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Abstracts

Chairmen:
Guy T. Carter
Edward J. Kennelly

Issue Editor:
Mark O'Neil-Johnson

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ern Cross Plant Science, Southern Cross University, e 2480, Australia

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determination of absolute configurations hydroxy acids by the expansion of Marfey's od

K[1], Lim C[2], Kim S[2], Oh DC[1]
al Products Research Institute, College of Pharmacy, National University, Seoul 151 – 742; [2]College of acy, Seoul National University, Seoul 151 – 742, ic of Korea

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natural product chemists.

race level voltammetric determination of mercury and toxic metals in tea matrices

li C[1], Melucci D[1], Locatelli M[2]
timento di Chimica "G. Ciamician", Università degli

(II) by square wave anodic stripping voltammetry (SWASV) in matrices involved in food chain as tea leaves is proposed. The digestion of each matrix was carried out using a concentrated HCl-HNO_3-H_2SO_4 acidic attack mixture. 0.01 mol/L EDTA-Na_2 + 0.06 mol/L NaCl + 2.0 mol/L $HClO_4$ was employed as the supporting electrolyte. The voltammetric measurements were carried out using a conventional three electrode cell, employing, as working electrodes, a gold electrode (GE) and a stationary hanging mercury drop electrode (HMDE). The analytical procedure has been verified on the standard reference materials Spinach Leaves NIST-SRM 1570a, Tomato Leaves NIST-SRM 1573a and Apple Leaves NIST-SRM 1515. For all the elements, the precision as repeatability, expressed as relative standard deviation (s_r) was of the order of 3 – 5%, while the accuracy, expressed as relative error (e) was of the order of 3 – 7%. Once set up on the standard reference materials, the analytical procedure was applied to commercial tea leaves samples. A critical comparison with spectroscopic measurements is also discussed.

PI35 Mutual interference problems in the simultaneous voltammetric determination of ultra-trace total mercury(ii) and toxic metals in medicinal herbs matrices

Locatelli C[1], Melucci D[1], Locatelli M[2]
[1]Dipartimento di Chimica "G. Ciamician", Università degli Studi di Bologna, Via F. Selmi 2, I-40126 Bologna, Italy; [2]Dipartimento di Scienze del Farmaco, Università degli Studi "G. d'Annunzio" Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy

The work describes the the voltammetric determination of mercury (II), copper (II), lead (II), cadmium (II), zinc (II) by square wave anodic stripping voltammetry (SWASV) in medicinal herbs. The digestion of each matrix was carried out using a concentrated HCl-HNO_3-H_2SO_4 acidic attack mixture. 0.01 mol/L EDTA-Na_2 + 0.06 mol/L NaCl + 2.0 mol/L $HClO_4$ was employed as the supporting electrolyte. The voltammetric measurements were carried out using a conventional three electrode cell, employing, as working electrodes, a gold electrode (GE) and a stationary hanging mercury drop electrode (HMDE). The analytical procedure has been verified on the standard reference materials Spinach Leaves NIST-SRM 1570a, Tomato Leaves NIST-SRM 1573a and Apple Leaves NIST-SRM 1515. For all the elements, the precision as repeatability, expressed as relative standard deviation (s_r) was of the order of 3 – 6%, while the accuracy, expressed as relative error (e) was of the order of 3 – 7%. Once set up on the standard reference materials, the analytical procedure was applied to commercial medicinal herbs samples. A critical comparison with spectroscopic measurements is also discussed.

PI36 Analysis of phenolic compounds in flowers from wild medicinal plants from northeastern Portugal

Barros L[1,2], Dueñas M[2], Carvalho AM[1], Ferreira ICFR[1], Santos-Buelga C[2]
[1]CIMO-ESA, Polytechnic Institute of Bragança, Portugal; [2]GIP, Faculty of Pharmacy, University of Salamanca, Spain

This study aimed to analyse phenolic compounds in wild medicinal flowers of *Crataegus monogyna*, *Cytisus multiflorus*, *Malva sylvestris* and *Sambucus nigra*, by HPLC-DAD-ESI/MS. Flavonols and flavones were the main groups in almost all the studied samples. *C. multiflorus* sample gave the highest levels of flavonoids, being a chrysin derivative the most abundant flavone. *C. monogyna* revealed the highest concentration in phenolic acids that were not found in *C. multiflorus*; 5-*O*-caffeoylquinic acid was the most abundant phenolic acid found in the first species, being a procyanidin trimer also found. Kaempferol-3-*O*-rutinoside and quercetin-3-*O*-rutinoside were the main flavonols present in *M. sylvestris* and *S. nigra*, respectively. The studied flowers could be selected for processing extracts with health-promoting properties or to be incorporated into functional beverages or products with bioactive properties related to oxidative stress. Acknowledgements: PEst-OE/AGR/UI0690/2011, SFRH/BPD/4609/2008 (L. Barros), Ramón y Cajal (M. Dueñas).

peripheral neuropathy (DPN) as well as diabetes mellitus. We carry on the study on bioactive fraction and compounds of Radix Astragali for DN and DPN. The bioactive fraction was identified by LC/MS^3 on the basis of separated active compounds, besides astragaloside IV (ASI) was focus on in our long term study. 16 peaks in the HPLC spectrum were determined by analysis their ESI-MS^3 spectra and retention time with isolated compounds and referring to literatures. Astragalosides and flavonoides are the main constituents in bioactive fractions.

PJ32 A simple HPLC method for detecting adulteration of ginkgo extracts with flavonol aglycones

Wohlmuth H[1], Savage K[1], Dowell A[1], Mouatt P[1]
[1]Southern Cross Plant Science, Southern Cross University, Lismore 2480, Australia

Ginkgo leaf contains more than 45 flavone glycosides, most of which are based on the aglycones quercetin, kaempferol and isorhamnetin [1]. Adulteration with rutin, quercetin and other plant extracts has been reported [2,3]. USP and EP/BP monographs for ginkgo extract stipulate 22 – 27% flavonoids, but pharmacopoeial methods cannot effectively detect adulteration with aglycones, because calculation of glycoside content is based on the aglycone content after acid hydrolysis. We developed a modification to the pharmacopoeial methods that enables quantification of both glycosides and free aglycones and applied it to 5 leaf samples and 8 retail ginkgo products. Free flavonol aglycones were not present in leaf samples or in two products. Most products largely met their label claim for flavonol glycoside content and relative aglycone content by USP (Identification Test B), but our method revealed high levels of free quercetin and kaempferol in three products, suggestive of adulteration. The presence of free aglycones in these products meant that the pharmacopoeial methods for calculating flavonol glycosides overestimated the glycoside content by up to 40%. We suggest the USP and EP/BP monographs for ginkgo extract be modified to increase their ability to detect adulteration with flavonol aglycones. References: 1. Lin, L.-Z. et al. (2008) *J Food Agric Chem* 56:6671 – 9. 2. Franz, C. et al. (2011) *Food Funct* 2:720 – 30. 3. Liu, C. et al. (2005) *Analyst* 130:325 – 9.

PJ33 Facile determination of absolute configurations of α -hydroxy acids by the expansion of Marfey's method

Moon K[1], Lim C[2], Kim S[2], Oh DC[1]
[1]Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 151 – 742; [2]College of Pharmacy, Seoul National University, Seoul 151 – 742, Republic of Korea

α-Hydroxy acids are often biosynthetically incorporated in natural small molecules such as depsipeptides from nonribosomal peptide synthetase pathways. While the absolute configurations of α-amino acids are conveniently determined by the LC/MS-based analysis of Marfey's derivatives of amino acids, the current methods to determine the absolute configurations of corresponding α-hydroxy acids require more complicated steps. So we expanded Marfey's method and developed a facile procedure determining the absolute configurations of α-hydroxy acids. The method was evaluated with the LC/MS analysis of the reaction products of various L,D-α-hydroxy acids coupled with Marfey's reagent (L-FDAA). This new method is operationally simple and applicable at a submilligram scale without any purification of the reaction mixture. We applied this facile procedure to a natural depsipeptide, zygosporamide, which bears L-phenylalanine, L,D-leucine, and L-leucic acid, and successfully determined the absolute configurations of its α-amino acids and α-hydroxy acid simultaneously. We believe that our approach may be practically useful for natural product chemists.

PJ34 Ultratrace level voltammetric determination of total mercury and toxic metals in tea matrices

Locatelli C[1], Melucci D[1], Locatelli M[2]
[1]Dipartimento di Chimica "G. Ciamician", Università degli Studi di Bologna, Via F. Selmi 2, I-40126 Bologna, Italy; [2]Dipartimento di Scienze del Farmaco, Università degli Studi "G. d'Annunzio" Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy

An analytical procedure regarding the voltammetric determination of mercury (II) and copper (II), and copper (II), lead (II), cadmium (II), zinc (II) by square wave anodic stripping voltammetry (SWASV) in matrices involved in food chain as tea leaves is proposed. The digestion of each matrix was carried out using a concentrated HCl-HNO_3-H_2SO_4 acidic attack mixture. 0.01 mol/L EDTA-Na_2 + 0.06 mol/L NaCl + 2.0 mol/L $HClO_4$ was employed as the supporting electrolyte. The voltammetric measurements were carried out using a conventional three electrode cell, employing, as working electrodes, a gold electrode (GE) and a stationary hanging mercury drop electrode (HMDE). The analytical procedure has been verified on the standard reference materials Spinach Leaves NIST-SRM 1570a, Tomato Leaves NIST-SRM 1573a and Apple Leaves NIST-SRM 1515. For all the elements, the precision as repeatability, expressed as relative standard deviation (s_r) was of the order of 3 – 5%, while the accuracy, expressed as relative error (e) was of the order of 3 – 7%. Once set up on the standard reference materials, the analytical procedure was applied to commercial tea leaves samples. A critical comparison with spectroscopic measurements is also discussed.

PJ35 Mutual interference problems in the simultaneous voltammetric determination of ultra-trace total mercury(ii) and toxic metals in medicinal herbs matrices

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[1]Dipartimento di Chimica "G. Ciamician", Università degli Studi di Bologna, Via F. Selmi 2, I-40126 Bologna, Italy; [2]Dipartimento di Scienze del Farmaco, Università degli Studi "G. d'Annunzio" Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy

The work describes the the voltammetric determination of mercury (II), copper (II), lead (II), cadmium (II), zinc (II) by square wave anodic stripping voltammetry (SWASV) in medicinal herbs. The digestion of each matrix was carried out using a concentrated HCl-HNO_3-H_2SO_4 acidic attack mixture. 0.01 mol/L EDTA-Na_2 + 0.06 mol/L NaCl + 2.0 mol/L $HClO_4$ was employed as the supporting electrolyte. The voltammetric measurements were carried out using a conventional three electrode cell, employing, as working electrodes, a gold electrode (GE) and a stationary hanging mercury drop electrode (HMDE). The analytical procedure has been verified on the standard reference materials Spinach Leaves NIST-SRM 1570a, Tomato Leaves NIST-SRM 1573a and Apple Leaves NIST-SRM 1515. For all the elements, the precision as repeatability, expressed as relative standard deviation (s_r) was of the order of 3 – 6%, while the accuracy, expressed as relative error (e) was of the order of 3 – 7%. Once set up on the standard reference materials, the analytical procedure was applied to commercial medicinal herbs samples. A critical comparison with spectroscopic measurements is also discussed.

PJ36 Analysis of phenolic compounds in flowers from wild medicinal plants from northeastern Portugal

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PJ37 Qualitative and quantitative analysis of flavonoids in Saba Senegalensis P. leaves by HPLC-DAD-ESI-MS/MS and HPTLC-UV

Mervoyer C, Portet B, Giboulot J, Lubrano C
Centre de Recherche Yves Rocher, 101 Quai Roosevelt, 92444 Issy les Moulineaux Cedex France

Saba senegalensis P. is a tendrilled liana widespread throughout Tropical West Africa, from Senegal to Nigeria. Leaves are essentially used in traditional medicine of several countries as an antiseptic and wound healing agent. To provide major information about the chemical content of the leaves, we performed analysis of secondary metabolites by HPTLC-UV. On our samples collected in Mali, the polyphenolic profile is mainly represented by two flavonoids. Liquid chromatography (LC) coupled to electrospray ionsation (ESI) and tandem mass spectrometry (MS/MS) was used for the identification of these two compounds. Comparison of retention time, UV and MS spectral data of standard compounds allowed us to characterize unambiguously: quercitrin and myricitrin. Quantification was achieved by HPLC-DAD and myricitrin was the main component (average 80%) regardless the date of harvest. Moreover, we optimized a rapid quantitative analysis of quercitrin and myricitrin by thin-layer chromatography with densitometric detection. The results obtained were compared to those of HPLC-DAD ones. This present study described for the first time a qualitative and quantitative fingerprint of *Saba senegalensis* P. and could be helpful in the chemotaxonomic study of this genus and for medicinal purposes.

PJ38 Fractal dimension in mass spectra from herbal extracts: Hypothesis for a new method of phytocomplex characterization

Mattoli L[1], Burico M[1], Mercati V[1], Traldi P[2], Ragazzi E[3]
[1]Research Area, Aboca S.p.A. Società Agricola, Loc. Aboca 20, Sansepolcro, Italy; [2]CNR-ISTM, Corso Stati Uniti 4, Padova, Italy; [3]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via Marzolo 5, Padova, Italy

In order to optimize the quality control of phytochemical products, we propose a non-conventional method of analysis of complex systems, called *fractal analysis*, applied to ESI (*Electrospray Ionisation*) mass spectra. The ESI spectra obtained with phytochemical commercial products (Mattoli et al., *J. Mass Spectrom.* **41:** 1534, 2006; Mattoli et al., *Metabolomics* **7:** 437, 2011) were submitted to fractal analysis using the "box counting" method. Subsequent cluster analysis permitted to determine a distinctive fractal dimension (D_B) for single plant extracts, as well as for mixtures of plant extracts contained in commercial herbal products. On several replicates obtained with different batches, D_B tended to display a normal distribution around a mean value, which might be suggested as a typical reference tag for that product. The fractal approach permitted to characterize the repeatability of the instrumental measure too. Changes in D_B following thermal treatment of samples, to simulate ageing, indicated the ability of the method also to identify appropriate conditions of storage and to suggest stability control interventions. In conclusion, evaluation of mass spectra D_B might be proposed as a new promising technique to be used as a summary measurement of the complexity of the overall composition of a phytochemical product.

PJ39 Analysis of phenolic, polysaccharidic and lipidic fractions of mushrooms from northeast Portugal

Heleno SA[1,2], Barros L[1,3], Martins A[1], Queiroz MJRP[2], Santos-Buelga C[3], Ferreira ICFR[1]
[1]CIMO-ESA, Polytechnic Institute of Bragança, Portugal; [2]Centre of Chemistry, University of Minho, Braga, Portugal; [3]GIP, Faculty of Pharmacy, University of Salamanca, Spain

Mushrooms consumption continues to increase due to their functional benefits and presence of bioactive compounds. Herein, phenolic, polysaccharidic and lipidic fractions of wild mushrooms from Northeast Portugal (*Coprinopsis atramentaria*, *Lactarius bertillonii*, *Lactarius vellereus*, *Rhodotus palmatus* and *Xerocomus chrysenteron*) were analysed. Protocatechuic, *p*-hydroxybenzoic, *p*-coumaric and cinnamic acids were found in the phenolic fraction; ramnose, xylose, fucose, arabinose, fructose, glucose, manose, mannitol, sucrose, maltose and trehalose were quantified in polysaccharidic fraction; linoleic and stearic (only in *Lactarius* sp.) acids, and β- and γ-tocopherols were the main compounds in the lipidic fraction. Acknowledgements: PEst-OE/AGR/UI0690/2011, FCT BD/70304/2010 (S.A. Heleno), BPD/4609/2008 (L. Barros).

PJ40 Characterization of flavonoid glycosides in traveller's tree (Ravenala Madagascariensis S.) leaves by HPLC-DAD-ESI-MS^n

Mervoyer C, Portet B, Giboulot J, Lubrano C
Centre de Recherche Yves Rocher, 101 Quai Roosevelt, 92444 Issy les Moulineaux Cedex France

Flavonoids from the leaves of *Ravenala madagascariensis* S. were characterized for the first time by high performance liquid chromatography method coupled to electrospray ionization (ESI) and mass spectrometry (MS^n experiments). A total of seven flavonols glycosides derived from quercetin and isorhamnetin aglycones were identified. The comparison of retention time, UV and MS spectral data of standard compounds allowed us to assign: quercetin-3-O-rutinoside (rutin), quercetin-3-O-glucoside, isorhamnetin-3-O-rutinoside and isorhamnetin-3-O-glucoside. Identification of quercetin-3-O-robinobioside, isorhamnetin-3-O-robinobioside and isorhamnetin-3-O-galactoside was carried out by interpretation of the MS^2 and MS^3 spectra obtained in positive and negative ionization mode and by preliminary reported studies. Quantification was performed by HPLC-DAD and on our samples collected in Madagascar, rutin was the main compound (average 42%) and quantitative repartition of flavonols glycosides was variable depending on the date of harvest. The phytochemical profile obtained would be a powerful tool to establish analytical specifications in order to assess the quality control of traveller's tree extracts, for cosmetic applications.

PJ41 From drupes to olive oil: How do bioactives variate during a single production procedure?

Kanakis P, Termentzi A, Michel T, Gikas E, Halabalaki M, Skaltsounis AL
National and Kapodistrian University of Athens, School of Pharmacy, Laboratory of Pharmacognosy and Medicinal Chemistry, Athens 15771, Greece

It has been well established, that the beneficial effects of virgin olive oil (VOO) are related to its content in polyphenols and secoiridoid derivatives. Several factors, such as fruit variety, ripening stage, malaxation time, temperature etc, have been mentioned to play key role in the quality of the final product and literature data are contradictory. In the present study we monitored the qualitative and quantitative alterations of numerous bioactive polyphenols and secoiridoids, throughout VOO production from a rich in polyphenols olive variety Koroneiki, at a two-phase oil mill in Greece. The compounds were monitored, with the application of LC-DAD-ESI(-)-HRMS (LTQ-Orbitrap) platform, out of the four main steps of the production procedure: drupes, olive paste, first oil, final refined oil. All initial materials were obtained simultaneously, during a single production line and were similarly extracted with methanol, after de-fatting. The extracts were finally enriched through Diol SPE cartridges before the LC injections. The chemical profiles of extracts, pure compounds and internal standards, were monitored in full scan mode and by ion extraction, in a post-acquisition analysis. Results showed an significant increase in the dialdehydic derivatives, oleacin and oleocanthal from drupes to the oil with a simultaneous decrease in oleuropein and ligstroside, which were absent from the final product. Hydroxytyrosol content was also increased but a great quantity seems to be lost during the final oil refinement processing.

PJ42 A monoclonal antibody-based elisa for the hedgehog inhibitors cyclopamine and cyclopamine-KAAD

Lee ST[1], Panter KE[1], Gardner DR[1], Green BT[1], Welch KD[1], Zhang J[2], Chang CWT[2]
[1]Poisonous Plant Research Laboratory, Agricultural Research Service, United States Department of Agriculture, 1150 East 1400 North, Logan, Utah 84341, USA. [2]Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, Utah 84322, USA

In the late 1960's cyclopamine was isolated from the plant *Veratrum californicum* and identified as the teratogen responsible for craniofacial birth defects including cyclops in the offspring of sheep grazing on mountain ranges in the western United States. More recently, cyclopamine was found to inhibit the hedgehog (Hh) signaling pathway which plays a critical role in embryonic development and is implicated in several types of cancer. Thus, cyclopamine and cyclopamine derivatives have been targeted as potential treatments for certain cancers and other