance with USP recommendations, where acceptable results were obtained. Applying to pharmaceutical formulation showed no interference from tablet excipients.

nehalfayek@gmail.com

A novel mesogenic ether crown stationary phase for reversed-phase liquid chromatography

Ouassila Ferroukhi[1], **Nihad Mermat**[1], **Saliha Guermouche**[1], **Jean-Pierre Bayle**[2] and **Moulay Hassene Guermouche**[1]
[1]University of Science and Technology Houari Boumediene, Algeria
[2]University of Paris-Sud, France

The development of new bonded stationary phases is an important aspect in reversed-phase liquid chromatography (RPLC) the most used mode in high-performance LC. A novel mesogenic ether crown stationary phase has been synthesized and characterized (figure 1). The phase was obtained by coupling between Lichrospher Si 100 NH2 and the mesogenic carboxylic ether crown acid liquid crystal ECLC. Characterization of ECLC was made with proton NMR, and the nematic state was determined by DSC. Thermal study of the new material exhibit transitions in Van't Hoff plots indicating changes of the structure of the phase during heating. Analytical chromatographic behaviors of the new bonded liquid crystal stationary phase BLCSP were investigated by reversed phase LC. Separation of poly-aromatic hydrocarbons (PAHs) is described using high water content mobile phase. Bonded materials exhibit a liquid crystal-like behavior and molecular shape recognition toward planar and non-planar solutes probably due to the mesogenic state. The long nematic chain combined with a terminal ether crown imparted the new stationary phase fine selectivity towards PAH isomers. The shape selectivity demonstrated by the BLCP is higher before the transition occurring around 40°C. Using acetonitrile/water (35/65), reversed phase data of polyaromatic hydrocarbons showed significant solute planarity recognition of anthracene/o-terphenyl. The shape discrimination was evaluated by some pairs of isomers like phenanthrene/anthracene and chrysene/tetracene. The linear solute with the high length to bread ratio L/B is more retained on the new stationary phase.

ferroukhiouassila@yahoo.fr

J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Novel combinatorial ligand for antibody separation: Peptide ligand with hydrophobic charge-induction group

Shan-Jing Yao, Hui-Li Lu, Qi-Lei Zhang and **Dong-Qiang in**
Zhejiang University, China

Affinity biomimetic chromatography with peptide ligands is a novel technology for antibody purification, which has the advantages of high specificity, stable ligand structure and low cost. However, due to high affinity of specially-designed ligands for antibody molecule, harsh conditions often have to be used to elute the antibody from affinity resins, which certainly cause some damages on the antibody structure and biologic activity. Hydrophobic charge-induction chromatography (HCIC) is also one of new techniques for antibody purification with dual-mode ligands that combine hydrophobic and electrostatic interactions. Target proteins can be adsorbed on uncharged ligands at neutral pH via hydrophobic interactions, and eluted via electrostatic repulsion between target protein and the charged ligand at acidic condition. HCIC process has shown the advantages of salt-tolerance, mild elution and flexible CIP, but the binding selectivity was limited due to relatively simple chemical structure of HCIC ligands. In the present work, peptide ligands were combined with hydrophobic charge-induction groups to developed new type of combinatorial ligands for antibody separation. On one hand, the structure of peptide ligands was designed via the molecular simulation to ensure high affinity to antibody. On the other hand, the hydrophobic charge-induction groups were introduced to enhance the antibody binding and benefit the elution via electrostatic repulsion with the charge-induction effects. The combinatorial ligands were synthesized and coupled onto agarose beads to prepare new affinity resins. The adsorption behaviors of IgG were investigated, and typical pH-dependent adsorption was found. New resins were evaluated with antibody purification from CHO cell culture broth, and high process efficiency was obtained. The results demonstrated that novel combinatorial ligands integrated high affinity of peptide ligand to improve binding selectivity and charge-induction effects for convenient elution, which is promising for cost-effective and large-scale purification of antibodies.

yaosj@zju.edu.cn

Liquid chromatographic enantioseparation of (RS)-mexiletine and (RS)-fluoxetine using chiral derivatizing reagents

Sonika Batra and **Ravi Bhushan**
GD Goenka University, India

Enantiomeric separation of racemic mexiletine and fluoxetine was achieved using three chiral derivatizing reagents (CDRs) based on (S)-naproxen. Diastereomers were synthesized by reaction of mexiletine or fluoxetine with the CDRs and were separated on a C18 column under reversed-phase conditions using a binary mixture of acetonitrile and triethylammonium phosphate/water, with UV detection at 230 and 226 nm. The results obtained for enantioseparation of the two drugs using the three CDRs were compiled and compared. The conditions for derivatization and chromatographic separation were optimized. The method was validated for linearity, repeatability, limit of detection and limit of quantification.

sonikabatra211@gmail.com

J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Gas chromatography-mass spectrometry determination of o-phthalates in water coupled with liquid-liquid micro-extraction

Valentin A Krylov and **VeraV Nesterova**
Nizhny Novgorod State University, Russia

Esters of o-phthalic acid are the high toxic compounds. The plasticized polymers are the main source of the emergence of esters of o-phthalic acid in the environment. Gas chromatography-mass spectrometry method coupled with liquid-liquid micro-extraction pre-concentration was used for high sensitive determination of o-phthalates in water. The optimal extractant volume (10 μL) was calculated from dependence of the impurities recovery on partition coefficient of impurities between the extractant (n-octane) and water. It was shown that the ultrasound assisted micro-extraction is an efficient method for pre-concentration of o-phthalates. Application of extract capillary collection solved the problem of the light extractant sampling. The following sources of systematic errors of the determination of o-phthalates have been found: Leaching of di-alkyl-o-phthalates from chromatographic septum; o-phthalates impurities in solvents; the hydrolytic lability of esters of o-phthalic acid. It was shown that the uncontrolled impact of these factors could lead to changes in the actual concentration of impurities determined at 1-2 orders of magnitude. The methods of accounting and elimination of systematic errors are proposed. Rayleigh distillation method was recommended for solvents purification. The storage time of water samples should not exceed three days. The lowering of o-phthalates leaching was achieved using merlin septa. The expanded uncertainty was calculated. It included precision, uncertainty of standards preparation, calibration, sample introduction, enrichment factor. The relative expanded uncertainty was at the level of 12.8–29.6%. The limits of detection and quantification of o-phthalates achieved were at the level of 10-5–10-6 mg L^{-1} and were highly competitive with the best world results.

k658995@mail.ru

Synthesis of two new derivatizing reagents and their application to separation of chiral drug

Vinod Kumar Vashistha[1] and **Ravi Bhushan**[2]
[1]GLA University, India
[2]Indian Institute of Technology Roorkee, India

Under appropriate reaction conditions of reaction time, temperature and solvent, the chlorine atoms in cyanuric chloride (2,4,6- trichloro-1,3,5-triazine; CC) can be substituted by nucleophiles in a controlled sequential manner, resulted into MCT (monochloro triazine) and DCT (dichloro triazine) based CDRs. Commercial availability and economic suitability makes the CC a more attractive starting material for the synthesis of CDRs. By these characteristics, synthesis of two MCT reagents, namely, N-(4-chloro-6-piperidinyl-[1,3,5]- triazine-2-yl)-L-Isoleucine; (CDR-1) and N-(4-chloro-6-piperidinyl-[1,3,5]- triazine-2-yl)-L-Methionine; (CDR-2) were carried out by nucleophilic substitution of one of the chlorine atoms by a piperidinyl group and the second with L-amino acids (as the chiral auxiliary). These reagents were characterized and used for derivatization of (RS)-isoprenaline (spiked in human plasma). The diastereomeric derivative were separated on a reversed-phase C18 column with a mobile phase consist of acetonitrile and 0.1% TFA under gradient mode from 35-65% of acetonitrile at a flow rate of 1.0 mL min-1 and UV detection at 254 nm. The method was validation according to ICH guidelines. The separation mechanism and elution order of the diastereomeric derivative were proposed and supported by developing the geometry optimized lowest energy structures of the two diastereomers using a DFT based program, Gaussian 09 Rev. A.02 and hybrid density functional B3LYP with 6-31G basis set. In L-(R)-diastereomer, the bulky moieties, alkyl group on the stereogenic center of amino acid (present in the CDR), and phenyl group on stereogenic center of Ipn, are oriented on the same side with respect to triazine moiety, i.e., cis orientation. In L-(S)-diastereomer, these groups are oriented in a manner anti to each other with respect to triazine moiety and thus have a trans-type arrangement. Therefore, being less hydrophobic, it is L-(S)-diastereomer which eluted before its counterpart.

vinod.vashistha@gla.ac.in

J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Identifying natural synergist from *Pongamia pinnata* using high-speed counter-current chromatography combined with isobolographic analysis

Weihong He and **Hao Yin**
South China Sea Institute of Oceanology, China

For identifying the synergistic compounds from *Pongamia pinnata*, an approach based on high-speed counter-current chromatography (HSCCC) combined with isobolographic analysis was designed to detect the synergistic effects in the complex mixture. In the approach, the complex mixture was considered as the combination of two individual samples for isobolographic analysis: the target compound and the mixture with complete removal of the target compound (subtracted residue). The two samples were prepared by HSCCC, and were used for the calculation of the expected effect of their combination. Using this approach, three compounds representing the major peaks in the HPLC of the brine shrimp toxic extract from P. pinnata (brine shrimp lethality test (BST) LC50 36.5 μg/mL), pinnatin (1), 3,7-Dimethoxy-3,4'-methylenedioxy flavone (2), and karanjin (3), were prepared from the extract, and were tested for their synergistic potency by BST. The two-phase solvent system containing n-hexane-ethyl acetate–MeOH–water (14:7:10:10, v/v/v/v) was selected for the one-step HSCCC separation according to the partition coefficient values (K). The extract was separated into seven fractions (Fr1–7) by HSCCC with a total mass recovery of 96.3%. Fr2, 4, and 6 were the peak fractions corresponding to compounds 3, 2, and 1, respectively. The purities and recoveries of the target compounds after the chromatographic analysis were 95.9%–97.5% and 92.2%–96.1%, respectively. The subtracted residue of each target compound was performed by recombining all HSCCC fractions except the fraction containing the target compound. Isobolographic analysis disclosed a significant synergistic effect between karanjin and its subtracted residue (potency ratio of 0.47), which gave clear evidence that the toxicity of the extract results from synergistic interactions, and karanjin was one of the synergists participating in the interaction. The other two compounds were excluded from the synergism because these two compounds showed additive effects with their subtracted residues.

weihonghe@scsio.ac.cn

Analysis of free fatty acids in olive oils by UPHLC–MS

Zeid Abdullah Alothman
King Saud University, Saudi Arabia

A simple, fast, highly efficient and direct method using ultra-performance liquid chromatography coupled to mass spectrometry has been established for the simultaneous separation, identification and quantitation of a few saturated and unsaturated fatty acids in olive oils from various countries. Many methods have already been found in the literature for the analysis of fatty acids. No sample pretreatment techniques were employed such as extraction or derivatization for the analysis of target acids from oil samples, as the oil samples were just diluted, filtered and then directly injected to the instrument. The chromatographic separations of all target fatty acids were achieved on a Hypersil Gold C18 column of particle size 1.9 μm, 50×2.1 mm I.D, while the gradient elution using a binary mobile phase mixture of acetonitrile and water at a flow rate of 1.5 ml/min was adopted for achieving optimum separations. The identification and quantitation of target compounds was accomplished using selected ion reaction monitoring mode. The recoveries of the fatty acids were obtained higher than 89% with good validation parameters; linearity (r2>0.992), detection limit between 0.09 and 0.24 μg/ml, run to run and day to day precisions with percent relative standard deviation lower than 2.4% at both low (1 μg/ml) and medium (10 μg/ml) concentration levels. The total content of fatty acids in each individual oils was found in the range of 472.63–7751.20 μg/ml of olive oil, while oleic acid was found to be the major fatty acid among all analyzed oils with the amount 3785.94 μg/ml (maximum) in Syrian olive oil. The obtained validation parameters confirm that the proposed analytical method is rapid, sensitive, reproducible and simple and it could be applied for the successful evaluation of fatty acids in various oils and other matrices. All the fatty acids were efficiently eluted in a time of less than 8 min with well resolved peaks by employing the proposed method.

zaothman@ksu.edu.sa

J Chromatogr Sep Tech 2017, 8:5(Suppl)
DOI: 10.4172/2157-7064-C1-032

Identify histone acetylation in acute lymphoblastic leukemia with liquid chromatography

Zhong J F, Zhang C, Stucky A, Chen X L, Press M F and **Zhang X**
University of Southern California, USA

High-performance liquid chromatography (HPLC) has been used for the study of proteins and the characterization of their post-translational modifications. Acute lymphoblastic leukemia (ALL) is generally considered as a genetic disease (disorder) with an aggressive tumor entity of highly proliferative malignant lymphoid cells. However, in recent years, significant advances have been made in the elucidation of the ALL-associated processes. Histone acetylation is involved in the permanent changes of gene expression controlling ALL developmental outcomes. Identifying histone acetylation with HPLC could potentially provide a method for ALL diagnosis and prognosis. Here, we used HPLC to profile histone acetylation from ALL bone marrow samples and correlated the result to clinical outcomes. Initial results suggested that histone acetylation could be used for ALL prognosis and treatment evaluation.

jzhong@usc.edu