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Optimization and Validation of a Fast UPLC Method for Simultaneous Determination of Hydroquinone, Kojic Acid, Octinoxate, Avobenzone, BHA, and BHT

Galimany-Rovira, Fanny; Pérez-Lozano, Pilar; García-Montoya, Encarna; Miñarro-Carmona, Montse; Ticó-Grau, Josep R.; Suñé-Negre, Josep M

ABSTRACT

A previously published HPLC method for the simultaneous determination of six major components (hydroquinone, kojic acid, octinoxate, avobenzone, butylated hydroxyanisole, and butylated hydroxytoluene) in a skin-whitening cream was transferred and optimized to an ultra-performance LC system. Separation was achieved in a ZORBAX SB-Phenyl Rapid-Resolution High Throughput column (2.1 × 100 mm, 1.8 μm), using a mobile phase consisting of water with 0.1% acetic acid and acetonitrile at a flow rate of 0.7 mL/min. The column was maintained at 40°C, and detection was carried out at 230 nm using a diode-array detector. These chromatographic conditions allow the separation of the six compounds in 3 min instead of 14 min. The extraction procedure was optimized, reducing the time and demonstrating its suitability. The method was validated according to International Conference on Harmonization guidelines, with respect to specificity, precision, accuracy, and linearity. Selectivity was found to be satisfactory. Linear regression analysis data for all compounds showed a good linear relationship, with r2 > 0.999 in the concentration range of 50–120% of the label claim for each compound. The RSD for precision and accuracy of the method was found to be less than 2% for all compounds. Comparison of system performance with the previously published HPLC method was made with respect to analysis time, efficacy, and resolution. The proposed method is faster and consumes less solvent and was applied in the determination of six major compounds in batches of skin-whitening cream manufactured during the validation process.

Determination of Mitragynine in Mitragyna speciosa Raw Materials and Finished Products by Liquid Chromatography with UV Detection: Single-Laboratory Validation

Mudge, Elizabeth M.; Brown, Paula N

ABSTRACT

Mitragyna speciosa (kratom) is a tree indigenous to Southeast Asia, and its leaves are used in herbal formulations because they contain indole alkaloids mitragynine and 7-hydroxy (7-OH) mitragynine. An HPLC method was developed, optimized, and validated using single-laboratory validation guidelines to quantify mitragynine in kratom raw materials and finished products. The method optimization evaluated several extraction parameters including solvent type, solvent volume, time, and extraction method. The separation of the mitragynine alkaloids was achieved in 18 min with a fused-core C18 EVO column using gradient separation with ammonium bicarbonate (pH 9.5) and acetonitrile. The calibration range for mitragynine was 1.0–500 μg/mL with correlation coefficients of ≥99.9% throughout method development and validation. The method detection limit and LOQ were 0.2 and 0.6 μg/mL, respectively for mitragynine. Eight test samples were obtained to evaluate method repeatability. RSDr ranged from 0.4 to 1.0%, whereas intermediate precision ranged from 3.7 to 7.3%, with HorRat values from 0.68 to 1.96. 7-OH mitragynine was below the LOQ for all samples, therefore, spikes repeatability sample RSD values were <1%. The validation data presented meet the Standard Method Performance Requirements as specified by the AOAC INTERNATIONAL Kratom Working Group.

Analysis of Closely Related Antioxidant Nutraceuticals Using the Green Analytical Methodology of ANN and Smart Spectrophotometric Methods

Korany, Mohamed A.1; Gazy, Azza A.2; Khamis, Essam F.1; Ragab, Marwa A.A.1; Kamal, Miranda F.3

ABSTRACT

Two new, simple, and specific green analytical methods are proposed: zero-crossing first-derivative and chemometric-based spectrophotometric artificial neural network (ANN). The proposed methods were used for the simultaneous estimation of two closely related antioxidant nutraceuticals, coenzyme Q10 (Q10) and vitamin E, in their mixtures and pharmaceutical preparations. The first method is based on the handling of spectrophotometric data with the first-derivative technique, in which both nutraceuticals were determined in ethanol, each at the zero crossing of the other. The amplitudes of the first-derivative spectra for Q10 and vitamin E were recorded at 285 and 235 nm respectively, and correlated with their concentrations. The linearity ranges of Q10 and vitamin E were 10–60 and 5.6–70 μg·mL−1, respectively. The second method, ANN, is a multivariate calibration method and it was developed and applied for the simultaneous determination of both analytes. A training set of 90 different synthetic mixtures containing Q10 and vitamin E in the ranges of 0–100 and 0–556 μg·mL−1, respectively, was prepared in ethanol. The absorption spectra of the training set were recorded in the spectral region of 230–300 nm. By relating the concentration sets (x-block) with their corresponding absorption data (y-block), gradient-descent back-propagation ANN calibration could be computed. To validate the proposed network, a set of 45 synthetic mixtures of the two drugs was used. Both proposed methods were successfully applied for the assay of Q10 and vitamin E in their laboratory-prepared mixtures and in their pharmaceutical tablets with excellent recovery. These methods offer advantages over other methods because of low-cost equipment, time-saving measures, and environmentally friendly materials. In addition, no chemical separation prior to analysis was needed. The ANN method was superior to the derivative technique because ANN can determine both drugs under nonlinear experimental conditions. Consequently, ANN would be the method of choice in the routine analysis of Q10 and vitamin E tablets. No interference from common pharmaceutical additives was observed. Student's t-test and the F-test were used to compare the two methods. No significant difference was recorded.

Quantitative Determination of Anthraquinones and Resveratrol in Polygonum Cillinerve (Nakai) Ohwi by HPLC-PAD

Wu, Yanfang1; Wang, Xinsheng2; Liu, Pu2; Niu, Qingshan2; Wu, Qinan3

ABSTRACT

A simple, sensitive, and validated high-performance liquid chromatography-photodiode array method has been established for the simultaneous determination of anthraquinones and resveratrol in Polygonum Cillinerve (Nakai) Ohwi (Zhushaqi in Chinese). The evaluation was performed using a Sunfire C18 reversed-phase column with 30°C column temperature. The mobile phase was composed of a gradient elution of 0.5% acetic acid (solvent A) and methanol (solvent B) with flow rate of 1.0 mL/min. The detection wavelength was at 254 nm. The method developed and validated is simple, shows good linearity, sensitivity, precision, and recovery, and is applied to analyze anthraquinones and resveratrol in 13 batches of Zhushaqi. The results show that the target compounds of Zhushaqi are significantly different among these samples. Based on results of the statistical analysis, the samples collected from Funiu Mountain were clustered together, and the samples obtained from Bozhou Market were close together. The developed method can be a useful tool in quality control and used to evaluate difference and to identify the geographical area of Zhushaqi, and also to provide technical support for the pharmacological and clinical research of related drugs.

Chromatographic Fingerprints Combined with Chemometric Methods Reveal the Chemical Features of Authentic Radix Polygalae

Xin, Zhongquan1; Ren, Dabing1; Zhang, Xiaojuan2; Yi, Zhibiao3; Yi, Lunzhao1

ABSTRACT

GC-MS fingerprints of Radix Polygalae (RP) were measured for deliberately collected samples. A total of 88 volatile components were identified and quantified by subwindow factor analysis, heuristic evolving latent projection, and retention index. Next, an efficient discrimination model based on partial least-squares (PLS) discriminant analysis (DA) was developed to distinguish the superior RP samples from the inferior ones, and the reliability and predictive ability of the model was evaluated by cross-validation and permutation tests. Furthermore, four components (1-octanol, shyobunone, isobornyl acetate, and α-asarone) were screened by coefficient β of PLS-DA. They represented the important chemical features of authentic RP and could be applied to the accurate discrimination and QC of RP in the future. Our results suggest that chromatographic fingerprints coupled with chemometric methods provide an effective and convenient strategy for QC of RP and are helpful for revealing the chemical features of a complex analytical sample.

Simultaneous Estimation of Ofloxacin, Clotrimazole, and Lignocaine Hydrochloride in Their Combined Ear-Drop Formulation by Two Spectrophotometric Methods

Bodiwala, Kunjan; Shah, Shailesh; Patel, Yogini; Prajapati, Pintu; Marolia, Bhavin; Kalyankar, Gajanan

ABSTRACT

Two sensitive, accurate, and precise spectrophotometric methods have been developed and validated for the simultaneous estimation of ofloxacin (OFX), clotrimazole (CLZ), and lignocaine hydrochloride (LGN) in their combined dosage form (ear drops) without prior separation. The derivative ratio spectra method (method 1) includes the measurement of OFX and CLZ at zero-crossing points (ZCPs) of each other obtained from the ratio derivative spectra using standard LGN as a divisor, whereas the measurement of LGN at the ZCP of CLZ is obtained from the ratio derivative spectra using standard OFX as a divisor. The double divisor-ratio derivative method (method 2) includes the measurement of each drug at its amplitude in the double divisor-ratio spectra obtained using a standard mixture of the other two drugs as the divisor. Both methods were found to be linear (correlation coefficients of >0.996) over the ranges of 3–15, 10–50, and 20–100 μg/mL for OFX, CLZ, and LGN, respectively; precise (RSD of <2%); and accurate (recovery of >98%) for the estimation of each drug. The developed methods were successfully applied for the estimation of these drugs in a marketed ear-drop formulation. Excipients and other ingredients did not interfere with the estimation of these drugs. Both methods were statistically compared using the t-test.

Bioanalytical Method Validation for Dronedarone and Duloxetine in Blood Serum

Chadha, Renu1; Bali, Alka1; Bansal, Gulshan2

ABSTRACT

The present work relates to the development and validation of reversed-phase HPLC–UV-photodiode array methods for the estimation of two drugs in blood serum: dronedarone hydrochloride (DDN), a class III antiarrhythmic drug, and duloxetine hydrochloride (DLX), an antidepressant. Chromatographic analysis of DLX was carried out on a Nucleodur C18 column (250 × 4.6 mm, 5 μm) using ammonium acetate buffer (32 mM, pH 5.5) and acetonitrile (40 + 60, v/v; flow rate of 1.0 mL/min; detection wavelength of 290 nm) as the mobile phase. A Waters XTerra C18 column (250 × 4.6 mm, 5 μm) was used for the chromatographic analysis of DDN using an acetonitrile–ammonium formate buffer (20 mM, pH 3.0, with formic acid; 45 + 55, v/v; flow rate 1.0 mL/min) as the mobile phase. Pentazocine and bupropion HCl were used as the internal reference standards for DLX and DDN, respectively. Excellent linearity was observed for DLX (r2 = 0.9996; concentration range 0.2–10.0 μg/mL) and DDN (r2 = 0.9997; concn. range 2.0–50.0 μg/mL). The LODs for DLX and DDN were 0.022 and 0.78 μg/mL, respectively, and the LOQs 0.066 and 2.4 μg/mL, respectively.

TLC-Densitometric and RP-HPLC Methods for Simultaneous Determination of Dexamethasone and Chlorpheniramine Maleate in the Presence of Methylparaben and Propylparaben

Farid, Nehal F.1; Naguib, Ibrahim A.2; Moatamed, Radwa S.3; El Ghobashy, Mohamed R.4

ABSTRACT

Validated simple, sensitive, and highly selective methods are applied for the quantitative determination of dexamethasone and chlorpheniramine maleate in the presence of their reported preservatives (methylparaben and propylparaben), whether in pure forms or in pharmaceutical formulation. TLC is the first method, in which dexamethasone, chlorpheniramine maleate, methylparaben, and propylparaben are separated on silica gel TLC F254 plates using hexane–acetone–ammonia (5.5 + 4.5 + 0.5, v/v/v) as the developing phase. Separated bands are scanned at 254 nm over a concentration range of 0.1–1.7 and 0.4–2.8 μg/band, with mean ± SD recoveries of 99.12 ± 0.964 and 100.14 ± 0.962%, for dexamethasone and chlorpheniramine maleate, respectively. Reversed-phase HPLC is the second method, in which a mixture of dexamethasone and chlorpheniramine maleate, methylparaben, and propylparaben is separated on a reversed-phase silica C18 (5 μm particle size, 250 mm, 4.6 mm id) column using 0.1 M ammonium acetate buffer–acetonitrile (60 + 40, v/v, pH 3) as the mobile phase. The drugs were detected at 220 nm over a concentration range of 5–50 μg/mL, 2–90 μg/mL, 4–100 μg/mL, and 7–50 μg/mL, with mean ± SD recoveries of 100.85 ± 0.905, 99.67 ± 1.281, 100.20 ± 0.906, and 99.81 ± 0.954%, for dexamethasone, chlorpheniramine maleate, methylparaben paraben, and propylparaben, respectively. The advantages of the suggested methods over previously reported methods are the ability to detect lower concentrations of the main drugs and to show better resolution of interfering preservatives; hence, these methods could be more reliable for routine QC analyses.

Simultaneous Determination of Ursodeoxycholic Acid and Chenodeoxycholic Acid in Pharmaceutical Dosage Form by HPLC–UV Detection

Khairy, Mostafa A.; Mansour, Fotouh R

ABSTRACT

A reversed-phase HPLC method was developed for the simultaneous determination of ursodeoxycholic acid (UDCA) and the epimeric isomer, chenodeoxycholic acid (CDCA), in their synthetic mixtures and in tablet dosage form. The proposed HPLC method uses a C18 column and mobile phase consisting of an acetonitrile–phosphate buffer mixture (pH 2.3, 100 mM; 50 + 50, v/v) at a flow rate of 2.0 mL/min with UV detection at 210 nm. The method was validated according to the International Conference on Harmonization guidelines; and linearity, range, accuracy, precision, robustness, and system suitability were studied. The LOD and LOQ were also calculated and found to be 1.23 and 3.73 μg/mL for UDCA and 0.83 and 2.52 μg/mL for CDCA, respectively. The method was adapted for UHPLC, in which baseline separation was achieved in <2.5 min. The assay results of Ursomix tablets by the developed method were statistically compared with those obtained by the reference method using t- and F-tests, and no significant differences were observed.

Development and Validation of a Reversed-Phase Chiral HPLC Method to Determine the Chiral Purity of Bulk Batches of (S)-Enantiomer in Afoxolaner

Padivitage, Nilusha; Kumar, Satish; Rustum, Abu

ABSTRACT

Afoxolaner is a new antiparasitic molecule from the isoxazoline family that acts on insect acarine g-aminobutyric acid and glutamate receptors. Afoxolaner is a racemic mixture, which has a chiral center at the isoxazoline ring. A reversed-phase chiral HPLC method has been developed to determine the chiral purity of bulk batches of (S)-enantiomer in afoxolaner for the first time. This method can also be used to verify that afoxolaner is a racemic mixture, which was demonstrated by specific rotation. ChromSword, an artificial intelligence method development tool, was used for initial method development. The column selected for the final method was CHIRALPAK AD-RH (150 × 4.6 mm, 5 μm particle size), maintained at 45°C, and isocratic elution using water–isopropanol–acetonitrile (40 + 50 + 10, v/v/v) as the mobile phase with a detection wavelength of 312 nm. The run time for the method was 11 min. The resolution and selectivity factors of the two enantiomers were 2.3 and 1.24, respectively. LOQ and LOD of the method were 1.6 and 0.8 μg/mL, respectively. This method was appropriately validated according to International Conference on Harmonization guidelines for its intended use.

Evaluation of 3M Molecular Detection Assay (MDA) 2–Listeria for the Detection of Listeria Species in Select Foods and Environmental Surfaces: Collaborative Study, First Action 2016.07

Bird, Patrick1; Flannery, Jonathan1; Crowley, Erin1; Agin, James1; Goins, David1; Monteroso, Lisa2

ABSTRACT

3M Molecular Detection Assay (MDA) 2–Listeria uses loop-mediated isothermal amplification and bioluminescence detection to rapidly detect Listeria species in a broad range of food types and environmental surfaces. Using an unpaired study design, MDA 2–Listeria was compared with the U.S. Department of Agriculture, Food Safety and Inspection Service's Microbiology Laboratory Guidebook Chapter 8.09 “Isolation and identification of Listeria monocytogenes from red meat, poultry and egg products, and environmental samples” reference method for the detection of Listeria in deli turkey and raw chicken breast fillet. Technicians from 13 laboratories located within the continental United States and Canada participated in the collaborative study. Each matrix was evaluated at three levels of contamination: uninoculated control (0 CFU/test portion), low inoculum (0.2–2 CFU/test portion), and high inoculum (2–5 CFU/test portion). Statistical analysis was conducted according to the probability of detection (POD) statistical model. Results obtained for the low-inoculum-level test portions produced a difference between two laboratory POD values (dLPOD) with 95% confidence intervals of 0.04 (–0.08, 0.17) for deli turkey, indicating the difference between the methods was not statistically significant at the P = 0.05. For raw chicken breast fillet, a dLPOD value with 95% confidence interval of 0.16 (0.04, 0.28) indicated a statistically significant difference between the two methods, with an observed higher proportion of positive results by the candidate method than the reference method.

Development of Loop-Mediated Isothermal Amplification (LAMP) Assay for the Rapid Detection of Alternaria alternata

Zhang, Xinyue; Xu, Guojie; Tang, Huaqi; Li, Yanpeng; Liu, Chunsheng

ABSTRACT

Fungi of the Alternaria genus are associated with allergic diseases, with Alternaria alternata being one of the most prevalent species. A. alternata has been frequently reported as the etiologic agent of hypersensitivity pneumonitis, allergic rhinosinusitis, bronchial asthma, and other diseases. In this study, we developed a loop-mediated isothermal amplification (LAMP) assay and a real-time PCR assay to detect low levels of A. alternata in herbal tea samples. The LAMP assay can detect as little as 3 pg/μL of A. alternata genomic DNA with high specificity. In addition, both the LAMP assay and the real-time PCR assay can be used for quantification of A. alternata. Although the newly developed LAMP assay is more rapid and specific in A. alternata identification, the real-time PCR assay is more precise in quantitation analysis.

Analysis of Illicit Liquor by Headspace Gas Chromatography-Mass Spectrometry (HS-GC-MS): A Preliminary Study

Punia, Bhupinder Singh; Yadav, Praveen Kumar; Bumbrah, Gurvinder Singh; Sharma, Rakesh Mohan

ABSTRACT

Illicit liquors are illegally manufactured to evade taxes and represent the majority of unrecorded liquors in developing countries. Because there are no standards, the composition of illicit liquors varies greatly from sample to sample. In the current study, we analyzed the volatile components of 27 different illicit liquors via samples collected from various locations in the northern region of India. Ethanol content varied drastically and methanol was not present in any of the samples. The components found can be categorized into different groups, namely alcohols, esters, acids, nitrogen-containing components, ketones, and aldehydes. Some components—such as 1-propanol; 1-pentanol; 1-butanol; d-limonene; phenylethyl alcohols; anethole; and decanoic, octanoic, and pentanoic acids—were frequently encountered.

Survey of Thai Commercial Food Products That Have Been Reported to Contain No Wheat, Rye, Barley, or Gluten According to Their Labels

Surojanametakul, Vipa1; Srikulnath, Sirinrat1; Chamnansin, Pailin1; Shoji, Masahiro2; Tamura, Hirotoshi3

ABSTRACT

Celiac disease (CD) and gluten-related disorders are significant health and social issues in Western countries, and CD individuals need to exclude gluten from their diets. The adverse health impacts of CD have extended to Asian countries in which CD was not a problem previously. Thai commercial food products that do not contain wheat, rye, barley, or gluten on their labels were surveyed as to whether they were suitable for CD individuals by examining the absence of gluten or the presence of gluten <20 ppm. In Thailand, ELISA tested for gluten content in 129 commercial food products that contained neither wheat, rye, barley, nor gluten on their labels. One hundred nineteen of these 129 products included <20 ppm gluten, and 10 products contained >20 ppm gluten. Surprisingly, four products showed gluten levels >1%. In these 10 products, wheat presence was confirmed by PCR analysis. Our survey suggests that CD individuals can consume most of the examined Thai food products, and the survey showed the potential of these Thai products as new diets for CD patients so as to expand the limited food choices from different food cultures, and ultimately to improve the quality of life for all CD individuals globally. The appropriate gluten management strategies need to be implemented by Thai food manufacturers to ensure accurate labeling and to protect the safety of consumers with CD.

Determination of Bovine Lactoferrin in Food by HPLC with a Heparin Affinity Column for Sample Preparation

Zhang, Yin1; Lou, Fei2; Wu, Wei1; Dong, Xin1; Ren, Jia1; Shen, Qiuguang2

ABSTRACT

An HPLC method was developed for the quantitative determination of bovine lactoferrin (bLF) in sterilized milk, modified milk, fermented milk, infant formula, adult formula, rice cereal, vitamin function drink, and protein powder products. bLF was first extracted with a phosphate buffer (pH 8), underwent cleanup in a heparin affinity column, and was detected by HPLC with a C4 column and diode-array detector at a wavelength of 280 nm. The proposed method provided a linear detection range of 10.0–1000 μg/mL with an LOD of 0.6 mg/100 g in liquid samples and 3 mg/100 g in solid samples and an LOQ of 2 mg/100 g in liquid samples and 10 mg/100 g in solid samples. In addition, the method showed good recovery for various samples, ranging from 76 to 96%. The method had several remarkable advantages, including ease of handling, high sensitivity and accuracy, good reproducibility, and low-cost detection. Based on the distinctive properties presented here, we believe the proposed HPLC assay holds great promise for the oversight and detection of bLF in testing organizations, dairy enterprises, and regulatory authorities.

Vitamin C in Infant Formula and Adult/Pediatric Nutritional Formula by Liquid Chromatography with UV Detection: Collaborative Study, Final Action 2012.22

Giménez, Esther Campos; Martin, Frédéric

ABSTRACT

To determine the repeatability and reproducibility values of the AOAC INTERNATIONAL First Action Method 2012.22, Vitamin C in Infant Formula and Adult/Pediatric Nutritional Formula by Liquid Chromatography with UV Detection, a collaborative study was organized. The study was divided into two parts: method setup and qualification of participants (part 1) and collaborative study participation (part 2). During part 1, each laboratory was asked to analyze two practice samples using the aforementioned method. Laboratories that provided results within a range of expected levels were qualified for part 2, where they analyzed 10 samples in blind duplicates. Two of the samples were suspected of spoilage during the test and new cans of the same type of product were analyzed by a subset of laboratories in part 3. The results were compared with Standard Method Performance Requirement (SMPR®) 2012.012 established for vitamin C. The precision results were within the requirements stated in the SMPR: 1.4–7.3% and 3.2–11.4% respectively, for repeatability and reproducibility. Finally, Horwitz ratio values were all <2 (0.5–1.7). The Expert Review Panel for Stakeholder Panel for Infant Formula and Adult Nutritionals Nutrient Methods determined that the data presented met the SMPR and therefore recommended the method be granted Final Action status.

Determination of Biotin in Infant, Pediatric, and Adult Nutritionals by High-Performance Liquid Chromatography and Fluorescence Detection: Single-Laboratory Validation, First Action 2016.11

Lin, Qi; Ding, Yi; Poh, Fiona; Zhang, Chunyan; Pan, Shang-Jing; Schimpf, Karen J.

ABSTRACT

A reversed-phase HPLC method with postcolumn protein conjugation and fluorescence detection for the quantitative determination of biotin in infant, pediatric, and adult nutritionals was developed and evaluated in a single-laboratory validation (SLV). Sample of appropriate size is mixed with 2% metaphosphoric acid to precipitate out the protein. The filtrate is injected onto a C18 HPLC column in which biotin and riboflavin are separated with an appropriate mobile phase. The biotin, after eluting from the column, binds with the streptavidin fluorescein to become a fluorescent conjugate. The conjugate is then detected by fluorescence at λex = 495 nm and λem = 518 nm. A column switch is used in the method as an option to shorten the run time from 30 to 15 min, by eluting out riboflavin at a higher flow rate. In this SLV, a total of 19 AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals matrixes representing a range of infant, pediatric, and adult formulas were evaluated for their biotin content. The analytical range was 1.66–142 μg/100 g reconstituted final product. The repeatability and intermediate precision ranged from 0.5 to 3.0% RSDr and from 1.3 to 4.5% RSDiR, respectively. Recovery from spiked matrixes varied from 95 to 111%, and accuracy of quantification using Standard Reference Material 1849a ranged from 99 to 105%. The LOQ in reconstituted product was estimated to be 0.8 μg/100 g. The method was approved by the Expert Review Panel as First Action at the 2016 AOAC INTERNATIONAL Mid-Year Meeting.

Investigation of the Virulence Factors and Molecular Characterization of the Clonal Relations of Multidrug-Resistant Acinetobacter baumannii Isolates

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ABSTRACT

Multidrug-resistant (MDR) Acinetobacter baumannii infections are a great public health concern and demand continuous surveillance and antibiotic stewardship. Virulence traits and the pathogenicity of Acinetobacter are less studied compared with the molecular epidemiological and antibiotic resistance profile of this organism. In our present study, we investigated the primary characteristics contributing to the virulence of MDR A. baumannii isolates and compared them with avirulent isolates. A total of 32 well-characterized MDR A. baumannii clinical isolates and 22 avirulent isolates from a healthy individual were subjected to multilocus sequence typing and polymerase chain reaction (PCR) for a variety of biofilm-associated genes. Additionally, a number of in vitro tests were performed to determine virulence properties. Isolates were found to relate to six sequence types (STs) in which the dominant sequence was ST557 in clinical isolates, followed by ST195 and ST208. However, ST557 and ST222 were absent in avirulent isolates. All STs belonged to clonal complex 2 and clonal lineage 2, which is considered to be a universal clone. PCR analysis showed that most clinical isolates were positive for biofilm-forming genes, such as csu and bap, and also carried pga and ompA genes, which were less common in avirulent isolates. Biofilm formation, phospholipase C production, hemolytic activity, and acinetobactin production occurred significantly more frequently in clinical isolates compared with avirulent isolates. Though A. baumannii clonal lineages showed common virulence traits, they differed in virulent phenotype expression. These findings further support previous studies indicating that A. baumannii is a versatile pathogen with an ability to acquire iron and survive in iron-limiting conditions, highlighting the acinetobactin-mediated iron acquisition mechanisms involved in the pathogenesis of A. baumannii infections.

Development of a Liquid Chip Technique to Simultaneously Detect Spring Viremia of Carp Virus, Infectious Hematopoietic Necrosis Virus, and Viral Hemorrhagic Septicemia of Salmonids

Tong, Guixiang1; Wei, Xinxian1; Yin, Weili2; Liao, Xiaoguang3; Yang, Kai3; Fang, Zhishan3; Sun, Tao2; Yue, Zhiqin2; Li, Xiaozheng1

ABSTRACT

A liquid chip technique was developed to detect spring viremia of carp virus (SVCV), infectious hematopoietic necrosis virus (IHNV), and viral hemorrhagic septicemia virus (VHSV) of salmonids simultaneously. Sequences of the G gene of SVCV, N gene of IHNV, and G gene of VHSV were used to design SVCV-, IHNV-, and VHSV-specific primers, which were labeled with biotin and subjected to amination modification. They were then coupled with fluorescence-coded microspheres and used for hybridization with reverse-transcription PCR products of SVCV, IHNV, and VHSV. A BD FACSArray was used to detect fluorescence signal in the reaction system. This assay system had a high sensitivity to SVCV, VHSV, and IHNV, with LODs of 10, 10, and 100 pg/μL, respectively. Moreover, the assay was specific for the detection of SVCV, IHNV, and VHSV and was not susceptible to cross-detection of other viruses, including pike fry rhabdovirus, hirame rhabdovirus, infectious pancreatic necrosis virus, viral nervous necrosis virus, yellowtail ascites virus, grass carp reovirus, red sea bream iridovirus, and koi herpesvirus. The liquid chip assay technique established in this study provides a novel, convenient, and rapid approach for the detection of SVCV, IHNV, and VHSV.

Crystal Diagnostics Xpress™ LM Kit for the Rapid Detection of Listeria monocytogenes from Environmental Surfaces

Stumpf, Curtis H.; Bullard, Brian; Zhoa, Weidong; Kuzenko-Hentosh, Stephanie; Niehaus, Gary D

ABSTRACT

The Crystal Diagnostics (CDx) Xpress™ LM kit is used for rapid screening of low concentrations of Listeria monocytogenes on environmental surfaces such as stainless steel, plastic, and ceramic tile. In addition to the Xpress LM kit, the CDx Xpress System comprises an automatic Xpress Reader, a BioCassette™ that incorporates antibody-coupled microspheres, and liquid crystal for selective identification of the intended microbe. All 56 of the 56 tested L. monocytogenes strains evaluated were detected, and 50 of the 50 nontarget bacterial strains were excluded when the test was conducted under the described kit conditions. Shelf-life testing of the antibody-coated microspheres and other CDx consumables indicated that all materials were stable for a minimum of 6 months (ongoing), and lot-to-lot testing demonstrated no significant differences among lots. The internal and independent laboratory tests on stainless steel, plastic, and ceramic tile surfaces demonstrated that the method is equivalent to the U.S. Department of Agriculture (USDA) reference method, and there were no significant differences between the CDx Xpress LM kit presumptive and confirmed results for any of the matrixes. Overall, the CDx Xpress LM kit is one of the fastest to provide the sensitivity and specificity equivalent to the USDA reference method in screening low levels of L. monocytogenes surface contamination and, when combined with chromogenic culturing of presumptive positives, provides a streamlined confirmation process to rapidly and accurately differentiate L. monocytogenes from other microbes.

Multiresidue Analysis of Five Neonicotinoid Insecticides and Their Primary Metabolite in Cucumbers and Soil Using High-Performance Liquid Chromatography with Diode-Array Detection

Abdel-Ghany, Maha F.; Hussein, Lobna A.; El Azab, Noha F

ABSTRACT

A sensitive, selective, and validated HPLC–diode-array detection method was developed for the simultaneous determination of five neonicotinoid insecticides—acetamiprid, imidacloprid, nitenpyram, flonicamid, and thiacloprid—and their primary metabolite, 6-chloronicotinic acid, in cucumbers and soil based on the quick, easy, cheap, effective, rugged, and safe (QuEChERS) technique as a pretreatment procedure. In the QuEChERS procedure, cucumber samples were extracted with acetonitrile and cleaned using C18, whereas soil samples were extracted with an acetonitrile–dichloromethane mixture (1 + 2). The HPLC conditions were optimized by separating neonicotinoids using an acetonitrile–water mixture (25 + 75) and a Synergi Hydro RP C18 column. Matrix-matched calibration standards were prepared in cucumber and soil to eliminate any matrix interference. RSDs were ≤9% in all recovery tests. LODs and LOQs for the five neonicotinoids were in the ranges of 0.006–0.122 and 0.018–0.366 μg/g, respectively. This method was successfully applied to determine residues, the rate of disappearance of the five neonicotinoids from cucumber and soil, and the half-lives of the neonicotinoids.

LC-MS/MS Validation of a Residue Analysis Method for Penicillin G and Its Metabolites in Commercial Orange Juice

Aldeek, Fadi; Canzani, Daniele; Standland, Matthew; Hammack, Walter; Cook, Jo-Marie; Crosswhite, Mark R.; Gerard, Ghislain

ABSTRACT

Florida citrus depends on a breakthrough in the fight against citrus greening disease. Of the antibiotics used to treat this disease, penicillin G has been one of the most effective. Because orange fruit grown in the state of Florida are mainly used to produce orange juice, we have validated an ultra-HPLC tandem MS method to screen for penicillin G and its metabolites (penillic and penilloic acids) at the chemical residue level after treatment. In this method, three spike levels (0.25, 1, and 20 ng/g) were tested in triplicate. Absolute recoveries for penillic and penilloic acids were 60–75% depending on the matrix used, whereas corrected recoveries of penicillin G using an isotopically labeled internal standard were ~100%. Two product ion transitions per analyte were required for identification, which contributes to a high degree of selectivity.

Modified Magnetic Nanoparticles as a Novel Sorbent for Dispersive Magnetic Solid-Phase Extraction of Triazine Herbicides in Aqueous Media

Feizbakhsh, Alireza; Ehteshami, Shokooh

ABSTRACT

In this study, a polythiophene/chitosan polymer and electrospinning polymer nanofibers as modifier compounds were used, and magnetic nanocomposites as a novel adsorbent were proposed, for the preconcentration of some triazines, including atrazine, ametryn, and terbutryn, in aqueous samples before GC. The synthesized magnetic nanoparticles, magnetic polymer nanofibers, and polythiophene magnetic nanocomposite were characterized by scanning electron microscopy. The separation of the target analytes from the aqueous solution containing the triazines and different magnetic nanocomposites were simply achieved by applying an external magnetic field. The extraction efficiency of magnetic polyamide nanofibers was enhanced as compared with other modified magnetic nanoparticles. The main factors affecting the extraction efficiency, including desorption conditions, nanocomposite component ratio, electrospinning time, sorbent amount, extraction time, ionic strength, and sample pH, were optimized. The developed method proved to be convenient and offers sufficient sensitivity and good reproducibility. Under optimized conditions, the method's LOD (S/N = 3) and LOQ (S/N = 10) were 1–5 and 15 ng L−1, respectively; good linearity was obtained within the range of 15–2000 ng L−1 for triazines, with correlation coefficients >0.9997. The RSD at the concentration level of 100 ng L−1 was 9–14% (n = 3). Furthermore, the method was successfully applied to the determination of triazines in real samples, in which relative recoveries of 98–103% were obtained. Compared with other methods, the current method is characterized by its ease, fast separation, and low detection limits.

A Validated Capillary Electrophoretic Method for the Determination of Olopatadine and Its Application to a Pharmaceutical Preparation of Eye Drops

Güray, Tufan1; Turan, Tugba1; Tunçel, Muzaffer2; Uysal, Ulku Dilek3

ABSTRACT

A validated rapid and sensitive capillary zone electrophoretic method for the determination of olopatadine hydrochloride (OLO) is described. Optimum conditions were found: 20 mmol/L sodium tetraborate buffer, acetonitrile 15% (v/v), 10 mmol/L NaCl at pH 9.5, with 25 kV of applied potential, injection time of 10 s at 5 × 103 N/m2, at a wavelength of 205 nm, and fixed temperature of 30°C. The calibration curve was linear in the range of 1.13 × 10−5 mol/L (4.22 μg/mL) to 5.65 × 10−5 mol/L (21.12 μg/mL), with R = 0.9995 for interday precision. LOD and LOQ values were 1.58 × 10−6 (0.58 μg/mL) and 4.78 × 10−6 mol/L (1.75 μg/mL), respectively. Precision values were 1.10–1.97% for intraday and 1.41% for interday RSDs. Accuracy was tested by preparing a synthetic mixture whose composition was similar to the pharmaceutical preparation for Patanol. The RSDs of the recovery values (98.2%) were between 0.42 and 0.65% and the amount of OLO found was 1.09 mg/mL. The result was within the requirements of USP 31-NF 26. Therefore, this validated method is suggested for routine analysis for the determination of OLO in laboratories.

Determination of Diphenylether Herbicides in Water Samples Using Dispersive Liquid–Liquid Microextraction Combined with High-Performance Liquid Chromatography

Liu, Yung-Hao1; Chen, Pai-Shan2; Huang, Shang-Da1

ABSTRACT

Shaker-assisted dispersive liquid−liquid microextraction (SA-DLLME) and surfactant dispersive liquid–liquid microextraction (SDLLME) have been developed to determine five diphenylether herbicides in water samples using high-performance liquid chromatography with photodiode array detection (HPLC-PDA). For SA-DLLME, an up-and-down shaker-assisted emulsification was used. Extraction was complete in 3 min. Only 30 μL of decyl acetate was required, without a dispersive solvent. The linear range was from 2 to 1000 μg L−1, the coefficient of determination (r 2) was better than 0.9992, and the limit of detection (LOD) was from 0.62 to 1.74 μg L−1. The relative recovery (RR) ranged from 90 to 102% for river water, 88 to 104% for lake water, and 93 to 102% for irrigating water. In SDLLME, a microsyringe was used to withdraw and discharge a mixture consisting of an extraction solvent and 1 mg L−1 Tween 60 as a surfactant four times within 10 s to form an emulsified solution. The linear range for the target compounds was from 2 to 1000 μg L−1. The LODs were between 0.72 and 1.38 μg L−1. The RR ranged from 95 to 108% for river water, 96 to 109% for lake water, and 86 to 114% for irrigating water.

Ionic Liquid-Bonded Fused Silica as a New Solid-Phase Microextraction Fiber for the Liquid Chromatographic Determination of Bisphenol A as an Endocrine Disruptor

Mohammadnezhad, Nasim1; Matin, Amir Abbas2; Samadi, Naser1; Shomali, Ashkan2; Valizadeh, Hassan2

ABSTRACT

Linear ionic liquid bonded to fused silica and its application as a solid-phase microextraction fiber for the extraction of bisphenol A (BPA) from water samples were studied. After optimization of microextraction conditions (15 mL sample volume, extraction time of 40 min, extraction temperature of 30 ± 1°C, 300 μL acetonitrile as the desorption solvent, and desorption time of 7 min), the fiber was used to extract BPA from packed mineral water, followed by HPLC–UV on an XDB-C18 column (150 × 4.6 mm id, 3.5 μm particle) with a mobile phase of acetonitrile–water (45 + 55%, v/v) and flow rate of 1 mL . min−1). A low LOD (0.20 μg. L−1) and good linearity (0.9977) in the calibration graph indicated that the proposed method was suitable for the determination of BPA.

Development of a Dispersive Liquid–Liquid Microextraction Method Combined with UV-Visible Spectrophotometry for Determination of Trace Aluminum (III) in Water, Wastewater, Food, Biological, and Pharmaceutical Samples

Birgani, Nasrin Taghipour; Elhami, Shahla

ABSTRACT

A simple and sensitive method was proposed for the preconcentration of trace levels of Al(III) prior to its determination by spectrophotometry, based on dispersive liquid–liquid microextraction. The complexation of the Al(III) was performed by chelation with Eriochrome Cyanine R (ECR). In this method, cetyltrimethyl ammonium bromide (CTAB) as a dispersant was dissolved in chloroform as an extractant solvent, and then the solution was rapidly injected by a syringe into the samples containing Al(III), which had already been complexed by ECR at optimized pH. Various parameters were studied and optimized for a 10 mL sample volume. Under the optimum conditions, the LOD (3 times the SD of 10 replicate readings of the reagent blank) and the dynamic range of the calibration obtained were 0.2 ng mL−1 (7 nM) and 1.0–80.0 ng mL−1, respectively. The RSDs for eight replicate determinations of 10 and 60 ng mL−1 of Al(III) were 3.3 and 1.8%, respectively. This strategy was successfully applied to determine the Al concentration in water, wastewater, yogurt, apple, carrot, celery, bread, potato, urine, and Al–Mg syrup samples.

Determination of Organic Impurities in Anthraquinone Color Additives D&C Violet No. 2 and D&C Green No. 6 by Ultra-High Performance Liquid Chromatography

Author: Yang, H. H. Wendy

ABSTRACT

A new practical and time-saving ultra-high performance liquid chromatography (UHPLC) method has been developed for determining the organic impurities in the anthraquinone color additives D&C Violet No. 2 and D&C Green No. 6. The impurities determined are p-toluidine, 1-hydroxyanthraquinone, 1,4-dihydroxyanthraquinone, and two subsidiary colors. The newly developed UHPLC method uses a 1.7-μ particle size C-18 column, 0.1 M ammonium acetate and acetonitrile as eluents, and photodiode array detection. For the quantification of the impurities, six-point calibration curves were used with correlation coefficients that ranged from 0.9974 to 0.9998. Recoveries of impurities ranged from 99 to 104%. Relative standard deviations ranged from 0.81 to 4.29%. The limits of detection for the impurities ranged from 0.0067% to 0.216%. Samples from sixteen batches of each color additive were analyzed, and the results favorably compared with the results obtained by gravity-elution column chromatography, thin-layer chromatography, and isooctane extraction. Unlike with those other methods, use of the UHPLC method permits all of the impurities to be determined in a single analysis, while also reducing the amount of organic waste and saving time and labor. The method is expected to be implemented by the U.S. Food and Drug Administration for analysis of color additive samples submitted for batch certification.

Analysis of Means (ANOM) as a Tool for Comparison of Sample Treatment Methods: Testing Various Mineralization Procedures for Selenium Determination in Biological Materials

Prokeš, Lubomir1; Hegrová, Jitka2; Kanický, Viktor1

ABSTRACT

Several mineralization methods for the determination of selenium using hydride generation optical emission spectrometry with inductively coupled plasma in biological samples (whole egg powder and pork liver) were compared using the analysis of means (ANOM) method. This statistical tool is suitable for graphical representation of testing on simple comparative experiments. The results yielded by ANOM are identical with those obtained with the commonly used analysis of variance (ANOVA) method; however, the graphical output of ANOM is more illustrative in comparison to ANOVA. Both methods indicated a significant discrepancy between the results obtained using muffle furnace ashing mineralization and the results provided by other mineralization methods. This is probably due to the loss of volatile selenium compounds during the decomposition of organic matter.

Application of an Accuracy Profile Strategy Based on the β-Expectation Tolerance Interval for the Validation of a Liquid Chromatography Analytical Method for the Quantification of Benzoic Acid and Its Salts in Different Foodstuffs

Tighrine, Abderrahmane1; Amir, Youcef2; Mamou, Marzouk1

ABSTRACT

This paper presents the validation of a method for the quantification of benzoic acid and its salt preservatives, which are extensively used in the preservation of foodstuffs. The Joint Expert Committee on Food Additives at the Food Agricultural Organization and World Health Organization has established maximum permitted limits for these compounds in different foodstuffs because of the harmful effects of benzoic acid and its salt preservatives when they exceed certain limits. Therefore, a reliable and simple method to quantify these preservatives was validated. The developed method used a combination of extracted external calibration standards, a simple extraction procedure, and reversed-phase HPLC. This method was validated by applying a new approach in which the total error was based on the β-expectation tolerance interval developed by the Society of Pharmaceutical Science and Techniques Commission with an acceptability limit fixed at a λ of ±15%. The results demonstrated that the method is accurate, with repeatability between 1.096 and 1.986% and intermediate precision between 1.133 and 2.005% in the considered concentration range. The LOD was 0.1597 μg/mL.

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NMR Metabolomics Investigates the Influence of Flavonoid-Enriched Rations on Chicken Plasma

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ABSTRACT

The use of flavonoids as dietary supplements is well established, mainly due to their intense antioxidant and anti-inflammatory properties. In the present study, hesperidin, naringin, and vitamin E were used as additives at different concentrations in poultry rations in order to achieve meat of improved quality. NMR metabolomics was applied to chicken blood serum samples to discern whether and how the enriched rations affected the animals' metabolic profile. Variations in the metabolic patterns according to sustenance consumption were traced by orthogonal projections to latent structures discriminant analysis (OPLS-DA) models and were attributed to specific metabolites by using S-line plots. In particular, serum samples from chickens fed with vitamin E displayed higher concentrations of glycine and succinic acid compared to control samples, which were mainly characterized by betaine, formic acid, and lipoproteins. Samples from chickens fed with hesperidin were characterized by increased levels of lactic acid, citric acid, creatine, carnosine, creatinine, phosphocreatine, anserine, glucose, and alanine compared to control samples. Lastly, naringin samples exhibited increased levels of citric and acetic acids. Results verify the scalability of NMR metabolomics to highlight metabolite variations among chicken serum samples in relation to food rations.

Determination of Polyphenols in White Wines by Liquid Chromatography: Application to the Characterization of Alella (Catalonia, Spain) Wines Using Chemometric Methods

Larrauri, Alexander; Núñez, Oscar; Hernández-Cassou, Santiago; Saurina, Javier

ABSTRACT

The determination of polyphenols in wines is of great interest in the field of food analysis due to health and organoleptic implications. In addition, the applicability of polyphenols as food descriptors to be used for characterization, classification, and authentication purposes is gaining popularity. In this work, a simple and reliable method based on HPLC separation in reversed-phase mode with UV-Vis detection was developed and applied to determine polyphenolic compounds in white wines. The chromatographic separation was performed using a C18 column under a methanol elution gradient and assessed by an experimental design approach. Analytical parameters were established under the optimal experimental conditions. LOD values were between 3 and 220 µg/L, and repeatability values were better than 1% for most of the analyzed polyphenols. Compositional data were further exploited to characterize white wines based on principal component analysis to discriminate among mono- and polyvarietal compositions.

Time-Domain Nuclear Magnetic Resonance (TD-NMR) and Chemometrics for Determination of Fat Content in Commercial Products of Milk Powder

Nascimento, Paloma Andrade Martins1; Barsanelli, Paulo Lopes1; Rebellato, Ana Paula2; Pallone, Juliana

ABSTRACT

This study shows the use of time-domain (TD)-NMR transverse relaxation (T2) data and chemometrics in the nondestructive determination of fat content for powdered food samples such as commercial dried milk products. Most proposed NMR spectroscopy methods for measuring fat content correlate free induction decay or echo intensities with the sample's mass. The need for the sample's mass limits the analytical frequency of NMR determination, because weighing the samples is an additional step in this procedure. Therefore, the method proposed here is based on a multivariate model of T 2 decay, measured with Carr-Purcell-Meiboom-Gill pulse sequence and reference values of fat content. The TD-NMR spectroscopy method shows high correlation (r = 0.95) with the lipid content, determined by the standard extraction method of Bligh and Dyer. For comparison, fat content determination was also performed using a multivariate model with near-IR (NIR) spectroscopy, which is also a nondestructive method. The advantages of the proposed TD-NMR method are that it (1) minimizes toxic residue generation, (2) performs measurements with high analytical frequency (a few seconds per analysis), and (3) does not require sample preparation (such as pelleting, needed for NIR spectroscopy analyses) or weighing the samples.

Efficiency of Rosemary and Basil Essential Oils on the Shelf-Life Extension of Atlantic Mackerel (Scomber scombrus) Fillets stored at 2°C

Karoui, Romdhane; Hassoun, Abdo

ABSTRACT

The objective of the present study was to evaluate the impact of rosemary and basil essential oils (EOs) on the quality of Atlantic mackerel fillets stored at 2°C up to 15 days. Atlantic mackerel (Scomber scombrus) fillets were periodically evaluated to assess their textural, color, physicochemical, and spectral characteristics. The results indicated that rosemary and basil treatments were effective for inhibiting the formation of total volatile basic nitrogen (TVB-N) and lipid oxidation products during storage. Based on TVB-N values, the shelf life of Atlantic mackerel fillets treated with rosemary and basil EOs was extended by 2 and 5 days, respectively, compared to the control group. Similar results were obtained with thiobarbituric acid–reactive substance analysis, which demonstrated an extended shelf life of Atlantic mackerel immersed with rosemary and basil EOs of 2 and 3 days, respectively, compared to the control group. The factorial discriminant analysis applied on the concatenated first five principal components corresponding to the physicochemical, textural, color, and fluorescence measurements allowed clear discrimination of the three groups, because a correct classification rate of 93.3% was obtained. Therefore, treatment with basil and rosemary EOs, as natural biopreservative compounds, could present a high-potential application in the seafood industry.

Fast-HPLC Fingerprinting to Discriminate Olive Oil from Other Edible Vegetable Oils by Multivariate Classification Methods

Jiménez-Carvelo, Ana M.; González-Casado, Antonio; Pérez-Castaño, Estefanía; Cuadros-Rodríguez, Luis

ABSTRACT

A new analytical method for the differentiation of olive oil from other vegetable oils using reversed-phase LC and applying chemometric techniques was developed. A 3 cm short column was used to obtain the chromatographic fingerprint of the methyl-transesterified fraction of each vegetable oil. The chromatographic analysis took only 4 min. The multivariate classification methods used were k-nearest neighbors, partial least-squares (PLS) discriminant analysis, one-class PLS, support vector machine classification, and soft independent modeling of class analogies. The discrimination of olive oil from other vegetable edible oils was evaluated by several classification quality metrics. Several strategies for the classification of the olive oil were used: one input-class, two input-class, and pseudo two input-class.

Classification of Edible Oils Based on ATR-FTIR Spectral Information during a Long Heating Treatment

Mahboubifar, Marjan1; Hemmateenejad, Bahram2; Yousefinejad, Saeed3

ABSTRACT

Identification of oil type and its QC are important concerns in food control laboratories. Classifying edible oils that have not been used (i.e., unheated) with the aid of vibrational spectroscopy has previously been reported. However, the classification of used (i.e., heat-treated) oils needs special attention. The effect of long heating times on the classification of four kinds of edible oils (canola, corn, frying, and sunflower) based on attenuated total reflectance (ATR)-FTIR spectra was surveyed. The sampling was done on the oils during a 36 h heating process (at 170°C). The ATR-FTIR spectra of the samples were collected in the range of 4000–550 cm−1. Interval extended canonical variates analysis (ECVA), as a variable selection and classification tool, was used to determine the best intervals during the heating procedure for classification. Principal component analysis discriminate analysis, partial least-squares discriminate analysis, and ECVA were performed on the selected intervals and on the total heating time. The effect of autoscaling and mean-centering, as data preprocessing methods, was also investigated. The ECVA method resulted in the best performances for classification, with a 94% cross-validated nonerror rate (one misclassification) for the heating process times of 24–27 and 33–36 h.

Chemometrics Expertise in the Links between Ecotoxicity and Physicochemical Features of Silver Nanoparticles: Environmental Aspects

Nedyalkova, Miroslava A.; Donkova, Borjana V.; Simeonov, Vasil D.

ABSTRACT

Studies of the ecotoxicological aspects of nanomaterials in aquatic environments are scarce. Given the growing variety of nanoparticles (NPs), along with the diversity of aquatic species and environments, the key to promoting sound risk assessment in nanoecotoxicology is under standing the mechanisms that govern the fate of NPs in aquatic environments and their behavior at the NP–biota interface. In this paper, data collected from the literature on ecotoxicological effects observed in aquatic species is discussed and analyzed using multivariate statistics techniques. We expand the knowledge of the environmental impact of silver NPs (AgNPs) by testing the acute toxicity of 47 AgNPs on crustacean eukaryotic organisms (Daphnia magna, Thamnocephalus platyurus, and D. galeata). Physicochemical properties, stabilization agents, toxicological end points, and test media were monitored as adding-outcome factors for the evaluation of environmental effects due to exposure to NPs. The chemometrics expertise performed by the use of hierarchical and nonhierarchical cluster analysis and principal component analysis revealed specific links between the ecotoxicology and the physicochemical features of NPs and helped in creating specific patterns of NPs discriminated by ecotoxicity levels and physicochemical parameters.

Application of Multivariate Classification Protocols in Research Focusing on Food, Environmental Samples, and Wastewater Technological Processes

Świderska-Dąbrowska, Renata; Piaskowski, Krzysztof; Baran, Michał J.

ABSTRACT

Analysis and quantification of multiple analytes in complex samples originating from food and environmental matrixes generate large data sets that can be difficult to analyze and interpret. Multivariate analysis and related computation protocols provide an effective platform and enable such problems to be dealt with. This review illustrates the effective application of chemometrics protocols used to improve quantification techniques and the interpretation of raw data from complex samples.

Chemometrics-Assisted Fast-Elution HPLC–DAD for the Quantification of Selected UV Filters and Parabens in Suncare Formulations

Vosough, Maryam1; Shekari, Nafiseh1; Salemi, Amir2; Heidar, Koorosh Tabar1

ABSTRACT

In the present study, a fast LC method coupled with multivariate curve resolution (MCR) alternating least-squares (ALS) and alternating trilinear decomposition (ATLD) was developed for the determination of the resolution of and quantitation of benzophenone-3, 4-methylbenzylidene camphor, octocrylene, ethylhexyl dimethyl para-aminobenzoic acid, butyl methoxydibenzoilmethane, and methyl and propyl parabens in suncare products. Chromatographic separation was optimized using full factorial and Box–Behnken designs. MCR-ALS and ATLD performance in quantitating the analytes in synthetic mixtures (which were randomly prepared in ultra-pure water) and blank sunscreen products was studied, and satisfying results were obtained. Acceptable qualification and quantification results were also achieved in the presence of matrix interferences via a short chromatographic runtime (5 min), and the second-order advantage was fully exploited, with MCR-ALS clearly emerging as the superior model. Average recoveries ranged from 98.0 to 112.5%, and RSD values were lower than 6.5%. LODs between 0.066 and 0.243 μg/g were achieved. In addition to acceptable precision and accuracy, the merits of the proposed method are that the analysis is fast and there is minimal solvent consumption. Moreover, coelution of analytes and interference from components in the sample matrixes were overcome with multivariate analysis.

Molecular and Quantum Mechanical Study for the Separation of Cefprozil in the Presence of Its Alkaline Degradation Product Using RP-HPLC with UV Detection

Attia, Khalid A.M.1; Nassar, Mohammed W.I.1; El-Zeiny, Mohamed B.2; Serag, Ahmed1

ABSTRACT

A reversed-phase HPLC method (RP-HPLC) with UV detection was developed and validated for the quantitative determination of cefprozil, a second-generation cephalosporin. Due to β-lactam ring instability under alkaline conditions, this RP-HPLC method was applied for the determination of cefprozil in the presence of its possible degradation product. The interactions that govern the separation process with stationary phase were investigated at both molecular and quantum mechanical levels. Moreover, electrostatic potential maps were generated to determine the sites of interaction with mobile phase. The suggested method was validated in compliance with International Conference on Harmonization guidelines and successfully applied for the determination of cefprozil in its commercial pharmaceutical formulation.

Analytical Stability-Indicating Methods for Alogliptin in Tablets by LC–CAD and LC–UV

Bertol, Charise Dallazem1; Friedrich, Maria Tereza2; Carlos, Graciela3; Froehlich, Pedro Eduardo1

ABSTRACT

Stability-indicating LC methods using a UV detector and a charged aerosol detector (CAD) simultaneously were validated for the assessment of alogliptin (ALG) in tablets. The analysis was performed on a C8 column (250 × 4.6 mm, 5 μm) at a flow of 0.8 mL/min, using acetonitrile–10 mM ammonium acetate buffer (pH 3.5; 90 + 10, v/v) as mobile phase and UV detection at 275 nm. Validation followed the International Conference on Harmonization guidelines. The method was linear over the range of 25–200 μg/mL. Normality of the residuals showed a normal distribution, no autocorrelation, and homoscedasticity. LODs were 6.25 and 2.65 μg/mL and LOQs were 20.85 and 8.84 μg/mL for the CAD and the UV detector, respectively. The methods were precise and accurate. Excipients and degradation products did not interfere in the methods in studies of specificity. None of the factors studied in the analysis of robustness had a significant effect on the quantification of the ALG by the Pareto chart. The results of the assay obtained with LC–CAD and LC–UV were similar. The methods could be considered interchangeable and stability-indicating, and can be applied as an appropriate QC tool for analysis of ALG in tablets.

Validated Chromatographic Methods for the Analysis of Two Binary Mixtures Containing Pyridoxine Hydrochloride

Habib, Neven M.; Abdelrahman, Maha M.; Abdelwhab, Nada S.; Ali, Nourudin W.

ABSTRACT

Accurate and precise TLC-densitometric and HPLC–diode-array detector (DAD) methods have been developed and validated to resolve two binary mixtures containing pyridoxine hydrochloride (PYH) with either cyclizine hydrochloride (CYH) or meclizine hydrochloride (MEH). In the developed TLC-densitometric method, chromatographic separation of the three studied drugs was carried out on silica gel 60 F254 plates using a developing system containing methylene chloride + acetone + methanol (7 + 1 + 0.5, v/v/v) scanning separated bands at 220 nm. Beer–Lambert law was obeyed in the ranges of 0.2–5, 0.2–4, and 0.2–4 μg/band for PYH, CYH, and MEH, respectively. On the other hand, the developed HPLC–DAD method depended on chromatographic separation on a Zorbax Eclipse C18 column using methanol–KH2PO4 (0.05 M; 90 + 10, v/v; pH 5, with H3PO4 and KOH) as the mobile phase, a flow rate of 1 mL/min, and UV scanning at 220 nm. A linear relationship was obtained between the integrated peak area and the concentration in the ranges of 10–50, 10–50, and 7–50 μg/mL for PYH, CYH, and MEH, respectively. The proposed methods were successfully applied for the determination of the cited drugs in their pharmaceutical formulations. Statistical comparison with the reported methods using Student's t- and F-tests found there were no significant differences between the proposed and reported methods for accuracy and precision.

Label-Free Classification of a Nasopharyngeal Carcinoma Tissue Test at Different Stages Based on Raman Spectroscopy

Liu, Mingyu; Lin, Jinyong; Qiu, Sufang; Wu, Weilin; Liu, Gaoqiang; Li, Yan; Gong, Haiming; Chen, Rong; Chen, Guannan

ABSTRACT

Raman spectroscopy (RS) of nasopharyngeal carcinoma (NPC) tissue provides substantial biomolecular information and various biomedicine features for tissue at different stages of cancer development. This study suggested an automatic and quick method for the classification of Raman spectra at different stages of NPC by multivariate statistical analysis. During RS measurement, Raman spectra were acquired from all NPC tissues in two groups of samples: 30 early-stage NPC patients (stages I and II) and 46 advanced-stage NPC patients (stages III and IV). In addition, a tentative diagnostic algorithm comprising principal components analysis and support vector machine was used to effectively classify multivariate data from the Raman spectra to yield sensitivities (70%; 21 of 30 samples) and specificities (91%; 42 of 46 samples) by the leave-one-out cross-validation method. Meaningful chemical compositions in the classification process were then deduced by analyzing the classified mathematical model. This beneficial work provides a great potential clinical method for the automatic classification of NPC stages and the speculation of the chemical compositions for NPC staging.

Chromatographic Determination of Cyclopentolate Hydrochloride and Phenylephrine Hydrochloride in the Presence of Their Potential Degradation Products

Rezk, Mamdouh R.; Fayed, Ahmed S.; Marzouk, Hoda M.; Abbas, Samah S.

ABSTRACT

Two sensitive, selective, and precise stability-indicating methods have been developed for the simultaneous determination of the active pharmaceutical ingredients cyclopentolate hydrochloride (CLO) and phenylephrine hydrochloride (PHE) in their pure forms and in the presence of their degradation products. The methods were applied for the determination of CLO and PHE in a pharmaceutical formulation. Method A was based on isocratic elution HPLC determination. Separation was achieved using a Waters Spherisorb ODS2 C18 analytical column (5 μm particle size) and a mobile phase of 0.1% heptane-1-sulphonic acid sodium salt in methanol–water (80 + 20, v/v). The flow rate was 1.0 mL/min and detection was performed at 210 nm. Method B was an HPTLC-densitometric method using HPTLC silica gel 60 F254 plates and an optimized mobile phase of ethyl acetate–methanol–ammonia (8 + 2 + 0.1, v/v/v). The separated spots were densitometrically scanned at 210 nm. Polynomial equations were used for regression. The developed methods are suitable for the determination of CLO and PHE in their binary mixture and in the presence of their corresponding degradation products. The two methods were validated in compliance with International Conference on Harmonization guidelines and successfully applied for the determination of CLO and PHE as synthetically prepared in laboratory mixtures and in the presence of their possible degradation products. CLO alkaline degradation products were stated as potential impurities in British Pharmacopoeia. The degradation products were separated and identified by mass spectra. Postulation of a PHE oxidative degradation pathway was suggested. The obtained results were statistically analyzed and compared with those obtained by applying the official methods for both drugs.

Detection, Enumeration, and Isolation of Vibrio parahaemolyticus and V. vulnificus from Seafood: Development of a Multidisciplinary Protocol

Banerjee, Swapan K.; Farber, Jeffrey M.

ABSTRACT

Vibrio parahaemolyticus and V. vulnificus are bacterial foodborne pathogens that can cause illnesses in humans after ingestion or exposure to contaminated seafood or coastal waters. A procedure that combines microbiological, biochemical, and molecular methods was designed and optimized for the detection, enumeration, isolation, and characterization of these clinically significant Vibrio spp. Initially, microbiological culturing is used to resuscitate and isolate presumptive Vibrio spp. from chilled seafood samples. Biochemical tests are then used to analyze and select presumptive isolates at the species level, and, lastly, molecular methods, such as PCR targeting species-specific hemolysin genes, are used to confirm identification and assess the potential pathogenicity of presumptive isolates. By using artificially contaminated molluscan homogenates with known numbers of V. parahaemolyticus, this method yielded, on average, 90% recovery on complete agar media and 88% recovery on selective media. For V. vulnificus, the recovery rates were 86% (complete media) and 84% (selective media). Linearity of recovery of Vibrio spp. from artificially contaminated seafood homogenates supported the applicability of this method. Overall, this performance-tested protocol is easy to use, cost-effective, and fit-for-purpose, with potential for routine use in basic microbiological facilities.

Evaluation of TA10 Broth for Recovery of Listeria monocytogenes from Ground Beef

Kamisaki-Horikoshi, Naoko1; Okada, Yukio1; Takeshita, Kazuko1; Takada, Makoto1; Kawamoto, Shinichi2; Kawasaki, Susumu2

ABSTRACT

In 2009, the enrichment broth TA10 was released for simultaneous recovery of Salmonella spp., Listeria monocytogenes, and Escherichia coli O157:H7. This medium was compared with other Salmonella enrichment broths [lactose (LAC) broth, buffered peptone water (BPW), and universal pre-enrichment (UP) broth] for the recovery of heat- and freeze-injured Salmonella spp. in beef by the conventional culture method. There was a significant difference between TA10 and LAC enrichment broths for detecting injured Salmonella spp. In this study, the International Organization for Standardization Listeria pre-enrichment broth (Half-Fraser/Fraser) was compared with TA10 broth for the recovery of L. monocytogenes from ground beef. Ground beef samples were contaminated with single Listeria serovars at levels of 0.096 to 0.001 most probable number/g. Twenty 25 g test portions of the contaminated ground beef were pre-enriched in each broth, and the ISO-11290-1 Listeria official isolation protocol was used thereafter. There was a significant difference between TA10 broth (48 h) and Half-Fraser/Fraser broth (72 h) in the recovery of L. monocytogenes. In addition, the incubation time for TA10 broth was shorter than for Half-Fraser/Fraser broth. The results indicate that TA10 broth should be used instead of Half-Fraser/Fraser broth for analysis of beef that may be contaminated with very low levels of L. monocytogenes.

The Celiac Patient Antibody Response to Conventional and Gluten-Removed Beer

Allred, Laura K.1; Lesko, Katherine2; McKiernan, Diane3; Kupper, Cynthia1; Guandalini, Stefano4

ABSTRACT

Enzymatic digestion, or hydrolysis, has been proposed for treating gluten-containing foods and beverages to make them safe for persons with celiac disease (CD). There are no validated testing methods that allow the quantitation of all the hydrolyzed or fermented gluten peptides in foods and beverages that might be harmful to CD patients, making it difficult to assess the safety of hydrolyzed products. This study examines an ELISA-based method to determine whether serum antibody binding of residual peptides in a fermented barley-based product is greater among active-CD patients than a normal control group, using commercial beers as a test case. Sera from 31 active-CD patients and 29 nonceliac control subjects were used to assess the binding of proteins from barley, rice, traditional beer, gluten-free beer, and enzymatically treated (gluten-removed) traditional beer. In the ELISA, none of the subjects' sera bound to proteins in the gluten-free beer. Eleven active-CD patient serum samples demonstrated immunoglobulin A (IgA) or immunoglobulin G (IgG) binding to a barley extract, compared to only one nonceliac control subject. Of the seven active-CD patients who had an IgA binding response to barley, four also responded to traditional beer, and two of these responded to the gluten-removed beer. None of the nonceliac control subjects' sera bound to all three beer samples. Binding of protein fragments in hydrolyzed or fermented foods and beverages by serum from active-CD patients, but not nonceliac control subjects, may indicate the presence of residual peptides that are celiac-specific.

Effect of Source of DNA on the Quantitative Analysis of Genetically Engineered Traits Using Digital PCR and Real-Time PCR

Demeke, Tigst1; Malabanan, Jemima2; Holigroski, Michelle1; Eng, Monika1

ABSTRACT

Seven commercially available DNA extraction kits were compared with a cetyltrimethylammonium bromide (CTAB) method to determine the suitability of the extracted DNA for RainDrop digital PCR (dPCR) and real-time PCR (RT-PCR) quantification of OXY235 canola, FP967 flax, and DP305423 soybean (spiked at the 0.1% level). For the kits, the highest amount of DNA extracted from a 0.2 g sample was obtained using OmniPrep for Plant for flax and DNeasy mericon Food for canola and soybean. For canola, DNA extracted with the Fast ID Genomic DNA Extraction Kit, FastDNA Spin Kit, GM Quicker 2, NucleoSpin Food, and DNeasy mericon Food was suitable for dPCR and RT-PCR. For flax, DNA extracted with Fast ID, FastDNA Spin Kit, OmniPrep for Plant, and NucleoSpin Food was suitable for RT-PCR. However, only Fast ID yielded DNA suitable for dPCR. For soybean, DNA extracted with five and six of the seven DNA extraction kits was suitable for dPCR and RT-PCR, respectively. Overall, Fast ID provided reliable results regardless of species or analysis method used. Canola, flax, and soybean DNA extracted with the CTAB method and then purified were suitable for both dPCR and RT-PCR. This is the first report showing the effect of different DNA extraction methods on the absolute quantification of genetically engineered traits using dPCR.

Characterization of the Thermal Degradation of Vinegar and the Construction of an Identification Model for Chinese Geographical Indication Vinegars by the Py-GC-MS Technique

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ABSTRACT

The pyrolysis (Py)-GC-MS technique was first introduced for the identification of two kinds of Chinese geographical indication vinegars because its advantages are that it is a simple and convenient sample pretreatment and inlet method. Abundant Py information about vinegars was obtained using Py-GC-MS; 21 common peaks were selected. With the help of the classical partial least-squares (PLS) modeling method for data analysis, two identification models for Shanxi extra-aged (SX) and Zhenjiang (ZJ) vinegars were established, respectively. An N-reducing method was used to select the variables. The variables were reduced one at a time to build the PLS models with the lowest number of misjudgments. Both models had good recognition rates, identifying over 90% of samples correctly. Thus, combining Py-GC-MS and PLS could be regarded as an effective method for the identification of SX and ZJ vinegars.

Characterization of Final Action Official Method SM 2011.19 and First Action Official Method 2015.06 Performance at Analyte Levels Corresponding to CODEX STAN 72 (1981) Minimum Levels

Thompson, Joseph J.; Pacquette, Lawrence H.

ABSTRACT

A limited single-laboratory validation (SLV) was conducted in the authors' laboratory to investigate the performance of AOAC Official Methods SM 2011.19 Determination of Chromium (Cr), Selenium (Se), and Molybdenum (Mo) in Infant Formula and Adult Nutritional Products by Inductively Coupled Plasma/Mass Spectrometry and 2015.06 Determination of Minerals and Trace Elements in Infant Formula and Adult/Pediatric Nutritional Formula by Inductively Coupled Plasma/Mass Spectrometry at analyte levels below the practical LOQs (PLOQs) already published for these Final Action Official Methods. This work was needed to verify that the actual LOQs were below the minimum requirements for minerals in infant formula as given in CODEX STAN 72 (1981). Linearity studies at low levels were conducted as well as the analysis of blanks over multiple days to establish the LOQs (as opposed to PLOQs) for these nutrients. Several placebo matrixes from the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) program were tested over multiple days at two different sample sizes to quantitate the effect of doubling the sample size given in the original publications. The SLV results indicate that both methods can meet the Codex minimum requirements as-is, without modification of the methods, albeit with a relaxation of the stringent precision criteria originally established for these methods by SPIFAN. Precision can be improved by doubling the sample size, but this step is not necessary to use the method for its intended purpose. A concurrent collaborative study of Method 2015.06 showed that the RSDR obtained across eight laboratories for several infant formula placebos containing mineral concentrations between the PLOQ and LOQ were indeed worse than SPIFAN expectations, but reasonable Horwitz ratios (HorRat) were nonetheless obtained for these analytes.

RAPD- and ERIC-Based Typing of Clinical and Environmental Pseudomonas aeruginosa Isolates

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ABSTRACT

Pseudomonas aeruginosa is a major cause of nosocomial infection in children and adults, resulting in significant morbidity and mortality due to its ability to acquire drug resistance. The ability of P. aeruginosa in the environment to cause infection in individuals has been reported previously; henceforth, surveillance of the emergence and transmission of P. aeruginosa strains among patients is important for infection control in a clinical setup. Various gene-typing methods have been used for epidemiological typing of P. aeruginosa isolates for the purpose of surveillance. In this work, the suitability and comparability of two typing methods, enterobacterial repetitive intergenic consensus (ERIC)-PCR and random amplification of polymorphic DNA (RAPD)-PCR fingerprinting, were studied to characterize P. aeruginosa strains isolated from clinical and environmental sources. Forty-four clinical and environmental bacterial isolates of P. aeruginosa were collected between October 2015 and January 2016. DNA extraction, ERIC-PCR and RAPD-PCR, agarose gel electrophoresis, and phylogenetic analyses were carried using the unweighted pair-group method with mean. RAPD typing revealed less clonality among clinical isolates, whereas the ERIC method showed greater similarity in comparison with RAPD. Environmental isolates, however, showed greater similarity using RAPD compared with ERIC typing. With only a few exceptions, most clinical isolates were distinct from environmental isolates, irrespective of the typing method. In conclusion, both the RAPD and ERIC typing methods proved to be good tools in understanding clonal diversity. The results also suggest that there is no relationship between clinical and environmental isolates. The absence of clonality among the clinical isolates may indicate that most P. aeruginosa infection cases could be endemic and not epidemic and that endemic infections may be due to nonclonal strains of P. aeruginosa.

Modified Activated Carbon Prepared from Acorn Shells as a New Solid-Phase Extraction Sorbent for the Preconcentration and Determination of Trace Amounts of Nickel in Food Samples Prior to Flame Atomic Absorption Spectrometry

Ebrahimi, Bahram

ABSTRACT

A new solid-phase extraction (SPE) sorbent was introduced based on acidic-modified (AM) activated carbon (AC) prepared from acorn shells of native oak trees in Kurdistan. Hydrochloric acid (15%, w/w) and nitric acid (32.5%, w/w) were used to condition and modify AC. The IR spectra of AC and AM-AC showed that AM lead to the formation of increasing numbers of acidic functional groups on AM-AC. AM-AC was used in the SPE method for the extraction and preconcentration of Ni+2 prior to flame atomic absorption spectrometric determination at ng/mL levels in model and real food samples. Effective parameters of the SPE procedure, such as the pH of the solutions, sorbent dosage, extraction time, sample volume, type of eluent, and matrix ions, were considered and optimized. An enrichment factor of 140 was obtained. The calibration curve was linear with an R2 of 0.997 in the concentration range of 1–220 ng/mL. The RSD was 5.67% (for n = 7), the LOD was 0.352 ng/mL, and relative recoveries in vegetable samples ranged from 96.7 to 103.7%.

Simultaneous Enrichment and On-line Detection of Low-Concentration Copper, Cobalt, and Nickel Ions in Water by Near-Infrared Diffuse Reflectance Spectroscopy Combined with Chemometrics

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ABSTRACT

Sensitive detection of heavy metal ions in water is of great importance considering the effects that heavy metals have on public health. A developed fluidized bed enrichment technique was used to concentrate and detect low concentrations of Cu2+, Co2+, and Ni2+ in water samples by near-IR diffuse reflectance (NIDR) spectroscopy (NIDRS) directly without using any chemicals or reagents. The NIDR spectra of adsorbent were measured on-line, and quantitative detection was achieved by applying a built partial least-squares chemometric model. Sensitivity and accuracy was improved significantly because large-volume mixture solutions were used in the enrichment process. Root mean square error of cross-validation values for Cu2+, Co2+, and Ni2+ were 0.29, 0.41, and 0.35 μg/mL, respectively, with mean relative error values in the acceptable range of 6.56–10.27%. This study confirms the potential application of fluidized bed enrichment combined with NIDRS and chemometrics for the simultaneous detection of trace heavy metal ions in water, with low relative error.

Rapid Measurement of Food Adulteration with Minimal Sample Preparation and No Chromatography Using Ambient Ionization Mass Spectrometry

Dalmia, Avinash

ABSTRACT

A rapid method, with minimal sample preparation and no chromatography, was developed for analyzing food samples such as olive oil and pomegranate juice to measure adulteration with cheaper ingredients using the novel Direct Sample Analysis™ (DSA) ion source in conjunction with a time-of-flight (TOF)-MS. In less than 30 s, with minimal sample preparation and method development, adulteration of olive oil and pomegranate juice with cheaper seed oils and fruit juices, respectively, was measured with DSA/TOF-MS.

Screening Natural Content of Water-Soluble B Vitamins in Fish: Enzymatic Extraction, HILIC Separation, and Tandem Mass Spectrometric Determination

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ABSTRACT

Despite the potential of LC with tandem MS (MS/MS) in improving sensitivity and selectivity, analytical methods are scarce for the determination of protein-bound and phosphorylated forms of B vitamins in food. This prompted us to develop a method for LC-MS/MS determination of naturally occurring nicotinamide, nicotinic acid, thiamine, pyridoxine, riboflavin, pantothenic acid, biotin, folic acid, and cyanocobalamin in fish. Baseline separation of the vitamins was achieved in a hydrophilic interaction LC condition. An ultrasonication-assisted enzymatic extraction protocol for sample preparation was optimized and validated. The time required for extraction was significantly reduced (to 4 h), while maintaining good extraction efficiency. Acetonitrile content (80%, v/v) in the prepared sample was found to be optimum for excellent peak shape and sensitivity. The dynamic linear range of the vitamins ranged from 2.5 to 500 ng/g, and the regression coefficient values were greater than 0.99. LOQ values ranged from 0.4 to 50 ng/g for the different vitamins. The spike recovery values at 50 and 100 ng/g ranged from 87.5 to 97.5%. The intra- and interday precision values were satisfactory. Accuracy of the developed method was determined by analysis of a Certified Reference Material. The method could also be used for unambiguous determination of the natural content of the target vitamins in fish.

Optimization of a Modified QuEChERS Method for Multiresidue Analysis of Pharmaceuticals and Personal Care Products in Sewage and Surface Water by LC-MS/MS

Authors: Kachhawaha, Akanksha S.1; Nagarnaik, Pranav M.1; Jadhav, Manjusha2; Pudale, Anjali3; Labhasetwar, Pawan K.1; Banerjee, Kaushik2

ABSTRACT

A quick, sensitive multiresidue method was developed for the analysis of 19 multiclass pharmaceuticals and personal care products (PPCPs) in surface water and sewage water. The proposed modified QuEChERS method involved the extraction of water samples (10 mL) with acetonitrile (10 mL) after the addition of 1% acetic acid, 4 g magnesium sulfate, and 0.2 g ammonium acetate, and was validated in distilled water, surface water, and sewage water with respect to linearity, LOD and LOQ, precision, and accuracy. The LOD and LOQ varied within the ranges of 0.001–0.167 and 0.002–0.25 ng/mL, respectively. Recoveries of the target compounds ranged from 73 to 125%, with precision RSD values <27%. The method provided a precise estimation of PPCPs in field samples, and acetaminophen, atenolol, metformin, sulfamethoxazole, carbamazepine, methylparaben, and triclosan were detected in concentrations ranging from 0.10 to 1.40 and 0.10 to 3.4 ng/mL in surface water and sewage water, respectively. This is an innovative application of the QuEChERS approach for estimation of PPCPs from aqueous matrixes. The method provides significantly higher output (preparation of 25–30 samples a day) compared to conventional SPE-based methods (<10 samples a day).

Purity Evaluation of Curcuminoids in the Turmeric Extract Obtained by Accelerated Solvent Extraction

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ABSTRACT

Curcuminoids, the active principle of Curcuma longa L, is one of the most researched subjects worldwide for its broad-spectrum biological activities. Being traditionally known for their anticancer properties and issues related to bioavailability, the curcuminoids, including diferuloylmethane (curcumin), have gained special attention. Thus, the current study focused on the purity profiling of curcuminoids when extracted by accelerated solvent extraction, which was run with turmeric rhizome powder (20 g) at 1500 psi and at 50°C, with a static time of 10 min and with three cycles. The performance of ethanol, ethyl acetate, and acetone as extraction solvents was comparatively evaluated. Once extracted, the individual curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) were purified by column chromatography, followed by preparative TLC, and the compounds were characterized by spectroscopic and chromatographic techniques. The HPLC method was standardized by using a gradient mobile phase of water and acetonitrile containing 0.1% formic acid. The LODs were calculated as 0.27, 0.33, and 0.42 μg/mL for curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively. Accuracy (relative percentage error) and precision RSD values of the developed HPLC method were below 5%. The intraday accuracy ranged between −0.9 and −3.63%. The physical yield was the highest in ethanol (8.4%) extraction, followed by ethyl acetate (7.4%) and acetone (6.6%). Maximum purity was recorded in acetone (46.2%), followed by ethanol (43.4%) and ethyl acetate (38.8%), with no significant differences across the individual curcuminoids. This research will be useful for future applications related to the extraction of curcuminoids at a commercial level and to their profiling in food matrixes.

Determination and Uncertainty Analysis of Inorganic Arsenic in Husked Rice by Solid Phase Extraction and Atomic Absorption Spectrometry with Hydride Generation

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ABSTRACT

This study enables the selective determination of inorganic arsenic (iAs) with a low detection limit using an economical instrument [atomic absorption spectrometer with hydride generation (HG)] to meet the regulatory requirements as per European Commission (EC) and Codex guidelines. Dry rice samples (0.5 g) were diluted using 0.1 M HNO3–3% H2O2 and heated in a water bath (90 ± 2°C) for 60 min. Through this process, all the iAs is solubilized and oxidized to arsenate [As(V)]. The centrifuged extract was loaded onto a preconditioned and equilibrated strong anion-exchange SPE column (silica-based Strata SAX 500 mg/6 mL), followed by selective and sequential elution of As(V), enabling the selective quantification of iAs using atomic absorption spectrometry with HG. In-house validation showed a mean recovery of 94% and an LOQ of 0.025 mg/kg. The repeatability (HorRatr) and reproducibility (HorRatR) values were <2, meeting the performance criteria mandated by the EC. The combined standard measurement uncertainty by this method was less than the maximum standard measurement uncertainty; thus, the method can be considered for official control purposes. The method was applied for the determination of iAs in husked rice samples and has potential applications in other food commodities.

Multiresidue Method for Targeted Screening of Pesticide Residues in Spice Cardamom (Elettaria cardamomum) by Liquid Chromatography with Tandem Mass Spectrometry

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ABSTRACT

A QuEChERS technique–based sample preparation method was optimized and validated in small cardamom to monitor the residues of 154 pesticides by LC with tandem MS. The proposed multiresidue method involved soaking powdered cardamom (2 g) in water (8 mL) for 30 min, followed by extraction with acetonitrile (10 mL). Cleanup by dispersive SPE was performed using primary secondary amine (25 mg/mL), C18 (25 mg/mL), and anhydrous magnesium sulfate (150 mg/mL). The method was validated as per the SANTE/11945/2015 guidelines at 5, 10, 50, and 100 ng/g spiking levels, and most of the analytes showed recoveries between 70 and 120% (with RSDs ≤20%). The LOQ of ≤10 ng/g was achieved for almost 90% of the target pesticides. The measurement uncertainties were evaluated at 100 ng/g, and the global uncertainty values were below 22% for all the analytes.

Fast Gas Chromatography with Tandem Mass Spectrometry Analysis of Selected Persistent Organic Pollutants and Organophosphorus and Synthetic Pyrethroid Pesticides in Indian Prawn (Fenneropenaeus indicus) in Compliance with the EU-MRLs

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ABSTRACT

A fast GC with tandem MS method was developed and validated for multiresidue determination of 95 chemical contaminants (24 synthetic pyrethroids, 17 organochlorines, 17 organophosphorus compounds, 18 polycyclic aromatic hydrocarbons, and 19 polychlorinated biphenyls) in Indian prawns (Fenneropenaeus indicus) as per the European Union maximum residual limit requirements. Chromatographic separation and MS determination were achieved within a short run time of 18 min, without compromising sensitivity and specificity. Our findings revealed a 2.5× reduction in the run time compared with conventional GC methods. Sample preparation involved a QuEChERS-based extraction of 10 g sample with 10 mL acidified acetonitrile (1% acetic acid) and phase separation with 6 g anhydrous magnesium sulfate and 1.5 g sodium acetate. The extract was cleaned in two steps, first by dispersive cleanup with primary secondary amine and then by C18 SPE cartridge. The regression coefficients of linearity (r2) for the concentration range of 5–50 ng/mL were >0.99 for all the compounds. Recoveries at 5 and 10 ng/g levels were within the acceptable range of 70–120%. The repeatability (RSDr) and within-laboratory reproducibility (RSDwR) precisions were ≤20%. The method was successfully applied for analysis of the real world samples for incurred residues.

Evaluation of Matrix Effects in Multiresidue Analysis of Pesticide Residues in Vegetables and Spices by LC-MS/MS

Chawla, Suchi; Patel, Hemlatta K.; Gor, Hetal N.; Vaghela, Kiran M.; Solanki, Priti P.; Shah, Paresh G.

ABSTRACT

The study was conducted to investigate matrix interferences using QuEChERS sample preparation to understand whether the dilution of matrix and/or the grouping of commodities can eliminate the need for selective individual matrix-matched standards in LC with tandem MS (MS/MS) analysis, and whether the calibration graph based on only one matrix can be used for quantification in the other matrixes. Matrix effects (MEs) were studied by comparing the slopes of calibration curves of the matrix-matched standards (diluted with mobile phase) vis-à-vis the solvent-based standards. The present study showed that MEs were dependent on the nature of both the commodity and the analyte. Among the test matrixes, the highest variability in ME was recorded in capsicum. Most of the pesticides showed signal suppression in tomato, capsicum, and cumin matrixes. In brinjal matrix, the signal of most of the pesticides showed slight enhancement. Due to the similar nature of the MEs in tomato and capsicum, these two commodities can be grouped together. Considering analyte variability, acetamiprid, 3-hydroxy carbofuran, dichlorvos, dimethoate, and spinosyn A and D showed no significant ME (≤20%) in tomato. Very high MEs (2360.9 and 1250.8%) were observed for quizalofop-p-tefuryl and tebuconazole, respectively. To check the effect of dilution in minimizing the ME, cucumber and brinjal matrixes were diluted 10×, and calibration curves were drawn with five concentration levels. It was found that about 60% of the total analyzed pesticides showed MEs ≤20%. In cumin, MEs ranged from −5.3% for triazophos to 661% for thiacloprid. Most of the pesticides showed recoveries in the acceptable range of 70–130% with calibration curves from both matrixes. To compensate for MEs, it is suggested that (1) tomato and capsicum matrixes, which show similar trends, can be grouped together; and (2) cucumber matrix, when diluted 10×, can be used to prepare calibration curves for the quantification of pesticides in various fruiting and cucurbit vegetable matrixes by LC-MS/MS.

A Rapid Method for the Quantitative Determination of 34 Pesticides in Nonalcoholic Carbonated Beverages Using Liquid–Liquid Extraction Coupled to Dispersive Solid-Phase Cleanup Followed by Gas Chromatography with Tandem Mass Spectrometry

Rai, Satyajeet1; Gullapalli, Madhuri Devi2; Srivastava, Anshuman1; Shaik, Hussain2; Siddiqui, Mohammed Haris3; Mudiam, Mohana Krishna Reddy2

ABSTRACT

An economical, rapid, and sensitive multiresidue method using liquid–liquid extraction (LLE) coupled with dispersive SPE (dSPE) cleanup was developed for the quantitative determination of 34 multiclass multiresidue (MCMR) pesticides (14 organochlorines, eight organophosphates, 10 synthetic pyrethroids, and two herbicides) in nonalcoholic carbonated beverages (cola, orange, lemon-lime, and citra) using GC with tandem MS. The procedure mainly involved LLE by dichloromethane and dSPE cleanup in the presence of magnesium sulfate, primary secondary amine, and C18. The RSD of the developed method was found to be less than 14%. The LOD and LOQ values for all the analyzed pesticides were found in the ranges of 0.001–0.027 μg/L and 0.004–0.088 μg/L, respectively. The LOQ levels of the pesticides analyzed were found to be well below the recommended limit by the European Union (0.1 μg/L in water). The mean recoveries of pesticides in different nonalcoholic carbonated beverages (cola, orange, lemon-lime, and citra) were found to be in the range of 79–111%, with RSDs less than 11%. The validation data prove that the method can be acceptable to regulatory agencies for the routine analysis of MCMR pesticides in nonalcoholic carbonated beverages.

Determination of Triazines and Triazoles in Grapes Using Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization High-Resolution Mass Spectrometry

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ABSTRACT

A chromatography-free atmospheric pressure matrix-assisted laser desorption/ionization high-resolution mass spectrometry (AP-MALDI HRMS) method is described for the simultaneous and quantitative detection of triazines and triazoles in grapes. The analytes were detected reproducibly with high mass accuracy (mass error within 5 ppm) and further confirmed by collision-induced dissociation fragmentation in tandem MS. The LODs and LOQs for all the analytes were found to be in the nanogram per gram level (15–20 ng/g LOQ). Internal standard–normalized high-resolution accurate mass–extracted (HR-AM) peak intensities of the detected ions were used to generate the concentration response curves. Linearity (with R2 values around 0.99) was obtained for these curves within a concentration range of 20–200 ng/g of the individual analytes. The accuracy and precision of the method were further established using QC samples. Validation and performance comparison of the AP-MALDI HRMS method with an existing standard method using LC with triple quadrupole MS was carried out (evaluating sensitivity, accuracy, precision, and analysis time) using 20 table-grape field samples after QuEChERS extraction.

Analysis of Numerous Androgen Disruptors in Fish by Gas Chromatography Tandem Mass Spectrometry

Liu, Shaoying1; Bian, Tianbin1; He, Huali1; Huang, Huang1; Jin, Quan1; Wang, Shuting1; Zhu, Guonian2

ABSTRACT

An improved analytical method was developed for the simultaneous quantification of numerous androgen disruptors—vinclozolin, dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes, chlordane, heptachlor, dieldrin, endrin, and aldrin—in fish, followed by GC with tandem MS (MS/MS). Extraction, cleanup, and MS/MS parameters of analytes were optimized. The LOQs of the analytes ranged from 0.3 to 3.7 μg/kg. Reasonable recoveries (73.7–119.2%) were demonstrated at different spike levels with RSDs lower than 12.2%. This method was applied for the analysis of the target analytes in fish samples sold in Hangzhou, China, and DDTs were found to be the predominant contaminants in fish samples.

Antioxidant Evaluation and Composition Analysis of Extracts from Fuzhuan Brick Tea and Its Comparison with Two Instant Tea Products

Zhang, Qing-An; Zhang, Xiao-Li; Yan, Yan-Ying; Fan, Xue-Hui

ABSTRACT

In this paper, the antioxidant capacities and compositions of two commercialized tea products and extracts from Fuzhuan brick tea (FBT) were investigated using three HPLC methods comparing the retention times of injected standards. Principal component analysis and DPPH-spiking HPLC analysis were used to analyze correlation between antioxidant capacity and the compounds detected to screen which compounds contribute to antioxidant activity. Results indicated that all samples contained high amounts of polysaccharides, phenols, and flavonoids and had good antioxidant activity and a high level of correlation among them. Furthermore, gallic acid, epigallocatechin, epicatechin, and epigallocatechin gallate were screened and found to be stronger antioxidant candidates. In summary, the quality of the FBT extracts was not inferior to that of commercialized tea products, suggesting the feasibility that extracts may directly act as instant tea products.

Quantitative Analysis of Aloins and Aloin-Emodin in Aloe Vera Raw Materials and Finished Products Using High-Performance Liquid Chromatography: Single-Laboratory Validation, First Action 2016.09

Kline, David1; Ritruthai, Vicha1; Babajanian, Silva1; Gao, Quanyin2; Ingle, Prashant2; Chang, Peter3; Swanson, Gary3

ABSTRACT

A single-laboratory validation study is described for a method of quantitative analysis of aloins (aloins A and B) and aloe-emodin in aloe vera raw materials and finished products. This method used HPLC coupled with UV detection at 380 nm for the aloins and 430 nm for aloe-emodin. The advantage of this test method is that the target analytes are concentrated from the sample matrix (either liquid or solid form) using stepwise liquid–liquid extraction (water–ethyl acetate–methanol), followed by solvent evaporation and reconstitution. This sample preparation process is suitable for different forms of products. The concentrating step for aloins and aloe-emodin has enhanced the method quantitation level to 20 parts per billion (ppb). Reversed-phase chromatography using a 250 × 4.6 mm column under gradient elution conditions was used. Mobile phase A is 0.1% acetic acid in water and mobile phase B is 0.1% acetic acid in acetonitrile. The HPLC run starts with a 20% mobile phase B that reaches 35% at 13 min. From 13 to 30 min, mobile phase B is increased from 35 to 100%. From 30 to 40 min, mobile phase B is changed from 100% back to the initial condition of 20% for re-equilibration. The flow rate is 1 mL/min, with a 100 μL injection volume. Baseline separation (Rs > 2.0) for aloins A and B and aloe-emodin was observed under this chromatographic condition. This test method was validated with raw materials of aloe vera 5× (liquid) and aloe vera 200× (powder) and finished products of aloe concentrate (liquid) and aloe (powder). The linearity of the method was studied from 10 to 500 ppb for aloins A and B and aloe-emodin, with correlation coefficients of 0.999964, 0.999957, and 0.999980, respectively. The test method was proven to be specific, precise, accurate, rugged, and suitable for the intended quantitative analysis of aloins and aloe-emodin in raw materials and finished products. The S/N for aloins A and B and aloe-emodin at 10 ppb level were 12, 10, and 8, respectively, indicating our conservative LOD level at 10 ppb (the typical LOD level S/N is about 3). The S/N for aloins A and B and aloe-emodin at the 20 ppb level were 17, 14, and 16, respectively, indicating our conservative LOQ level at 20 ppb (the typical LOQ level S/N is about 10). The stock standard solution of a mixture of aloins and aloe-emodin and a working standard solution were found to be stable for at least 19 days when stored refrigerated at 2–8°C, with a recovery of 100 ± 5%.

Development and Validation of an RP-HPLC Method for the Determination of Vinpocetine and Folic Acid in the Presence of a Vinpocetine Alkaline Degradation Product in Bulk and in Capsule Form

Elkady, Ehab F.1; Tammam, Marwa H.2; Mohamed, Ayman A.2

ABSTRACT

An alkaline-forced degradation hydrolytic product of vinpocetine was prepared and characterized by 1H-NMR, FTIR spectroscopy, and MS. Subsequently, a simple, selective, and validated reversed-phase HPLC method was developed for the simultaneous estimation of vinpocetine and folic acid in the presence of a vinpocetine alkaline degradation product. Chromatographic separation was achieved using an isocratic mobile phase consisting of acetonitrile–0.02 M KH2PO4 [containing 0.2% (v/v) triethylamine and adjusted to pH 6 with orthophosphoric acid; (80 + 20, v/v)] at a flow rate of 0.9 mL/min at ambient temperature on a Eurospher II C18 (250 × 4.6 mm, 5 μm) column, with UV detection at 280 nm for folic acid and 230 nm for vinpocetine and its alkaline hydrolytic product. Linearity, accuracy, and precision were found to be acceptable over a concentration range of 12.5–200 μg/mL for vinpocetine and 1–16 μg/mL for folic acid. The proposed method was successfully applied for the determination of both drugs and a vinpocetine hydrolysis product in a laboratory-prepared mixture and in a capsule containing both drugs.

Validated Spectrophotometric and RP-HPLC–DAD Methods for the Determination of Ursodeoxycholic Acid Based on Derivatization with 2-Nitrophenylhydrazine

El-Kafrawy, Dina S.1; Belal, Tarek S.2; Mahrous, Mohamed S.1; Abdel-Khalek, Magdi M.1; Abo-Gharam, Amira H.1

ABSTRACT

This work describes the development, validation, and application of two simple, accurate, and reliable methods for the determination of ursodeoxycholic acid (UDCA) in bulk powder and in pharmaceutical dosage forms. The carboxylic acid group in UDCA was exploited for the development of these novel methods. Method 1 is the colorimetric determination of the drug based on its reaction with 2-nitrophenylhydrazine hydrochloride in the presence of a water-soluble carbodiimide coupler [1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride] and pyridine to produce an acid hydrazide derivative, which ionizes to yield an intense violet color with maximum absorption at 553 nm. Method 2 uses reversed-phase HPLC with diode-array detection for the determination of UDCA after precolumn derivatization using the same reaction mentioned above. The acid hydrazide reaction product was separated using a Pinnacle DB C8 column (4.6 × 150 mm, 5 μm particle size) and a mobile phase consisting of 0.01 M acetate buffer (pH 3)–methanol–acetonitrile (30 + 30 + 40, v/v/v) isocratically pumped at a flow rate of 1 mL/min. Ibuprofen was used as the internal standard (IS). The peaks of the reaction product and IS were monitored at 400 nm. Different experimental parameters for both methods were carefully optimized. Analytical performance of the developed methods were statistically validated for linearity, range, precision, accuracy, specificity, robustness, LOD, and LOQ. Calibration curves showed good linear relationships for concentration ranges 32–192 and 60–600 μg/mL for methods 1 and 2, respectively. The proposed methods were successfully applied for the assay of UDCA in bulk form, capsules, and oral suspension with good accuracy and precision. Assay results were statistically compared with a reference pharmacopeial HPLC method, and no significant differences were observed between proposed and reference methods.

Development of a Terbium-Sensitized Fluorescence Method for Analysis of Silibinin

Ershadi, Saba1; Jouyban, Abolghasem2; Molavi, Ommoleila3; Shayanfar, Ali4

ABSTRACT

Silibinin is a natural flavonoid with potent anticancer properties, as shown in both in vitro and in vivo experiments. Various methods have been used for silibinin analysis. Terbium-sensitized fluorescence methods have been widely used for the determination of drugs in pharmaceutical preparations and biological samples in recent years. The present work is aimed at providing a simple analytical method for the quantitative determination of silibinin in aqueous solutions based on the formation of a fluorescent complex with terbium ion. Terbium concentration, pH, and volume of buffer, the important effective parameters for the determination of silibinin by the proposed method, were optimized using response surface methodology. The fluorescence intensity of silibinin was measured at 545 nm using λex = 334 nm. The developed method was applied for the determination of silibinin in plasma samples after protein precipitation with acetone. Under optimum conditions, the method provided a linear range between 0.10 and 0.50 mg/L, with a coefficient of determination (R2) of 0.997. The LOD and LOQ were 0.034 and 0.112 mg/L, respectively. These results indicate that the developed method is a simple, low-cost, and suitable analytical method for the quantification of silibinin in aqueous solution and plasma samples.

Novel Pure Component Contribution Algorithm (PCCA) and UHPLC Methods for Separation and Quantification of Amlodipine, Valsartan, and Hydrochlorothiazide in Ternary Mixture

Mowaka, Shereen1; Hegazy, Maha A.2; Lotfy, Hayam M.2; Mohamed, Ekram H.1

ABSTRACT

Two accurate and sensitive methods were developed and validated for the simultaneous determination of amlodipine (AML), valsartan (VAL), and hydrochlorothiazide (HCT) in their ternary mixture. The first method is a novel simple algorithm capable of extracting the contribution of each component from a mixture signal in which the components are partially or completely overlapped. It is based on the use of a coded function that eliminates the signal of interfering components using mean centering as a processing tool. Determination was performed at 237.6, 250.0, and 270.6 nm for AML, VAL, and HCT, respectively. Two fit values were developed and calculated for optimization of the method for each drug, one to test that the absorptivity values of the extracted spectra are within the confidence limits of the slope, and the other for correlation between the pure and extracted spectra. The fit values for AML, VAL, and HCT were α = 0.0449, 0.03981, and 0.07251, respectively, and r = 1 for each drug. The second method is an ultra-HPLC (UHPLC®) method in which separation of AML, VAL, and HCT was carried out on a UHPLC C18 column (100 × 2.1 mm, 2.2 μm) using a mobile phase of acetonitrile–methanol–phosphate buffer (pH 2.8; 25 + 50 + 25, v/v/v). The flow rate was 0.5 mL/min, and the detection was set at 255.0 nm. The proposed methods were successfully applied to the analysis of AML, VAL, and HCT in pharmaceutical formulations, without interference from the dosage-form additives. The results were statistically compared to a previously reported method, and no significant difference was found regarding accuracy or precision.

Computation-Assisted Molecularly Imprinted Polymer Synthesis for Extraction of Naltrexone from Urine Using Experimental Design and Determination by UPLC–DAD

Rahmani, Mahdiyeh Ebrahimi1; Ansari, Mehdi2; Nateghi, Mohammadreza3; Kazemipour, Maryam1

ABSTRACT

To design a molecularly imprinted polymer (MIP) for naltrexone, calculations were performed using Gaussian 03 software, and the interaction energy (ΔE) of template–monomer complexes was estimated using the density functional theory method with the B3LYP function and 6-311G (d) basis set. The effect of different solvents in the polymerization process was studied using the polarizable continuum model. It was shown that five molecules of methacrylic acid gave the largest ΔE with tetrahydrofuran as the polymerization solvent. Effective factors of the removal efficiency of naltrexone by the MIP were selected using a central composite design, and thereafter, the optimization of significant factors was performed by response surface methodology. The results predicted through these models showed good agreement with experimental values. The adsorption amount, selectivity distribution coefficient, and selectivity coefficient were found to be 11.60 mg/g, 35.31, and 2.27, respectively. Experiments of naltrexone adsorption onto the MIP were in accordance with the first-order and Langmuir-Freundlich adsorption models. By applying the data to the Scatchard equation, the K D and Q max were determined as 526.31 mg/L and 19.47 mg/g, respectively.

Ionic Liquid Dispersive Liquid–Liquid Microextraction Method for the Determination of Irinotecan, an Anticancer Drug, in Water and Urine Samples Using UV-Vis Spectrophotometry

Uysal, Deniz; Karadaş, Cennet; Kara, Derya

ABSTRACT

A new, simple, efficient, and environmentally friendly ionic liquid dispersive liquid–liquid microextraction method was developed for the determination of irinotecan, an anticancer drug, in water and urine samples using UV-Vis spectrophotometry. The ionic liquid 1-hexyl-3-methylimidazolium hexafluorophosphate was used as the extraction solvent, and ethanol was used as the disperser solvent. The main parameters affecting the extraction efficiency, including sample pH, volume of the ionic liquid, choice of the dispersive solvent and its volume, concentration of NaCl, and extraction and centrifugation times, were investigated and optimized. The effect of interfering species on the recovery of irinotecan was also examined. Under optimal conditions, the LOD (3σ) was 48.7 μg/L without any preconcentration. Because the urine sample was diluted 10-fold, the LOD for urine would be 487μg/L. However, this could be improved 16-fold if preconcentration using a 40 mL aliquot of the sample is used. The proposed method was successfully applied to the determination of irinotecan in tap water, river water, and urine samples spiked with 10.20 mg/L for the water samples and 8.32 mg/L for the urine sample. The average recovery values of irinotecan determined were 99.1% for tap water, 109.4% for river water, and 96.1% for urine.

Simultaneous Determination of Five Active Components in the Chinese Patent Medicine Niuhuang Jiangya Pill by HPLC-MS/MS

Xiong, Shan1; Lei, Shanshan2

ABSTRACT

Niuhuang Jiangya (NHJY) pill is one of the well-known Chinese patent medicines in China used in the treatment of high blood pressure. The primary purpose of this study was to establish and validate a method using HPLC with tandem MS for the quality evaluation of NHJY pill through simultaneous determination of the following five active components: baicalin, paeoniflorin, astragaloside IV, ferulic acid, and emodin. Chromatographic separation was carried out on a Hypersil GOLD HPLC C18 column (50 × 4.6 mm, 3 μm) with acetonitrile and water as mobile phase and gradient elution at a flow rate of 0.4 mL/min. The method established in this study was selective, linear, precise, and accurate and was successfully applied to evaluate five active components in NHJY pill collected from different production batches, which could be considered a good approach to control the quality of NHJY pill and other related botanical drugs.

Baseline Practices for the Application of Genomic Data Supporting Regulatory Food Safety

Lambert, Dominic1; Pightling, Arthur2; Griffiths, Emma3; Van Domselaar, Gary4; Evans, Peter5; Berthelet, Sharon1; Craig, Duncan6; Chandry, P. Scott7; Stones, Robert8; Brinkman, Fiona3; Angers-Loustau, Alexandre9; Kreysa, Joachim9; Tong, Weida10; Blais, Burton11

ABSTRACT

The application of new data streams generated from next-generation sequencing (NGS) has been demonstrated for food microbiology, pathogen identification, and illness outbreak detection. The establishment of best practices for data integrity, reproducibility, and traceability will ensure reliable, auditable, and transparent processes underlying food microbiology risk management decisions. We outline general principles to guide the use of NGS data in support of microbiological food safety. Regulatory authorities across intra- and international jurisdictions can leverage this effort to promote the reliability, consistency, and transparency of processes used in the derivation of genomic information for regulatory food safety purposes, and to facilitate interactions and the transfer of information in the interest of public health.

Determination of Ethanol in Kombucha Products: Single-Laboratory Validation, First Action 2016.12

Ebersole, Blake1; Liu, Ying2; Schmidt, Rich3; Eckert, Matt3; Brown, Paula N.2

ABSTRACT

Kombucha is a fermented nonalcoholic beverage that has drawn government attention due to the possible presence of excess ethanol (≥0.5% alcohol by volume; ABV). A validated method that provides better precision and accuracy for measuring ethanol levels in kombucha is urgently needed by the kombucha industry. The current study validated a method for determining ethanol content in commercial kombucha products. The ethanol content in kombucha was measured using headspace GC with flame ionization detection. An ethanol standard curve ranging from 0.05 to 5.09% ABV was used, with correlation coefficients greater than 99.9%. The method detection limit was 0.003% ABV and the LOQ was 0.01% ABV. The RSDr ranged from 1.62 to 2.21% and the Horwitz ratio ranged from 0.4 to 0.6. The average accuracy of the method was 98.2%. This method was validated following the guidelines for single-laboratory validation by AOAC INTERNATIONAL and meets the requirements set by AOAC SMPR 2016.001, "Standard Method Performance Requirements for Determination of Ethanol in Kombucha."

Molecular Tracing of the Origin of Six Different Plant Species in Bee Honey Using Real-Time PCR

Authors: Wu, Yajun; Yang, Yange; Liu, Mingchang; Wang, Bin; Li, Meige; Chen, Ying

ABSTRACT

The quality of honey is significantly influenced by floral origin. Mislabeling floral species occurs frequently in bee honey products. To protect consumers from economic fraud and maintain a fair market environment, methods to identify floral species in honey are necessary. In our study, real-time PCRs were established, targeting six honey types mainly produced in China (canola, Chinese milkvetch, Chinese chaste tree, locust tree, litchi, and longan). Sensitivity testing on DNA from plant tissues exhibited LODs of about 0.5–5 pg/μL. For DNA extracts of pollen sediments from different honey species, LODs ranged from 13.6 to 403.2 pg/μL. In an experiment to determine the practical LODs of honey in which adulterant honey was spiked in the genuine honey, adulterant honey as low as about 0.1–0.5% was detected in 90–100% in 10 parallel tests. Additionally, pollen was spiked in the honey and stored under various conditions to investigate the migration of pollen DNA into the honey supernatant. Finally, the efficiency of our method was investigated by testing honey samples of unknown compositions from different geographic regions. Of the 159 honey samples that were supposed to be monofloral that had been collected in five provinces, a small portion were found to be contaminated with foreign pollen (7%). The methods proved to be specific, sensitive, and reliable in identifying the six plant species in honey, which would be a useful tool during the market supervision and QC of honey products.

Dual-Laboratory Validation of a Method for the Determination of Fructans in Infant Formula and Adult Nutritionals: First Action 2016.14

Brunt, Kommer1; Sanders, Peter2; Spichtig, Véronique3; Ernste-Nota, Veronica2; Sawicka, Paulina3; Iwanoff, Kimberley4; Van Soest, Jeroen2; Lin, Paul Kong Thoo5; Austin, Sean3

ABSTRACT

Until recently, only two AOAC Official Methods SM have been available for the analysis of fructans: Method 997.08 and Method 999.03. Both are based on the analysis of the fructan component monosaccharides (glucose and fructose) after hydrolysis. The two methods have some limitations due to the strategies used for removing background interferences (such as from sucrose, α-glucooligosaccharides, and free sugars). The method described in this paper has been developed to overcome those limitations. The method is largely based on Method 999.03 and uses combined enzymatic and SPE steps to remove the interfering components without impacting the final analytical result. The method has been validated in two laboratories on infant formula and adult nutritionals. Recoveries were in the range of 86–119%, with most being in the range of 91–104%. RSDr values were in the range of 0.7–2.6%, with one exception when the fructan concentration was close to the LOQ, resulting in an RSDr of 8.9%. The performance is generally within the requirements outlined in the AOAC Standard Method Performance Requirements (SMPR® 2014.002), which specifies recoveries in the range of 90–110% and RSDr values below 6%.

Determination of Lutein and β-Carotene in Infant Formula and Adult Nutritionals by Ultra-High-Performance Liquid Chromatography: Single-Laboratory Validation, First Action 2016.13

Hostetler, Gregory L.

ABSTRACT

An ultra-HPLC method for the determination of lutein and β-carotene in infant formula and adult nutritionals was validated using both unfortified and fortified samples provided by the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN). All experiments showed separation of all-trans-lutein and β-carotene from their major cis isomers, apocarotenal, α-carotene, lycopene, and zeaxanthin. Samples spiked with all-trans-lutein and β-carotene showed no isomerization during sample preparation. Linearity of the calibration solutions correlated to approximately 0.8–45 μg/100 g (reconstituted basis) for samples prepared for the lowest sample concentrations. With dilutions specified in the method, the range can be extended to approximately 2250 μg/100 g. The LOD for both lutein and β-carotene was 0.08 μg/100 g, and the LOQ for both was 0.27 μg/100 g. For all measurements in the range of 1–100 μg/100 g, repeatability RSD was ≤5.8% for lutein and ≤5.1% for β-carotene. For measurements >100 μg/100 g, repeatability RSD was ≤1.1% for lutein and ≤1.7% for β-carotene. Accuracy was determined by recovery from spiked samples and ranged from 92.3 to 105.5% for lutein and from 100.1 to 107.5% for β-carotene. The data provided show that the method meets the criteria specified in the Standard Method Performance Requirements for carotenoids (SMPR 2014.014).

Evaluation of Mercury in Environmental Samples by a Supramolecular Solvent–Based Dispersive Liquid–Liquid Microextraction Method before Analysis by a Cold Vapor Generation Technique

Ali, Jamshed1; Tuzen, Mustafa1; Kazi, Tasneem G.2

ABSTRACT

Supramolecular solvent–based dispersive liquid–liquid microextraction was used as a preconcentration method for the determination of trace levels of Hg. This simple method accurately measured oxidized HgII content in claystone and sandstone samples obtained from the Thar Coalfield in Pakistan. Cold vapor atomic absorption spectrometry was used as the detection technique because it is reliable and accurate. The HgII in acidic media forms a complex with dithizone (DTz) in the presence of supramolecular solvent (tetrahydrofuran and 1-undecanol), forming reverse micelles. Formation of the Hg-DTz complex was achieved to increase the interactions with the supramolecular solvent phase at pH 2.5 under the optimized experimental conditions. After addition of the supramolecular solvent to the aqueous solution, the micelles were uniformly mixed using a vortex mixer. The cloudy solution was centrifuged, and the Hg-DTz complex was extracted into the supramolecular solvent phase. Under optimized experimental conditions, the LOD and enrichment factor were found to be 5.61 ng/L and 77.8, respectively. Accuracy of the developed method was checked with Certified Reference Materials. The developed method was successfully applied for the determination of HgII in claystone and sandstone samples from the Block VII and Block VIII areas of the Thar Coalfield on the basis of depth.

Determination of Diquat Residues in Potato Using Reversed-Phase Liquid Chromatography with Tandem Mass Spectrometry

Attallah, Emad R.1; Soliman, Mostafa1; Abo-Aly, Mohamed M.2

ABSTRACT

A simple, modified quick, easy, cheap, effective, rugged, and safe procedure was developed for the determination of diquat in potato using reversed-phase LC coupled with tandem MS (MS/MS) in a total run time of 10 min. Different sample preparation parameters (pH modifier type, sample size, and elevated temperature) were tested and optimized. Potato sample was extracted with acetonitrile in the presence of ammonium hydroxide at 80°C. Phase separation was obtained by shaking the extract with magnesium sulfate and sodium chloride, and analysis was done using LC-MS/MS. Matrix-matched standard calculations were used to compensate for matrix-induced suppression in LC-MS/MS determination. The precision and trueness of the method were determined from recovery experiments on five replicates of spiked blank potato samples at 0.01, 0.05, and 0.1 mg/kg. The range of the obtained recoveries was 74–110%, with RSD values <5% for all the concentrations.

Development and Validation of an HPLC Method for Determination of Thiamethoxam and Its Metabolites in Cotton Leaves and Soil

Jyot, Gagan; Singh, Balwinder

ABSTRACT

An easy and simple analytical method was standardized and validated for the estimation of residues of thiamethoxam and its metabolites in cotton. The samples were extracted with acetonitrile, water, and methanol; diluted with brine solution; partitioned into dichloromethane and ethyl acetate; dried over anhydrous sodium sulfate; and cleaned up by glass column chromatography. Final clear extracts were concentrated under vacuum and reconstituted into HPLC grade acetonitrile, and residues were estimated using an HPLC instrument equipped with a C18 column and photodiode array detector system. Acetonitrile–1% formic acid in HPLC grade water (30 + 70) was used as mobile phase at 0.2 mL/min. Consistent recoveries ranging from 82 to 97% for thiamethoxam and its metabolites were observed when samples were spiked at 0.05–1.0 mg/kg levels. The LOQ of the method was determined to be 0.05 mg/kg. The analytical method was validated in terms of the selectivity, linearity, precision, and accuracy of the detection system.

Determination of Organophosphorous Pesticides in Environmental Water Samples Using Surface-Engineered C18 Functionalized Silica-Coated Core-Shell Magnetic Nanoparticles–Based Extraction Coupled with GC-MS/MS Analysis

Srivastava, Neha; Kumari, Supriya; Nair, Kishore; Alam, Samsul; Raza, Syed K.

ABSTRACT

The present paper depicts a novel method based on magnetic SPE (MSPE) for the determination of organophosphorus pesticides (OPs) such as phorate, malathion, and chlorpyrifos in environmental water samples. In this study, C18 functionalized silica-coated core-shell iron oxide magnetic nanoparticles (MNPs) were used as a surface-engineered magnetic sorbent for the selective extraction of pesticides from aqueous samples, followed by GC-MS and GC–tandem MS analysis for confirmative determination of the analytes. Various important method parameters, including quantity of MNP adsorbent, volume of sample, effective time for extraction, nature of the desorbing solvent, and pH of the aqueous sample, were investigated and optimized to obtain maximum method performance. Under the optimized instrumental analysis conditions, good linearity (r2 value ≥0.994) was achieved at the concentration range of 0.5–500 μg/L. Recoveries were in the range of 79.2–96.3 and 80.4–97.5% in selective-ion monitoring and multiple reaction monitoring (MRM) modes, respectively, at the spiking concentrations of 1, 5, and 10 μg/L. MRM mode showed better sensitivity, selectivity, and low-level detection (0.5 μg/L) of analytes. The novel MSPE method is a simple, cheap, rapid, and eco-friendly method for the determination of OPs in environmental water samples.

Comments on a Method to Measure Sucralose Using UV Photodegradation Followed by UV Spectrophotometry

Fang, Te; Andrews, Susan A.; Hofmann, Ron

ABSTRACT

A simple and quick method to measure sucralose in aqueous solution at concentrations in the order of 0.1–1.2 g·L−1 proposed by Idris et al. uses UV irradiation prior to UV spectrophotometry. The photolysis of sucralose forms a photoactive compound characterized by maximum absorbance at approximately 270 nm. The conditions required for sucralose photolysis, however, had not been completely reported. In this work, the procedure described by Idris et al. was replicated using a low-pressure UV lamp to irradiate sucralose samples with a wider range of initial concentrations (0.04–10 g·L−1) with known fluences. It was determined that care must be taken to ensure that the same fluence is applied for both calibration and measurement steps because the absorbance of the sucralose photolysis product is a function of the applied fluence. The way the samples are irradiated also has an impact on the results in that the method exhibits a greater linear range if an apparatus is used that maximizes the fluence rate (e.g., by placing samples closer to the UV source or using a higher-intensity lamp).

Evaluation of Method-Specific Extraction Variability for the Measurement of Fatty Acids in a Candidate Infant/Adult Nutritional Formula Reference Material

Place, Benjamin J.

ABSTRACT

To address community needs, the National Institute of Standards and Technology has developed a candidate Standard Reference Material (SRM) for infant/adult nutritional formula based on milk and whey protein concentrates with isolated soy protein called SRM 1869 Infant/Adult Nutritional Formula. One major component of this candidate SRM is the fatty acid content. In this study, multiple extraction techniques were evaluated to quantify the fatty acids in this new material. Extraction methods that were based on lipid extraction followed by transesterification resulted in lower mass fraction values for all fatty acids than the values measured by methods utilizing in situ transesterification followed by fatty acid methyl ester extraction (ISTE). An ISTE method, based on the identified optimal parameters, was used to determine the fatty acid content of the new infant/adult nutritional formula reference material.

Determination of Tryptamines and β-Carbolines in Ayahuasca Beverage Consumed during Brazilian Religious Ceremonies

Santos, Mônica Cardoso1; Navickiene, Sandro1; Gaujac, Alain2

ABSTRACT

Ayahuasca is a potent hallucinogenic beverage prepared from Banisteriopsis caapi in combination with other psychoactive plants. N,N-dimethyltryptamine, tryptamine, harmine, harmaline, harmalol, and tetrahydroharmine were quantified in ayahuasca samples using a simple and low-cost method based on SPE and LC with UV diode-array detection. The experimental variables that affect the SPE method, such as type of solid phase and nature of solvent, were optimized. The method showed good linearity ($r > 0.9902$) and repeatability (RSD < 0.8%) for alkaloid compounds, with an LOD of 0.12 mg/L. The proposed method was used to analyze 20 samples from an ayahuasca cooking process from a religious group located in the municipality of Fortaleza, state of Ceará, Brazil. The results showed that concentrations of the target compounds ranged from 0.3 to 36.7 g/L for these samples.

Analytical Methods in Tracing Honey Authenticity

Trifković, Jelena1; Andrić, Filip1; Ristivojević, Petar2; Guzelmeric, Etil3; Yesilada, Erdem3

ABSTRACT

Honey is a precious natural product that is marketed with a wide range of nutritional and medicinal properties. However, it is also a product subjected to frequent adulteration through mislabeling and mixing with cheaper and lower-quality honeys and various sugar syrups. In that sense, honey authentication regarding its genuine botanical and geographical origins, as well as the detection of any adulteration, is essential in order to protect consumer health and to avoid competition that could create a destabilized market. Various analytical techniques have been developed to detect adulterations in honey, including measuring the ratios of stable isotopes (mostly 13C/12C) and the use of different spectroscopic, chromatographic, and electrochemical methods. This review aims to provide a cross-section of contemporary analytical methods used for the determination of honey authenticity in order to help the scientific community engaged in the field of honey chemistry make appropriate choices and select the best applications that should lead to improvements in the detection and elimination of fraudulent practices in honey manufacturing.

Physicochemical Parameters as a Tool for the Assessment of Origin of Honey

Lazarević, Kristina B.1; Jovetić, Milica S.1; Tešić, Živoslav Lj.2

ABSTRACT

Honey is a complex mixture of various substances, and its composition depends on both botanical and geographical origin, as well as anthropogenic factors. The accurate identification of honey origin guarantees the satisfaction of consumers' needs and has an impact on the honey market value. Physicochemical parameters, some of which are used in routine analysis of honey quality, could be useful for the assessment of its origin. In this review, special attention is paid to those studies that assessed the sugar and mineral composition of honey, whether they were investigated in terms of botanical or geographical origin, or for the characterization of honey type. The oligosaccharides present in honey and the electrical conductivity of honey correlate strongly with its botanical origin. Mineral content could be indicative for distinguishing honeys according to their botanical and geographical origins because it depends on both the soil composition and the floral type of melliferous plants. This review provides insight into the results obtained by various studies from approximately the last 10 years concerning the sugar profile and the mineral and trace element content of different types of honey. An attempt was made to statistically analyze the results regarding mineral and trace element content in order to identify indicators that could distinguish honey by origin.

Polyphenols as Possible Markers of Botanical Origin of Honey

Gašić, Uroš M.; Milojković-Opsenica, Dušanka M.; Tešić, Živoslav Lj.

ABSTRACT

In recent years, the botanical and geographical origin of food has become an important topic in the context of food quality and safety, as well as consumer protection, in accordance with international standards. Finding chemical markers, especially phytochemicals, characteristic for some kind of food is the subject of interest of a significant number of researchers in the world. This paper is focused on the use of polyphenols as potential markers for the determination of botanical origin of honey. It includes a review of the polyphenols present in various honey samples and the methods for their separation and identification. Special emphasis in this paper is placed on the identification of honey polyphenols using advanced LC-MS techniques in order to find specific markers of botanical origin of honey. In this regard, this study gives an overview of the literature that describes the use of LC-MS techniques for the isolation and determination of honey polyphenols. This review focuses on the research performed in the past two decades.

Mineral Content as a Tool for the Assessment of Honey Authenticity

Jovetić, Milica1; Trifković, Jelena2; Stanković, Dalibor3; Manojlović, Dragan2; Milojković-Opsenica, Dušanka2

ABSTRACT

The present work aims to provide a contribution to the overall investigation of European unifloral honeys with regard to authentication according to botanical and geographical origins. The mineral content of 206 monofloral honey samples of five botanical origins from six different regions in Serbia was investigated by inductively coupled plasma optical emission spectrometry. Chemometric techniques were applied for the classification and differentiation of acacia, sunflower, and linden honey according to botanical origin, as well as acacia honey samples according to regional origin. The highest influence on the differentiation of acacia honey samples was the presence of siderophile and chalcophile elements, whereas sunflower and linden honeys were determined by the presence of lithophile elements, indicating their origin from soil. However, due to the different bioaccumulation properties of plants, the presence of elements is not necessarily directly correlated to their presence in soil, which is confirmed by the results of the authentication of geographical origin of acacia honey.

Elemental Composition of Different Slovenian Honeys Using k 0- Instrumental Neutron Activation Analysis

Kropf, Urška1; Stibilj, Vekoslava2; Jaćimović, Radojko2; Bertoncelj, Jasna1; Golob, Terezija1; Korošec, Mojca1

ABSTRACT

The botanical origin of seven types of Slovenian honey was investigated by analysis of their elemental content using k 0-instrumental neutron activation analysis. A total of 28 representative samples were collected from beekeepers all over Slovenia in 2 consecutive years. Nineteen of the 37 elements measured were present in amounts above their LOD. The present study suggests that the determination of only alkali elements might be sufficient for the classification of Slovenian honeys according to their botanical origin. Linden and multifloral honeys can be differentiated on the basis of Na content. The differentiation of forest, spruce, and fir honeys is possible on the basis of differences in Cs, K, and Rb content. The difference between Na and Rb content can be used as a discriminating tool between light and dark honeys, because light honeys (black locust, linden, and multifloral) contained more Na than Rb, whereas it was the opposite for dark honeys (chestnut, forest, spruce, and fir). Statistically significant correlations were found between K and Rb and between K and Cs content. This study represents a considerable step in filling the knowledge gap concerning both the determination of elements present in low concentrations and the botanical origin of Slovenian honey.

Characterization of Croatian Rape (Brassica sp.) Honey by Pollen Spectrum, Physicochemical Characteristics, and Multielement analysis by ICP-OES

Rajs, Blanka Bilić1; Flanjak, Ivana1; Mutić, Jelena2; Vukojević, Vesna2; Đurđić, Slađana2; Primorac, Ljiljana1

ABSTRACT

Rape (Brassica sp.) unifloral honey from Croatia was characterized by certain physicochemical parameters, micro- and macroelement content, and pollen spectrum, as determined in 21 honey samples. The Brassica sp. pollen type was predominant in the analyzed samples and ranged between 60 and 98%, with Trifolium spp., Robinia pseudoacacia, Rosaceae, Helianthus annuus, Salix spp., and Taraxacum officinale as the main accompanying pollen types. The electrical conductivity mean value was 0.22 ± 0.05 mS/cm and the glucose/fructose ratio mean value was 1.1 ± 0.07, whereas sucrose was absent in the samples. The most abundant macroelement was potassium (K) (268.49 mg/kg), followed by phosphorus (P) (60.23 mg/kg), calcium (Ca) (54.02 mg/kg), sodium (Na) (22.52 mg/kg), sulfur (S) (15.79 mg/kg), and magnesium (Mg) (12.58 mg/kg). Toxic elements were mainly bellow the LODs; only arsenic (As) concentration was detectable in higher amount (0.233 mg/kg), which may be related to the high arsenic concentration in the soil and groundwater of eastern Croatia. The differences between the two harvesting seasons observed in a large number of elements could be related to climatic and soil conditions and different nectar yields originating from the associated plant species.

Development and Validation of a GC-MS Method for the Analysis of Homogentisic Acid in Strawberry Tree (Arbutus unedo L.) Honey

Brčić Karačonji, Irena1; Jurica, Karlo2

ABSTRACT

To confirm the botanical origin of strawberry tree (Arbutus unedo L.) honey, a liquid–liquid extraction followed by GC-MS method was developed for the quantitative determination of homogentisic acid (HGA), the main phenolic compound in this honey. Different parameters affecting extraction, such as the type and volume of extraction solvents, pH of the solution, and amount of salt, were optimized. The method showed good linearity (r2 = 0.9990) over the tested concentration range (50–500 mg/kg) and a low LOD (0.3 mg/kg). Precision expressed as RSD was <7%. The average accuracy was 95%. The optimized method was applied for determining the HGA content in strawberry tree honey samples from Croatia. The HGA content in analyzed samples (n = 7) ranged from 245.1 to 485.9 mg/kg. The proposed method provided reliable performance and can be easily implemented for the routine monitoring of HGA in strawberry tree honey in order to assure honey QC.

Aptamer–Magnetic Bead Quantum Dot Sandwich Assays for Foodborne Pathogen Detection: Pros, Cons, and Lessons Learned

Bruno, John G.; Sivils, Jeffrey C.; Phillips, Taylor

ABSTRACT

DNA and RNA aptamers have been extensively investigated as potential competitors for antibodies for a variety of applications including food safety testing. Ultrasensitive fluorescence detection of foodborne pathogenic bacteria as low as 1–10 cells/mL has been achieved using aptamers coupled to quantum dots in clear pristine buffers for environmental sample detection. Quantum dots offer other advantages, including single UV or blue light source multiplex (multicolored) detection. However, quantum dots can exhibit decreased fluorescence in some food matrixes and even completely fail to fluoresce in some fatty matrixes, as documented in this report. Given the need to detect substances in complex food matrixes (and from data reported elsewhere), aptamer–magnetic bead pull-down methods followed by enzymatic/fluorometric- or PCR-based detection methods may be more robust methods for testing in foods or enrichment cultures. Other lessons learned, including the initial choice of aptamer targets to enhance assay specificity, are also discussed.

Graphene Nanolayers as a New Method for Bacterial Biofilm Prevention: Preliminary Results

Dybowska-Sarapuk, Łucja1; Kotela, Andrzej2; Krzemiński, Jakub1; Wróblewska, Marta3; Marchel, Halina4; Romaniec, Magdalena5; Łęgosz, Paweł2; Jakubowska, Małgorzata1

ABSTRACT

Biofilms are microbial communities of surface-attached cells embedded in a self-produced extracellular matrix. They have been found to play a role in a wide variety of infections, including catheter-related urinary tract and bloodstream infections, and, therefore remain a significant source of morbidity and mortality among the world's population. Recently, much attention has been devoted to the prevention of biofilm formation on implant surfaces. Nanomaterials such as graphene, characterized by antibacterial activity and low toxicity to human cells, are promising candidates for biomedical applications. This study investigates the antibacterial efficiency of graphene and specially produced graphene decorated with silver nanoparticles, obtained by one of the methods of printed electronics (spray-coating system). These methods are not only economical, but also enable the printing of layers of various thicknesses on different types of materials, including flexible and nonplanar substrates. The aim of the study was to reveal the ability of graphene and graphene-nanosilver layers to prevent the formation of Staphylococcus epidermidis biofilm on the surface of a Foley catheter.

The Method of Coating Fe3O4 with Carbon Nanoparticles to Modify Biological Properties of Oxide Measured in Vitro

Niemiec, Tomasz1; Dudek, Mariusz2; Dziekan, Natalia3; Jaworski, Sławomir1; Przewozik, Aleksandra2; Soszka, Emilia2; Koperkiewicz, Anna2; Koczoń, Piotr4

ABSTRACT

The coating of nanoparticles on materials for medical application [e.g., the coating of Fe3O4 nanopowder (IONP) with a carbon nanolayer] serves to protect and modify the selected biological, physical, and chemical properties of the coated material. Increases in chemical stability, changes in biocompatibility, and a modified surface structure are examples of the effects caused by the formation of carbon coatings. In the current study, Fe3O4 nanoparticles were coated with a carbon nanolayer (IONP@C) in a plasmochemical reactor (using radio-frequency plasma-enhanced chemical vapor deposition methods) under various experimental conditions. Based on data from X-ray diffraction, Raman, and IR spectroscopy, the best processing parameters were determined in order to produce a carbon coating that would not change the structure of the IONP. The materials with the best cover, i.e., a uniform carbon nanolayer, were used in cytotoxic tests to investigate their biological properties using the human HepG2 hepatocarcinoma cell line and chicken embryo red blood cells as an in vitro model. The obtained results proved the low cytotoxicity of Fe3O4 micropowder and IONP in contrast to IONP@C, which reduced cell viability, increased hemolysis, and generally was more toxic than bare Fe3O4.

Micro Thin-Layer Chromatography Fingerprints of Selected Basil Species and Their Chemometric Analysis

Hawrył, Mirosław A.; Świeboda, Ryszard; Hawrył, Anna; Niemiec, Małgorzata; Waksmundzka-Hajnos, Monika

ABSTRACT

The dried aerial parts of 12 plants of Ocimum spp. were extracted with the Soxhlet apparatus using dichloromethane and methanol as solvents. A micro-TLC system with silica and a normal-phase solvent system (propan-2-ol–n-heptane–formic acid) was used for the chemometric analysis of 12 selected basil methanolic extracts. Some indices of similarity (Pearson's correlation coefficient, determination coefficient, congruence coefficient, and Euclidean, Manhattan (city-block), Chebyshev, and Hausdorff distances) were calculated on the basis of thin-layer chromatograms using ImageJ software. Principal component analysis was also performed. The method allowed the comparison of the analyzed extracts and confirmed the identities of two selected unknown plants.

The Thin-Layer Microchromatography (μTLC) and TLC–FID Technique as a New Methodology in the Study of Lubricating Oils

Nowak, Paulina; Kosińska, Judyta; Glinka, Marta; Kamiński, Marian

ABSTRACT

This paper concerns the possibility of using TLC coupled with a flame ionization detector (FID) and micro-TLC (μTLC) as precursors for microfluidized devices of analytical techniques to identify and determine the presence and content of the petroleum/vegetable oil base in the lubricating oils applied in cutting devices (chainsaws). This research is related to the problem of ensuring, in compliance with the requirements of environmental protection, a sufficient level of biodegradability of lubricating oils emitted to the environment during operation of equipment lubricated with these oils. Such oils include those mainly used in cutting devices and emitted in the form of a mist into the environment during the operation of those devices. When oil components are eco-toxic, contamination of the environment occurs. New methodologies for the identification and determination of the petroleum oil base, which is very difficult to biodegrade, as well as the easily biodegradable ingredients of vegetable origin in the lubricating oils, are presented. The described procedures indicate in an indisputable way whether the oil contains the oil base originating from crude oil and whether it contains adequate enriching additives. The procedures also allow the assessment of the content of particular groups of constituents (μTLC) or the determination of the group composition (TLC–FID).

Advances in the Analysis of Water and Wastewater Samples Using Various Sensing Protocols and Microfluidic Devices Based on PAD and μTAS Systems

Piaskowski, Krzysztof; Świderska-Dąbrowska, Renata; Kaleniecka, Aleksandra; Zarzycki, Paweł K.

ABSTRACT

The main goal of this review is to summarize practical approaches concerning the application of microfluidic systems for the analysis of various biomarkers and pollutants, as well as microbes, in water and wastewater matrixes. This problem involves multidisciplinary expertise combining research knowledge from various areas, including wet chemistry, biochemistry, physical chemistry, molecular biology, genetics, signal processing, microelectronics material science, and separation science. It has been documented that fairly primitive but fast and inexpensive screening methods involving paper-based analytical devices (PADs) and micro total analytical systems (μTAS) can be considered as serious alternatives to their more advanced counterparts such as GC, HPLC, and capillary electrophoresis coupled to various sophisticated detectors (e.g., multiwavelength spectrophotometers such as UV-Vis/DAD and mass spectrometers). The main advantage of PAD- and μTAS-driven technology is that such sensing devices may work under on-site and real-time conditions and measure a number of physical parameters and chemical factors simultaneously. Moreover, hybrid miniaturized analytical systems may combine sensing and data acquisition modules with common mobile phones and electronic devices working with global positioning systems and communicating through the Internet.

Development and Validation of a Rapid LC-MS/MS Method for Simultaneous Determination of Kaempferol and Quercetin in Thespesia populnea Extract

Panchal, Hiteksha1; Shah, Mamta B.2

ABSTRACT

In this study, a simple and rapid LC with tandem MS method was developed and validated for the simultaneous determination of kaempferol and quercetin in Thespesia populnea extract. The compounds were eluted using a Gemini C18 column (50 × 2.0 mm, 3 μm), with the mobile phase consisting of acetonitrile–0.3% formic acid in water at the flow rate of 0.400 mL/min. The assay exhibited a linear dynamic range of 25–2500 ng/mL for both kaempferol and quercetin. The values for intra- and interday precision and accuracy were well within the generally accepted criteria for analytical methods (<15%). Selectivity, linearity, LOD, LOQ, accuracy, and precision were evaluated for both analytes. The proposed method is accurate and sensitive and can be used for the routine quantification of kaempferol and quercetin in the herbal extract and in polyherbal formulations.

Spectrophotometric Methods for Simultaneous Determination of Sofosbuvir and Ledipasvir (HARVONI Tablet): Comparative Study with Two Generic Products

Abo-Talib, Nisreen F.1; El-Ghobashy, Mohamed R.2; Tammam, Marwa H.1

ABSTRACT

Sofosbuvir and ledipasvir are the first drugs in a combination pill to treat chronic hepatitis C virus. Simple, sensitive, and rapid spectrophotometric methods are presented for the determination of sofosbuvir and ledipasvir in their combined dosage form. These methods were based on direct measurement of ledipasvir at 333 nm (due to the lack of interference of sofosbuvir) over a concentration range of 4.0–14.0 µg/mL, with a mean recovery of 100.78 ± 0.64%. Sofosbuvir was determined, without prior separation, by third-derivative values at 281 nm; derivative ratio values at 265.8 nm utilizing 5.0 µg/mL ledipasvir as a divisor; the ratio difference method using values at 270 and 250 nm using 5.0 µg/mL ledipasvir as a divisor; and the ratio subtraction method using values at 261 nm. These methods were found to be linear for sofosbuvir over a concentration range of 5.0–35.0 µg/mL. The suggested methods were validated according to International Conference on Harmonization guidelines. Statistical analysis of the results showed no significant difference between the proposed methods and the manufacturer's LC method of determination with respect to accuracy and precision. These methods were used to compare the equivalence of an innovator drug dosage form and two generic drug dosage forms of the same strength.

Green Pharmaceutical Analysis of Drugs Coformulated with Highly Different Concentrations Using Spiking and Manipulation of Their Ratio Spectra

Ayoub, Bassam M.

ABSTRACT

Introducing green analysis to pharmaceutical products is considered a significant approach to preserving the environment. This method can be an environmentally friendly alternative to the existing methods, accompanied by a validated automated procedure for the analysis of a drug with the lowest possible number of samples. Different simple spectrophotometric methods were developed for the simultaneous determination of empagliflozin (EG) and metformin (MT) by manipulating their ratio spectra in their application on a recently approved pharmaceutical combination, Synjardy tablets. A spiking technique was used to increase the concentration of EG in samples prepared from the tablets to allow for the simultaneous determination of EG with MT without prior separation. Validation parameters according to International Conference on Harmonization guidelines were acceptable over a concentration range of 2–12 µg/mL for both drugs using derivative ratio and ratio subtraction coupled with extended ratio subtraction. The optimized methods were compared using one-way analysis of variance and proved to be suitable as ecofriendly approaches for industrial QC laboratories.

A Validated HPLC Method for Simultaneous Determination of Perindopril Arginine, Amlodipine, and Indapamide: Application in Bulk and in Different Pharmaceutical Dosage Forms

El-Bagary, Ramzia I.1; Elkady, Ehab F.1; Mowaka, Shereen2; Attallah, Maria A.2

ABSTRACT

A simple, accurate, and precise LC method with a reversed stationary phase was developed and validated for the determination of perindopril (PER) arginine, amlodipine (AML), and indapamide (IND) alone and in binary mixtures (PER arginine is found in two dosage forms, i.e., with either AML or IND). Chromatographic separation was carried out on a BDS Hypersil® C18 column (100 × 3 mm, 5 μm). The mobile phase, consisting of 0.05 M potassium dihydrogen phosphate buffer (pH 2.6)–methanol (50 + 50, v/v), was pumped through the column whose temperature was maintained at 50°C at a flow rate of 0.6 mL/min using isocratic elution, and UV detection at 215 nm was performed. Acceptable values of linearity, accuracy, and precision of the method were found over the concentration ranges of 5–80 μg/mL PER, 2.5–80 μg/mL AML, and 0.5–20 μg/mL IND. The proposed chromatographic method was statistically compared to that of reference methods using one-way analysis of variance. The results showed that there was no significant difference between the methods. The developed method proved reliable for use in accurate QC of the drugs in their pharmaceutical preparations.

Comparative Validation of the Determination of Sofosbuvir in Pharmaceuticals by Several Inexpensive Ecofriendly Chromatographic, Electrophoretic, and Spectrophotometric Methods

El-Yazbi, Amira F.

ABSTRACT

Sofosbuvir (SOFO) was approved by the U.S. Food and Drug Administration in 2013 for the treatment of hepatitis C virus infection with enhanced antiviral potency compared with earlier analogs. Notwithstanding, all current editions of the pharmacopeias still do not present any analytical methods for the quantification of SOFO. Thus, rapid, simple, and ecofriendly methods for the routine analysis of commercial formulations of SOFO are desirable. In this study, five accurate methods for the determination of SOFO in pharmaceutical tablets were developed and validated. These methods include HPLC, capillary zone electrophoresis, HPTLC, and UV spectrophotometric and derivative spectrometry methods. The proposed methods proved to be rapid, simple, sensitive, selective, and accurate analytical procedures that were suitable for the reliable determination of SOFO in pharmaceutical tablets. An analysis of variance test with P-value > 0.05 confirmed that there were no significant differences between the proposed assays. Thus, any of these methods can be used for the routine analysis of SOFO in commercial tablets.

Simultaneous Determination of First-Line 4-FDC Antituberculosis Drugs by UHPLC–UV and HPLC–UV: A Comparative Study

Franco, Pedro H. C.1; Chellini, Paula R.2; Oliveira, Marcone A. L.3; Pianetti, Gerson A.1

ABSTRACT

Tuberculosis is the second most deadly infectious disease, surpassed only by HIV/AIDS, and has resulted in over 1 billion deaths in the last 200 years. The World Health Organization estimates that in 2014, 9.6 million people were infected by this disease and 1.5 million had died. First-choice treatment consists of fixed-dose combination tablets containing rifampicin, isoniazid, pyrazinamide, and ethambutol hydrochloride (4-FDC). There are pharmacopeial protocols available to test 4-FDC, but they are prolonged, two-step methods. One single-step method in the literature performs the simultaneous determination by HPLC, but requires a long acquisition time. In this context, an ultra-HPLC (UHPLC) method was developed based on the HPLC method with the objective of reducing analysis time. A C18 column (1.9 μm particle size) was used with UV–diode-array detection at 238 and 282 nm. The method was found to be selective, linear, exact, precise, and robust. Samples from two batches were analyzed and the results compared with those obtained by the HPLC method, with no statistically significant differences observed ($P > 0.05$). This UHPLC method reduced the analysis time from 17 to 4 min, with a more than 90% reduction in sample and reagent consumption and a financial economy of almost 50-fold.

Determination of Four Nadolol Stereoisomers in Capsules by High-Performance Liquid Chromatography and Circular Dichroism

Prado Alexandre, Grazielle; Wandermuren, Márcio Nardelli; Peron, Adriano; Tavares, Vanessa Franco; Ramalho Padeiro, Denis Alexandre; Sakiara, Kelly Alessandra; Aurora-Prado, María Segunda; Singh, Anil Kumar; Kedor-Hackmann, Erika Rosa; Miritello Santoro, Maria Inês Rocha

ABSTRACT

Nadolol is a blocking agent with activity in β-adrenergic receptors. The objective of this research was to develop and validate an HPLC and circular dichroism method for the quantification of four nadolol stereoisomers in capsules. The HPLC method was validated using a Chiralcel OD column (250 × 4,6 mm, 10 μm). The mobile phase consisted of hexane–ethanol–diethylamine–acetic acid (86 + 14 + 0.3 + 0.3, v/v/v/v), with a flow rate of 0.7 mL/min and temperature of 25 ± 1°C and UV detection carried out at 220 nm. Solutions were prepared in ethanol containing 200 μg/mL nadolol. The method proved to be precise, selective, accurate, and robust and was successfully applied for the determination of the two homologs of four nadolol stereoisomers in capsules.

Selective Determination of Human Growth Hormone (Somatropin) in the Presence of Its Chemical Degradation Products

Rezk, Mamdouh R.1; Mohamed, Marwa F.2; Fathalla, Faten Abdelaziz2; Shehata, Mostafa A.1

ABSTRACT

A rapid and sensitive HPLC method was developed and validated for selective determination of the human growth hormone (hGH) somatropin in the presence of its deamidated and oxidized degradation products. Reversed-phase chromatography with an acetonitrile and ammonium bicarbonate mobile phase in gradient elution mode was used. A short run time of 15 min allowed rapid and cost-effective analysis, with an average retention time of 7.4 min for native hGH, 6.2 min for its deamidated form, and 4.3 and 6 min for its oxidized variants. The method was validated for selectivity, linearity, and intra- and interday variations according to International Conference on Harmonization guidelines. The proposed method was successfully applied for rapid evaluation of the quantity of hGH during downstream processing, formulation, and storage.

Identification, Characterization, and Quantification of Impurities of Safinamide Mesilate: Process-Related Impurities and Degradation Products

Zou, Liang1; Sun, Lili2; Zhang, Hui1; Hui, Wenkai1; Zou, Qiaogen1; Zhu, Zheying3

ABSTRACT

The characterization of process-related impurities and degradation products of safinamide mesilate (SAFM) in bulk drug and a stability-indicating HPLC method for the separation and quantification of all the impurities were investigated. Four process-related impurities (Imp-B, Imp-C, Imp-D, and Imp-E) were found in the SAFM bulk drug. Five degradation products (Imp-A, Imp-C, Imp-D, Imp-E, and Imp-F) were observed in SAFM under oxidative conditions. Imp-C, Imp-D, and Imp-E were also degradation products and process-related impurities. Remarkably, one new compound, identified as (S)-2-[4-(3-fluoro-benzyloxy) benzamido] propanamide (i.e., Imp-D), is being reported here as an impurity for the first time. Furthermore, the structures of the aforementioned impurities were characterized and confirmed via IR, NMR, and MS techniques, and the most probable formation mechanisms of all impurities proposed according to the synthesis route. Optimum separation was achieved on an Inertsil ODS-3 column (250 × 4.6 mm, 5 μm), using 0.1% formic acid in water (pH adjusted to 5.0) and acetonitrile as the mobile phase in gradient mode. The proposed method was found to be stability-indicating, precise, linear, accurate, sensitive, and robust for the quantitation of SAFM and its process-related substances, including its degradation products.

Crystal Diagnostics Xpress S Kit for the Rapid Detection of Salmonella spp. in Selected Food Matrixes

Bullard, Brian; Stumpf, Curtis H.; Zhao, Weidong; Kuzenko, Stephanie; Niehaus, Gary D.

ABSTRACT

The Crystal Diagnostics (CDx) Xpress S Kit is a rapid-screening assay for Salmonella spp. in whole raw tomatoes, whole chicken carcasses, raw ground beef, raw beef trim, and whole liquid pasteurized eggs with citric acid when present at levels of 1 CFU/portion size. The Xpress S system comprises an automatic CDx Xpress Reader and a single-use CDx BioCassette that incorporates antibody-coupled microspheres and liquid crystal for the selective identification of the intended microbe. In internal evaluations, the CDx Xpress S Kit detected all 142 Salmonella strains tested, including non-enterica subspecies, and excluded all non-Salmonella species assayed. Method-developer studies, as well as a third-party evaluation, demonstrated that 15 h single-stage enrichment permits rapid detection equivalent to the U.S. Department of Agriculture and U.S. Food and Drug Administration reference methods. The results demonstrate that the CDx Xpress S Kit is one of the fastest, most sensitive, and most accurate methods for detecting Salmonella in food matrixes.

Development of a Method for Determination of Buckwheat Allergens Using Liquid Chromatography with Tandem Mass Spectrometry

Nagai, Hiroyuki

ABSTRACT

An analysis technique using LC with tandem MS (MS/MS) has been developed for the determination of buckwheat proteins, including major allergens. A protein solution extracted from buckwheat was reduced, alkylated, and digested by trypsin. Peptide spectra were obtained using full-scan LC-MS/MS analysis, and peptide sequences were determined through a protein search. Nine peptides of the 13S globulin seed storage protein and one peptide of a 16 kDa allergen were selected as the marker peptides, and multiple reaction monitoring conditions were optimized. Using the conditions, different kinds of buckwheat noodles, powders, and other food ingredients were analyzed. As a result, buckwheat samples present all the fragment peaks, whereas other foods, including Sesamum indicum, wheat, and soybeans, are not detected at all. These findings indicate that LC-MS/MS analysis may be applied to the detection of buckwheat food allergens.

Solid Phase Extraction Preconcentration Method for Simultaneous Determination of Cadmium, Lead, and Nickel in Poultry Supplements

Baig, Jameel Ahmed1; Memon, Hina Daud1; Bukhari, Syed Ali Imran2; Kazi, Tasneem Gul1; Afridi, Hassan Imran1; Naseer, Hafiz Muhammad3; Elci, Latif4

ABSTRACT

A new simple, selective, and economical preconcentration method was developed for the determination of Cd, Pb, and Ni in poultry antibiotics and supplements. The proposed preconcentration procedure is based on SPE using 8-hydroxyquinoline and Amberlite IRC-50 resin as complex and adsorbent, respectively. The determination was carried out by microsample injection system (MIS) flame atomic absorption spectroscopy (FAAS). Several analytical parameters were examined, including pH, type of resin, amount of resin, type of eluent, eluent volume, flow rate, sample volume, and interference of diverse ions. Under optimum experimental conditions, LODs and LOQs were 0.017 and 0.055, 0.016 and 0.53, and 0.074 and 0.248 μg/L for Cd, Pb, and Ni, respectively, with RSDs < 2.50%. The accuracy of SPE-MIS-FAAS was successfully tested by the standard addition method, with obtained recoveries >99%. The proposed method was successfully applied for the determination of Cd, Pb, and Ni in poultry supplement and antibiotic samples.

Determination of Polycyclic Aromatic Hydrocarbons in Commercial Parenteral Formulations and Medications Using High-Performance Liquid Chromatography with Diode Array Detection

Barichello, Marcia M.1; Bohrer, Denise1; Viana, Carine1; Carvalho, Leandro M.2; Nascimento, Paulo C.2

ABSTRACT

HPLC coupled to UV diode array detection (DAD) is proposed for the determination of polycyclic aromatic hydrocarbons (PAHs) in pharmaceutical products for parenteral administration. Because rubber is a possible source of PAHs for these products, samples stored in containers with rubber parts were selected for the analysis. The basis for method optimization was EPA Method 8310, which determines 16 priority PAHs in ground water and wastewater by HPLC using both UV and fluorescence detection. Using DAD, two channels were selected for detection, with one operating at 254 nm for the detection of nine PAHs and the other at 225 nm for the detection of seven PAHs. This method allowed for the detection of PAHs using external calibration with LODs and LOQs ranging from 0.001 to 0.060 μg/mL and from 0.003 to 0.167 μg/mL, respectively. Within-day precision, expressed as RSD, varied from 1.24 to 7.76% for PAH concentrations from 0.05 to 0.50 μg/mL, and intraday precision varied from 3.10 to 9.40% for the same concentration range. Method accuracy was confirmed by recoveries of 75–120% of the spiked samples. This method was applied for the determination of PAHs in three commercial infusion solutions and in nine different medications stored in syringes prior to administration to patients. Twelve of 16 PAHs were found in these samples. Total PAH concentrations varied from 0.13 to 13.50 μg/mL. Pyrene was the most prevalent contaminant, being present in 11 of 12 samples in concentrations ranging from 0.17 to 4.80 μg/mL. This method presented good sensitivity for the measurement of PAH in the target samples, allowing for the determination of the 16 priority PAHs in one run and in 30 min.

Multilaboratory Validation of First Action Method 2016.04 for Determination of Four Arsenic Species in Fruit Juice by High-Performance Liquid Chromatography–Inductively Coupled Plasma–Mass Spectrometry

Kubachka, Kevin1; Heitkemper, Douglas T.1; Conklin, Sean2

ABSTRACT

Before being designated AOAC First Action Official Method SM 2016.04, the U.S. Food and Drug Administration's method, EAM 4.10 High Performance Liquid Chromatography–Inductively Coupled Plasma–Mass Spectrometric Determination of Four Arsenic Species in Fruit Juice, underwent both a single-laboratory validation and a multilaboratory validation (MLV) study. Three federal and five state regulatory laboratories participated in the MLV study, which is the primary focus of this manuscript. The method was validated for inorganic arsenic (iAs) measured as the sum of the two iAs species arsenite [As(III)] and arsenate [As(V)], dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) by analyses of 13 juice samples, including three apple juice, three apple juice concentrate, four grape juice, and three pear juice samples. In addition, two water Standard Reference Materials (SRMs) were analyzed. The method LODs and LOQs obtained among the eight laboratories were approximately 0.3 and 2 ng/g, respectively, for each of the analytes and were adequate for the intended purpose of the method. Each laboratory analyzed method blanks, fortified method blanks, reference materials, triplicate portions of each juice sample, and duplicate fortified juice samples (one for each matrix type) at three fortification levels. In general, repeatability and reproducibility of the method was ≤15% RSD for each species present at a concentration >LOQ. The average recovery of fortified analytes for all laboratories ranged from 98 to 104% iAs, DMA, and MMA for all four juice sample matrixes. The average iAs results for SRMs 1640a and 1643e agreed within the range of 96–98% of certified values for total arsenic.

Validation of Multiresidue Method for Analysis of 31 Pesticides in Rice Using Gas Chromatography-Tandem Mass Spectrometry

Mondal, Rahul; Kole, Ramen Kumar; Bhttacharyya, Anjan

ABSTRACT

Five modified QuEChERS were tested for the multiresidue analysis of 31 pesticides in rice. Rice was spiked with mixture solution of pesticides at 10 ng/g. Method selection was based on the LODs (sensitivity) and recovery tests (accuracy) of the pesticides. Analysis was done in GC-tandem MS in multiple reaction monitoring mode with a total run time of approximately 37 min. The selected method was validated after spiking rice at 20 and 100 ng/g in rice. The performance characteristics of the method impacted for all selected pesticides were acceptable according to the guidelines for method validation (recovery of 70–120% with an RSD of <20% and r2 value of ≥0.99). For rice, matrix effect on the signals of the compounds was corrected by using matrix-matched calibration standards. The LOQs met the requirements of the maximum residue limits for pesticides in rice. The developed method allowed for the simultaneous determination and confirmation of a large number of different groups of pesticides and was fast, simple, inexpensive, and useful for the routine analysis of rice.

Simultaneous Analysis of Oil-Soluble, Basic, and Acidic Illegal Dyes in Foods Using Liquid Chromatography–Diode-Array Detection

Uematsu, Yoko; Mizumachi, Toshiko; Monma, Kimio

ABSTRACT

A method for simutaneously detecting 8 oil-soluble and 10 water-soluble (3 basic and 7 acidic) illegal dyes in foods was developed. The sample was mixed with water, followed by methanol and tetrahydrofuran. Transesterification with sodium methoxide was applied to the mixture, which allowed the triglycerides in the sample to be converted to fatty acid methyl esters. This treatment resulted in a biphasic mixture. Oil-soluble dyes and fatty acid methyl esters were deposited in the upper organic phase, which was cleaned using a silica-gel solid-phase extraction (SPE) column to remove the fatty acid methyl esters from the solution. The water-soluble dyes were deposited in the aqueous phase, and an Oasis hydrophilic–lipophilic-balanced SPE column was used to remove polar matrix components from the solution. The resulting dyes were subsequently analyzed via LC–diode-array detection using a single method. The practical LODs of the samples were defined as the lowest spiked dye concentrations at which the similarity coefficient for the spectra of the LC test solution and the corresponding reference standard solution were greater than 0.99, thus affording LODs of 0.5–1.0 µg/g. Recoveries of the dyes at a spiking level of 5.0 µg/g from soft drink, chili sauce, and mustard were generally greater than 70%. Recoveries from paprika powder were between 33 and 103%.

The Analysis of Phenylbutazone and Its Active Metabolite, Oxyphenbutazone, in Equine Tissues (Muscle, Kidney, and Liver), Urine, and Serum by LC-MS/MS

Boison, Joe O.1; Dowling, Patricia2; Matus, Johanna L.3; Kinar, Jana3; Johnson, Ron4

ABSTRACT

This study reports the use of two validated LC with tandem MS (MS/MS) methods to study the residue depletion profile of phenylbutazone (PBZ) and its metabolite oxyphenbutazone (OXPBZ) from equine serum, urine, and muscle, kidney, and liver tissues. One LC-MS/MS method, with an LOQ of 1.0 ng/mL for PBZ and 2.0 ng/mL for OXPBZ, was used for the analysis of the two drugs in the biological fluids (equine urine and serum); the other LC-MS/MS method, with an LOQ of 0.5 ng/g for PBZ and OXPBZ, was used for the analysis of the drugs in the equine tissue samples. PBZ was administered intravenously to two horses dosed with 8.8 mg/kg PBZ once daily for 4 days and sacrificed humanely at a slaughter plant 7 days after the last drug administration. Urine, serum, and kidney, liver, and muscle tissues were collected from the two horses and shipped on ice to the laboratory and stored at −20°C until analysis. The concentrations of PBZ and OXPBZ residues in the biological fluid and tissue samples collected at slaughter were measured with the two validated LC-MS/MS methods using deuterated internal standards. The results demonstrate that the validated methods are fit for studying the depletion kinetics of PBZ residues in equine tissues and biological fluids.

Creating Best Practices for the Submission of Actionable Food and Feed Testing Data Generated in State and Local Laboratories

Wangsness, Kathryn1; Salfinger, Yvonne2; Randolph, Robyn2; Shea, Shari2; Larson, Kirsten2

ABSTRACT

Laboratory accreditation provides a level of standardization in laboratories and confidence in generated food and feed testing results. For some laboratories, ISO/IEC 17025:2005 accreditation may not be fiscally viable, or a requested test method may be out of the scope of the laboratory's accreditation. To assist laboratories for whom accreditation is not feasible, the Association of Public Health Laboratories Data Acceptance Work Group developed a white paper entitled "Best Practices for Submission of Actionable Food and Feed Testing Data Generated in State and Local Laboratories." The basic elements of a quality management system, along with other best practices that state and local food and feed testing laboratories should follow, are included in the white paper. It also covers program-specific requirements that may need to be addressed. Communication with programs and end data users is regarded as essential for establishing the reliability and accuracy of laboratory data. Following these suggested best practices can facilitate the acceptance of laboratory data, which can result in swift regulatory action and the quick removal of contaminated product from the food supply, improving public health nationally.

Highlight on Bottlenecks in Food Allergen Analysis: Detection and Quantification by Mass Spectrometry

Planque, Mélanie1; Arnould, Thierry2; Renard, Patricia2; Delahaut, Philippe1; Dieu, Marc2; Gillard, Nathlie3

ABSTRACT

Food laboratories have developed methods for testing allergens in foods. The efficiency of qualitative and quantitative methods is of prime importance in protecting allergic populations. Unfortunately, food laboratories encounter barriers to developing efficient methods. Bottlenecks include the lack of regulatory thresholds, delays in the emergence of reference materials and guidelines, and the need to detect processed allergens. In this study, ultra-HPLC coupled to tandem MS was used to illustrate difficulties encountered in determining method performances. We measured the major influences of both processing and matrix effects on the detection of egg, milk, soy, and peanut allergens in foodstuffs. The main goals of this work were to identify difficulties that food laboratories still encounter in detecting and quantifying allergens and to sensitize researchers to them.

Clinical, Research, and Public Health Implications of Poor Measurement of Vitamin D Status

Lucas, Robyn M.1; Gorman, Shelley2; Black, Lucinda3; Neale, Rachel E.4

ABSTRACT

There is widespread concern about the high prevalence of vitamin D deficiency amid evidence to support that such a state may increase the risk of a wide range of adverse health outcomes. Estimating the prevalence of deficiency, as well as establishing links to health outcomes, requires the accurate and precise measurement of 25-hydroxyvitamin D [25(OH)D] in serum or plasma. Accurate measurement of 25(OH)D underlies the definitions of vitamin D deficiency, insufficiency, and sufficiency and, thus, prevalence estimates. Imprecise measurement of 25(OH)D in epidemiological research can result in incorrect null findings of associations with disease. When associations with disease are found, the inaccuracy of measurement forestalls defining the absolute level of 25(OH)D that is associated with increased risk. For the clinician, both inaccuracy and imprecision are problematic, because clinical care is most often based on a single measurement to define vitamin D status. New initiatives to develop a standard reference method and the assignment of "true" values to samples provide a solution to these problems. The use of standardized assays in large population studies will allow comparisons to be made between populations and over time that have not previously been possible and will improve our understanding of the role of vitamin D in health and disease.

General Steps to Standardize the Laboratory Measurement of Serum Total 25-Hydroxyvitamin D

Sempos, Christopher T.1; Betz, Joseph M.1; Camara, Johanna E.2; Carter, Graham D.3; Cavalier, Etienne4; Clarke, Michael W.5; Dowling, Kirsten G.6; Durazo-Arvizu, Ramon A.7; Hoofnagle, Andrew N.8; Liu, Andy9; Phinney, Karen W.10; Sarafin, Kurtis11; Wise, Stephen A.1; Coates, Paul M.1

ABSTRACT

The Vitamin D Standardization Program (VDSP) has collaborated with numerous groups and agencies to assemble a set of tools, i.e., a reference measurement system, that can be used to establish the traceability of 25-hydroxyvitamin D [25(OH)D] assays to relevant reference measurement procedures and reference materials. This is done with the goal of verifying end-user laboratory performance using precise statistical criteria to determine whether a specific assay is standardized. The purpose of this paper was to outline a set of steps that routine clinical and research laboratories can use to standardize their 25(OH)D assays using these tools. These steps apply to laboratories using commercially developed immunoassay measurement systems as well as in-house assays, usually based on high HPLC or LC tandem MS measurement systems. The steps are (1) initial calibration, (2) initial assessment of accuracy and bias, (3) assessment of total percent CV and mean bias, (4) use of trueness controls, and (5) participation in accuracy-based performance testing and/or external quality assessment schemes. The goal of each laboratory assay is to have a total CV of ≤10% and mean bias of ≤5%. Rigorous and less rigorous but low-cost options for meeting these statistical criteria are provided. Research laboratories who infrequently measure 25(OH) D are advised to repeat steps 1–4 for every measurement cycle. For users of commercial immunoassays who have relatively little control over standardization, we present an option for using trueness controls to develop a master equation that can be used to standardize results to the reference methods.

The Vitamin D Standardization Program (VDSP) Manual for Retrospective Laboratory Standardization of Serum 25-Hydroxyvitamin D Data

Durazo-Arvizu, Ramon A.1; Tian, Lu2; Brooks, Stephen P.J.3; Sarafin, Kurtis3; Cashman, Kevin D.4; Kiely, Mairead4; Merkel, Joyce5; Myers, Gary L.6; Coates, Paul M.5; Sempos, Christopher T.5

ABSTRACT

Low concentrations of total 25-hydroxyvitamin D [25(OH)D], the principal biological measure of vitamin D status, have been associated with clinical and public health outcomes. The determination of levels under which there is an increase in the risk of disease, as well as comparisons across populations, have been difficult to establish due the large assay variability in measuring 25(OH)D. Accordingly, the Vitamin D Standardization Program (VDSP) includes the retrospective standardization of existing 25(OH)D values collected by epidemiological and clinical studies, as well as clinical trials, as one of its main objectives. We introduce methodology developed by the VDSP that can be used to standardize the measurement of time-stable analytes, including 25(OH)D, in samples that have been banked and maintained appropriately. Sample size estimation formulae are first applied to calculate the required number of banked blood samples to be reanalyzed using either of two approaches. In the first approach, existing samples are remeasured using the current measurement procedure, and an equation relating “old” to “current” measurements is obtained. A second set of sera, usually 40–50 single-donor serum samples, are measured with the current measurement procedure and an assay traceable to a reference measurement procedure and/or certified reference materials, which yields a second calibration equation. These two equations are combined to produce standardized levels from the original old values. This approach is necessary when study restrictions prevent serum samples from being shipped to an external laboratory and is illustrated with samples from the Canadian Health Measures Survey. When serum samples are permitted to be shared with other laboratories, or the study investigators can carry out the measurements with a traceable assay, a single calibration equation method is used. Existing samples are selected and remeasured using the available traceable assay. We outline the statistical theory supporting the VDSP protocol and provide implementation examples. The methods proposed are generalizable to any instance in which banked specimens have been properly prepared and stored and the analyte of interest is stable under those conditions.

Baseline Assessment of 25-Hydroxyvitamin D Assay Performance: A Vitamin D Standardization Program (VDSP) Interlaboratory Comparison Study

Wise, Stephen A.1; Phinney, Karen W.2; Tai, Susan S.-C.2; Camara, Johanna E.2; Myers, Gary L.3; Durazo-Arvizu, Ramon4; Tian, Lu5; Hoofnagle, Andrew N.6; Bachmann, Lorin M.7; Young, Ian S.8; Pettit, Juanita9; Caldwell, Grahame10; Liu, Andrew10; Brooks, Stephen P.J.11; Sarafin, Kurtis11; Thamm, Michael12; Mensink, Gert B.M.12; Busch, Markus12; Rabenberg, Martina12; Cashman, Kevin D.13; Kiely, Mairead13; Kinsella, Michael13; Galvin, Karen13; Zhang, Joy Y.13; Oh, Kyungwon14; Lee, Sun-Wha15; Jung, Chae L.15; Cox, Lorna16; Goldberg, Gail16; Guberg, Kate16; Prentice, Ann16; Carter, Graham D.17; Jones, Julia17; Brannon, Patsy M.18; Lucas, Robyn M.19; Crump, Peter M.20; Cavalier, Etienne21; Merkel, Joyce1; Betz, Joseph M.1; Sempos, Christopher T.1

ABSTRACT

The Vitamin D Standardization Program (VDSP) coordinated an interlaboratory study to assess the comparability of measurements of total 25-hydroxyvitamin D [25(OH)D] in human serum, which is the primary marker of vitamin D status. A set of 50 individual donor samples were analyzed by 15 different laboratories representing national nutrition surveys, assay manufacturers, and clinical and/or research laboratories to provide results for total 25(OH)D using both immunoassays (IAs) and LC tandem MS (MS/MS). The results were evaluated relative to bias compared with the target values assigned based on a combination of measurements at Ghent University (Belgium) and the U.S. National Institute of Standards and Technology using reference measurement procedures for the determination of 25(OH)D2 and 25(OH)D3. CV and mean bias for each laboratory and assay platform were assessed and compared with previously established VDSP performance criteria, namely CV $\leq$ 10% and mean bias $\leq$ 5%. Nearly all LC-MS/MS results achieved VDSP criteria, whereas only 50% of IAs met the criterion for a $\leq$10% CV and only three of eight IAs achieved the $\leq$5% bias. These results establish a benchmark for the evaluation of 25(OH)D assay performance and standardization activities in the future.

Value Assignment of Vitamin D Metabolites in Vitamin D Standardization Program Serum Samples

Phinney, Karen W.1; Camara, Johanna E.1; Tai, Susan S.-C.1; Sander, Lane C.1; Wise, Stephen A.1; De Grande, Linde A.C.2; Thienpont, Linda M.2; Possolo, Antonio M.3; Toman, Blaza3; Sempos, Christopher T.4; Betz, Joseph M.4; Coates, Paul M.4

ABSTRACT

Assay variability has been cited as an obstacle to establishing optimal vitamin D exposure. As part of the Vitamin D Standardization Program (VDSP) effort to standardize the measurement of total 25-hydroxyvitamin D [25(OH)D], the value assignment of total 25(OH)D in 50 single-donor serum samples was performed using two isotope-dilution LC with tandem MS methods. Both methods are recognized as reference measurement procedures (RMPs) by the Joint Committee for Traceability in Laboratory Medicine. These samples and their assigned values serve as the foundation for several aspects of the VDSP. To our knowledge, this is the first time that two RMPs have been used to assign 25(OH)D values to such a large number of serum samples.

Role of the National Institute of Standards and Technology (NIST) in Support of the Vitamin D Initiative of the National Institutes of Health, Office of Dietary Supplements

Wise, Stephen A.1; Tai, Susan S.-C.1; Burdette, Carolyn Q.1; Camara, Johanna E.1; Bedner, Mary1; Lippa, Katrice A.1; Nelson, Michael A.1; Nalin, Federica1; Phinney, Karen W.1; Sander, Lane C.1; Betz, Joseph M.2; Sempos, Christopher T.2; Coates, Paul M.2

ABSTRACT

Since 2005, the National Institute of Standards and Technology (NIST) has collaborated with the National Institutes of Health (NIH), Office of Dietary Supplements (ODS) to improve the quality of measurements related to human nutritional markers of vitamin D status. In support of the NIH-ODS Vitamin D Initiative, including the Vitamin D Standardization Program (VDSP), NIST efforts have focused on (1) development of validated analytical methods, including reference measurement procedures (RMPs); (2) development of Standard Reference Materials (SRMs); (3) value assignment of critical study samples using NIST RMPs; and (4) development and coordination of laboratory measurement QA programs. As a result of this collaboration, NIST has developed RMPs for 25-hydroxyvitamin D2 [25(OH)D2], 25(OH)D3, and 24R,25-dihydroxyvitamin D3 [24R,25(OH)2D3]; disseminated serum-based SRMs with values assigned for 25(OH)D2, 25(OH)D3, 3-epi-25(OH)D3, and 24R,25(OH)2D3; assigned values for critical samples for VDSP studies, including an extensive interlaboratory comparison and reference material commutability study; provided an accuracy basis for the Vitamin D External Quality Assurance Scheme; coordinated the first accuracy-based measurement QA program for the determination of 25(OH)D2, 25(OH)D3, and 3-epi-25(OH)D3 in human serum/plasma; and developed methods and SRMs for the determination of vitamin D and 25(OH)D in food and supplement matrix SRMs. The details of these activities and their benefit and impact to the NIH-ODS Vitamin D Initiative are described.

Establishing an Accuracy Basis for the Vitamin D External Quality Assessment Scheme (DEQAS)

Burdette, Carolyn Q.1; Camara, Johanna E.1; Nalin, Federica1; Pritchett, Jeanita1; Sander, Lane C.1; Carter, Graham D.2; Jones, Julia2; Betz, Joseph M.3; Sempos, Christopher T.3; Wise, Stephen A.1

ABSTRACT

Until recently, the Vitamin D External Quality Assessment Scheme (DEQAS) assessed the performance of various assays for the determination of serum total 25-hydroxyvitamin D [25(OH)D] by using a consensus mean based on the all-laboratory trimmed mean (ALTM) of the approximately 1000 participants' results. Since October 2012, the National Institute of Standards and Technology (NIST), as part of the Vitamin D Standardization Program, has participated in DEQAS by analyzing the quarterly serum sample sets using an isotope dilution LC-tandem MS (ID LC-MS/MS) reference measurement procedure to assign an accuracy-based target value for serum total 25(OH)D. NIST has analyzed 90 DEQAS samples (18 exercises × 5 samples/exercise) to assign target values. The NIST-assigned values are compared with the ALTM and the biases assessed for various assays used by the participants, e.g., LC-MS/MS, HPLC, and several ligand-binding assays. The NIST-value assignment process and the results of the analyses of the 90 DEQAS samples are summarized. The absolute mean bias between the NIST-assigned values and the ALTM was 5.6%, with 10% of the samples having biases >10%. Benefits of the accuracy-based target values are presented, including for sample sets with high concentrations of 25(OH)D2 and 3-epi-25(OH)D3.

Baseline Assessment of 25-Hydroxyvitamin D Reference Material and Proficiency Testing/External Quality Assurance Material Commutability: A Vitamin D Standardization Program Study

Phinney, Karen W.1; Sempos, Christopher T.2; Tai, Susan S.-C.3; Camara, Johanna E.3; Wise, Stephen A.2; Eckfeldt, John H.4; Hoofnagle, Andrew N.5; Carter, Graham D.6; Jones, Julia6; Myers, Gary L.7; Durazo-Arvizu, Ramon8; Miller, W. Greg9; Bachmann, Lorin M.9; Young, Ian S.10; Pettit, Juanita11; Caldwell, Grahame12; Liu, Andrew12; Brooks, Stephen P.J.13; Sarafin, Kurtis13; Thamm, Michael14; Mensink, Gert B.M.14; Busch, Markus14; Rabenberg, Martina14; Cashman, Kevin D.15; Kiely, Mairead15; Galvin, Karen15; Zhang, Joy Y.15; Kinsella, Michael15; Oh, Kyungwon16; Lee, Sun-Wha17; Jung, Chae L.17; Cox, Lorna18; Goldberg, Gail18; Guberg, Kate18; Meadows, Sarah18; Prentice, Ann18; Tian, Lu19; Brannon, Patsy M.20; Lucas, Robyn M.21; Crump, Peter M.22; Cavalier, Etienne23; Merkel, Joyce24; Betz, Joseph M.24

ABSTRACT

The Vitamin D Standardization Program (VDSP) coordinated a study in 2012 to assess the commutability of reference materials and proficiency testing/external quality assurance materials for total 25-hydroxyvitamin D [25(OH)D] in human serum, the primary indicator of vitamin D status. A set of 50 single-donor serum samples as well as 17 reference and proficiency testing/external quality assessment materials were analyzed by participating laboratories that used either immunoassay or LC-MS methods for total 25(OH)D. The commutability test materials included National Institute of Standards and Technology Standard Reference Material 972a Vitamin D Metabolites in Human Serum as well as materials from the College of American Pathologists and the Vitamin D External Quality Assessment Scheme. Study protocols and data analysis procedures were in accordance with Clinical and Laboratory Standards Institute guidelines. The majority of the test materials were found to be commutable with the methods used in this commutability study. These results provide guidance for laboratories needing to choose appropriate reference materials and select proficiency or external quality assessment programs and will serve as a foundation for additional VDSP studies.

Development of Standard Reference Material (SRM) 2973 Vitamin D Metabolites in Frozen Human Serum (High Level)

Tai, Susan S.-C.1; Nelson, Michael A.1; Bedner, Mary1; Lang, Brian E.1; Phinney, Karen W.1; Sander, Lane C.1; Yen, James H.2; Betz, Joseph M.3; Sempos, Christopher T.3; Wise, Stephen A.1

ABSTRACT

The National Institute of Standards and Technology (NIST), in collaboration with the National Institutes of Health Office of Dietary Supplements and the Vitamin D Standardization Program, has recently issued a new serum-matrix Standard Reference Material (SRM): 2973 Vitamin D Metabolites in Frozen Human Serum (High Level). SRM 2973 was designed to provide a serum material with a total 25-hydroxyvitamin D [25(OH)D] concentration near 100 nmol/L to complement the existing serum-based SRMs with values assigned for total 25(OH)D between 20 and 80 nmol/L. Values were assigned for 25-hydroxyvitamin D2 [25(OH)D2], 25-hydroxyvitamin D3 [25(OH)D3], 3-epi-25(OH)D3, and total 25(OH)D [the sum of 25(OH)D2 + 25(OH)D3] using the NIST isotope dilution LC with tandem MS (MS/MS) reference measurement procedure (RMP) and related methods. SRM 2973 has a certified value of 98.4 ± 2.1 nmol/L for 25(OH)D3 and reference values of 1.59 ± 0.05 nmol/L for 25(OH)D2 and 5.23 ± 0.20 nmol/L for 3-epi-25(OH)D3. In addition, a candidate RMP for 24R,25-dihydroxyvitamin D3 [24R,25(OH)2D3] based on LC-MS/MS was used to assign values to SRM 2973 and the existing SRM 972a Vitamin D Metabolites in Frozen Human Serum. Reference values for 24R,25(OH)2D3 were assigned to SRM 2973 (7.51 ± 0.26 nmol/L) and the four levels of SRM 972a: Level 1 (6.38 ± 0.23 nmol/L), Level 2 (3.39 ± 0.12 nmol/L), Level 3 (3.88 ± 0.013 nmol/L), and Level 4 (6.32 ± 0.22 nmol/L). The development of SRM 2973 [with a higher concentration of 25(OH)D3] and the addition of values for 24R,25(OH)2D3 assigned to both SRM 972a and SRM 2973 provide laboratories involved in vitamin D measurements with improved QA tools.

Technical Note: Determination of Cholecalciferol (Vitamin D3) in Standard Reference Material 3532 Calcium-Containing Solid Oral Dosage Form

Burdette, Carolyn Q.

ABSTRACT

Vitamin D is an important nutrient for many areas of human health and well-being, including improved bone strength, muscle movement, cognitive function, and immune health. The National Institute of Standards and Technology, in collaboration with the National Institutes of Health Office of Dietary Supplements, has developed SRM 3532 Calcium-Containing Solid Oral Dosage Form to help address the analytical challenges seen by the dietary supplement communities for the determination of vitamin D3 (cholecalciferol) and elements. Described here is the process to assess the homogeneity and stability of the material, as well as the value assignment of the vitamin D3 levels.

Interlaboratory Comparison for the Determination of 24,25-Dihydroxyvitamin D3 in Human Serum Using Liquid Chromatography with Tandem Mass Spectrometry

Wise, Stephen A.1; Tai, Susan S.-C.1; Nelson, Michael A.1; Burdette, Carolyn Q.1; Camara, Johanna E.1; Hoofnagle, Andrew N.2; Laha, Thomas J.2; Carter, Graham D.3; Jones, Julia3; Williams, Emma L.3; Barclay, Zoe J.3; Jones, Glenville4; Kaufmann, Martin4; Binkley, Neil5; Kapoor, Amita5; Ziegler, Toni5; Cashman, Kevin D.6; Dowling, Kirsten G.6; Sempos, Christopher T.7

ABSTRACT

Six laboratories associated with the Vitamin D Standardization Program (VDSP) participated in an interlaboratory comparison of LC with tandem MS (MS/MS) methods for the determination of 24,25-dihydroxyvitamin D3 [24,25(OH)2D3] in human serum. The laboratories analyzed two different serum-based Standard Reference Materials (SRMs) intended for use in the determination of 25-hydroxyvitamin D and 30 samples from the Vitamin D External Quality Assessment Scheme (DEQAS). All laboratory methods for 24,25(OH)2D3 were based on isotope dilution LC-MS/MS; three of the methods used derivatization of the vitamin D metabolites before LC-MS/MS. Laboratory results were compared to the National Institute of Standards and Technology (NIST) results, which were obtained using their newly developed candidate reference measurement procedure for 24,25(OH)2D3. Laboratory results for the SRM samples varied in comparability to the NIST results, with one laboratory in excellent agreement (−1.6% mean bias), three laboratories at 10–15% mean bias, and the remaining laboratory at 36% mean bias. For the 30 DEQAS samples, the mean bias for the five laboratories ranged from 6 to 15%; however, the SD of the bias ranged from 8 to 29%. As a result of this intercomparison study, one laboratory discovered and corrected a method calculation error and another laboratory modified and improved their LC-MS/MS method.

A Direct Assay for Measuring Free 25-Hydroxyvitamin D

Heureux, Nicolas1; Lindhout, Ernst2; Swinkels, Leon2

ABSTRACT

Recent studies suggest that the concentration and genotype of vitamin D binding protein (VDBP) are important factors that determine the bioavailability of 25-hydroxyvitamin D [25(OH)D] in blood. Accumulating data indicate that, e.g., in pregnant women, hemodialysis patients, chronic kidney disease, liver failure, and bladder and pancreatic cancers, the measurement of free 25(OH)D in serum provides more relevant diagnostic information than measurement of total 25(OH)D. The aim of this study was to develop and validate an ELISA for direct measurement of free 25(OH)D in serum. A simple and direct ELISA was developed, based on a two-step immunoassay procedure performed in a microtiter plate. The assay has been characterized in terms of precision (4–10% CV, according to concentration), sensitivity (limits of blank = 0.5–1.0 pg/mL and LODs = 1.3–1.8 pg/mL), accuracy (correlation to dialysis, ELISA = 0.99xdialysis-0.5 pg/mL, r2 = 0.74), cross-reactivity of the antibody for the D2 form (77%), and addition of both VDBP and albumin (35–38% recovery upon addition of VDBP, 53–58% upon addition of albumin). The assay has already been used in multiple studies, including its comparison with calculation methods and in studies of patients with liver failure, different ethnic groups, supplemented mice, respiratory diseases, and obesity. The free 25(OH)D ELISA can be used in studies as a valuable tool to establish the clinical relevance of free 25(OH)D.

Current Assays to Determine Free 25-Hydroxyvitamin D in Serum

Malmstroem, Sofie1; Rejnmark, Lars2; Imboden, John B.3; Shoback, Dolores M.1; Bikle, Daniel D.1

ABSTRACT

The 25-hydroxylated metabolite of vitamin D is the best clinical indicator of vitamin D status. For many years, emphasis has been on measuring total levels of 25-hydroxyvitamin D [25(OH)D], but recently, interest in measuring free 25(OH)D as a potentially better marker of vitamin D status has arisen. Since the 1980s when the first measurements of free 25(OH)D were made, little progress has been made in the development of rapid, reliable methods to determine the levels of free 25(OH)D. For many years, assessment of free 25(OH)D relied on calculations using levels of total 25(OH)D, albumin, and vitamin D binding protein (VDBP), for which many assays exist. However, because of vagaries in the measurement of VDBP in particular and the assumption of a constant affinity of VDBP for the vitamin D metabolites (which has been shown to be problematic), calculated values have proved suspect. This changed a few years ago when a new immunoassay was developed to measure free 25(OH)D directly. This review examines methods for determining free 25(OH)D, the different methods used in clinical studies, and the relationships between free 25(OH)D and other vitamin D metabolites and the physiologic functions affected by vitamin D metabolites, such as bone cell activity and turnover. The review also comments on the value of assessing free 25(OH)D and the efforts to standardize the assays.

Simplified 25-Hydroxyvitamin D Standardization and Optimization in Dried Blood Spots by LC-MS/MS

Makowski, Andrew J.1; Rathmacher, John A.1; Horst, Ronald L.1; Sempos, Christopher T.2

ABSTRACT

Previous studies have assessed vitamin D status based on the 25-hydroxyvitamin D [25(OH)D] concentration measured in samples from dried blood spots (DBSs). In 40 individuals participating in a clinical study, we compared 25(OH)D levels measured from DBSs and in serum using an LC-MS/MS reference procedure in collaboration with the Vitamin D Standardization Program. The main objective was to simplify and optimize current methods to produce an assay that can be used as a screening tool for 25(OH)D concentration assessment without derivatization. The DBS 25(OH)D levels, compared to serum concentrations, were found to have 101% accuracy overall, and the correlation coefficient (r) was 0.83 ($P < 0.0001$), with a significant linear relationship. Free 25(OH)D and vitamin D binding protein (VDBP) were assessed in the serum samples for potential correlations to the DBS calculations: the levels of free 25(OH)D had moderate to strong correlation to DBS and serum concentrations, with r values of 0.67 ($P < 0.0001$) and 0.76 ($P < 0.0001$), respectively. VDBP and hematocrit had no significant correlation to either DBS or serum sample types, with r values <0.1. In conclusion, the use of two DBSs and an increase in DBS sample size improved overall sample representation without the need for derivatization, and produced an accurate and robust method that can be used to screen 25(OH)D levels.

An Analysis of Factors Associated with 25-Hydroxyvitamin D Levels in White and Non-White Canadians

Brooks, Stephen P.J.1; Greene-Finestone, Linda1; Whiting, Susan2; Fioletov, Vitali E.3; Laffey, Patrick4; Petronella, Nicholas4

ABSTRACT

Vitamin D status was assessed in 19–79 year old whites (8351 participants of European ancestry) and non-whites (1840 participants encompassing all other ancestries) from cycles 1 to 3 (years 2007–2013) of the Canadian Health Measures Survey. Status was assessed using the U.S. Institute of Medicine (IOM) 25-hydroxyvitamin D [25(OH)D] cut point values of 30 and 40 nmol/L. Overall, median 25(OH)D concentrations were significantly higher in whites [58.9 (28.6, 100.1) nmol/L; 5th and 95th percentile] compared with non-whites [43.5 (19.0, 83.2); $P < 0.001$]. Values were higher in females [58.5 (27.5, 101.3) nmol/L] when compared with males [53.5 (24.2, 92.7) nmol/L] and increased with age. Non-whites were more likely to have 25(OH)D values below IOM established cut points for optimum bone health with 20.1 (16.0, 24.2) and 42.2% (36.8, 47.7) of non-whites having serum 25(OH)D concentrations <30 and <40 nmol/L, respectively. The corresponding values for whites were 5.9 (4.6, 7.2) and 16.1% (14.0, 18.3). Values were lower during the first quarter when compared with the third quarter. Supplement intake was an important factor in determining 25(OH)D levels, but it did not alone account for the difference in status. Equivalent increases in 25(OH)D levels were observed in whites and non-whites during the summer months, suggesting there was no functional difference in sun exposure response. It is apparent that a complex interaction of factors affect 25(OH)D values in free-living Canadians.

Comparative Study of NMR Spectral Profiling for the Characterization and Authentication of Cannabis

Wang, Xinyi1; Harrington, Peter de B.1; Baugh, Steven F.2

ABSTRACT

For the authentication of botanical materials, it is difficult to obtain representative reference materials because botanicals vary significantly with respect to cultivation conditions. Chemical profiling of plant extracts or spectral fingerprinting can differentiate botanicals and group them by their chemical profiles. NMR spectroscopy yields a powerful and useful method for profiling plant extracts. Both 500 MHz 1H and 1H-1H correlation NMR spectroscopy coupled with pattern recognition were used to discriminate among Cannabis samples. A rapid method of analysis was achieved by extracting directly into the deuterated solvent. Spectral ranges including or excluding the downfield region were compared to evaluate the effect on classification accuracy by projected difference resolution. Six classification methods—fuzzy rule-building expert system, linear discriminant analysis (LDA), super partial least-squares discriminant analysis, support vector machine (SVM), and SVM classification trees (SVMTrees)—all gave better classification performance for proton NMR spectra than for proton-proton correlation NMR spectra for seven Cannabis samples. Among the classification methods for a set of 25 Cannabis samples, the 0.5–7.2 plus 7.4–13.0 ppm ranges gave higher prediction rates of greater than 96% when compared to the reduced range of 0.5–7.2 ppm that excluded the downfield range. The LDA method had the best prediction accuracy of 99.8 ± 0.4%. SVMTree methods provide a robust tool, and classification trees are amenable to interpretation. Hence, NMR spectroscopy combined with chemometrics could be used as a fast screening method for the authentication of Cannabis samples.

Certified Reference Material for Use in 1H, 31P, and 19F Quantitative NMR, Ensuring Traceability to the International System of Units

Rigger, Romana; Rück, Alexander; Hellriegel, Christine; Sauermoser, Robert; Morf, Fabienne; Breitruck, Kathrin; Obkircher, Markus

ABSTRACT

In recent years, quantitative NMR (qNMR) spectroscopy has become one of the most important tools for content determination of organic substances and quantitative evaluation of impurities. Using Certified Reference Materials (CRMs) as internal or external standards, the extensively used qNMR method can be applied for purity determination, including unbroken traceability to the International System of Units (SI). The implementation of qNMR toward new application fields, e.g., metabolomics, environmental analysis, and physiological pathway studies, brings along more complex molecules and systems, thus making use of 1H qNMR challenging. A smart workaround is possible by the use of other NMR active nuclei, namely 31P and 19F. This article presents the development of three classes of qNMR CRMs based on different NMR active nuclei (1H, 31P, and 19F), and the corresponding approaches to establish traceability to the SI through primary CRMs from the National Institute of Standards and Technology and the National Metrology Institute of Japan. These TraceCERT® qNMR CRMs are produced under ISO/IEC 17025 and ISO Guide 34 using high-performance qNMR.

Comparative Study between Multivariate and Univariate Analysis of Two Antidiabetic Combinations

Abdel-Ghany, Maha F.; Abdel-Aziz, Omar; Ayad, Miriam F.; Tadros, Mariam M.

ABSTRACT

New multivariate and univariate methods were developed for the analysis of two novel gliptin combinations by manipulating the zero-order and ratio spectra of empagliflozin and linagliptin in combination, with application on Glyxambi® tablets, and of alogliptin and pioglitazone in combination, with application on Oseni® tablets. Linearity ranges for chemometric approaches using principal component regression and partial least-squares were found to be 2–10, 2.5–12.5, 5–15, and 5–25 μg/mL for empagliflozin, linagliptin, alogliptin, and pioglitazone, respectively, whereas the respective linearity ranges for the spectrophotometric approaches were found to be 5–15, 2–12, 5–15, and 5–15 μg/mL. The proposed spectrophotometric methods included ratio subtraction coupled with extended ratio subtraction, spectrum subtraction coupled with constant multiplication, and mean centering. Acceptable LOD and LOQ values were obtained by all methods. Statistical analysis showed no significant difference between multivariate and univariate methods in comparison with the reference methods. The optimized methods provide fast and economic determination of the recently approved antidiabetic combinations without the complex instrumentation or time-consuming mobile phase preparations that were used in the chromatographic techniques reported in the literature.

Application of an HPLC Method for Selective Determination of Phenazopyridine Hydrochloride: Theoretical and Practical Investigations

Attia, Khalid A.M.; El-Abasawi, Nasr M.; El-Olemy, Ahmed; Abdelazim, Ahmed H.

ABSTRACT

HPLC method was developed for the selective determination of phenazopyridine hydrochloride (PAP) in the presence of its computationally selected metabolite. Density functional theory was applied as a computational model to study the energy of PAP metabolites, and the results revealed that 2,3,6-triaminopyridine (TAP) is the most stable metabolite. Good resolution and separation of PAP from TAP was achieved using a reversed-phase BDS Hypersil C18 column with a mobile phase consisting of acetonitrile–water (75 + 25, v/v) at flow rate of 1 mL/min and with UV detection at 280 nm. The linear regression analysis data for the calibration plot of PAP showed a good linear relationship over the concentration range of 5–45 μg/mL, with an LOD of 0.773 μg/mL. Moreover, a theoretical investigation of the relationship between the stationary phase and the studied molecules was performed to confirm the experimental results. The proposed method was successfully applied for the selective determination of PAP in pharmaceutical formulation. In addition, the obtained results were statistically compared to a reported method, with no significant differences found between the investigated method and the reported one with respect to accuracy and precision.

Stability-Indicating UPLC Method for the Estimation of Nadifloxacin, Terbinafine Hydrochloride, Mometasone Furoate, Methyl Paraben, and Propyl Paraben in Topical Pharmaceutical Dosage Form

Authors: Bhosale, Dileep M.; Nikalje, Anna Pratima G.

ABSTRACT

A novel and simple ultra-performance LC method was developed for the estimation of nadifloxacin (NAD), terbinafine hydrochloride (TBH), mometasone furoate (MMF), methyl paraben (MP), and propyl paraben (PP) in a topical pharmaceutical dosage formulation. The analysis was carried out on a Waters Acquity UPLC ethylene bridged hybrid C18 column (50 × 2.1 mm, 1.7 μm) with a flow rate of 0.4 mL/min in gradient mode at a wavelength of 255 nm. Elution of all components was achieved within 9 min. The retention times of MP, NAD, PP, TBH, and MMF were observed at 1.5, 2.6, 3.4, 6.0, and 6.9 min, respectively. The proposed method was validated per current International Conference on Harmonization guidelines for specificity, precision, linearity, accuracy, range, LOD, LOQ, robustness, filter paper interference, and solution stability parameters. A complete method study was performed to determine the stability-indicating nature of the developed method.

All-Solid-State, PVC Membrane, and Carbon Paste Ion-Selective Electrodes for Determination of Donepezil Hydrochloride in Pharmaceutical Formulation

Khamees, Nesreen1; Mohamed, Tagreed Abdel-Fattah2; Derar, Abeer Rashad2; Aziz, Azza1

ABSTRACT

All-solid-state, polyvinyl chloride (PVC) membrane, and carbon paste potentiometric ion-selective electrodes (ISEs) were proposed for the determination of donepezil hydrochloride (DON) in the drug substance and a pharmaceutical formulation. The potentiometric response toward DON was based on the existence of donepezil-tetraphenyl borate (DON-TPB) in a PVC membrane or a carbon paste in the presence of dioctylphthalate. In contrast, the solid-state electrode was prepared by direct incorporation of DON-TPB into a commercial nail varnish without external additives. The electrodes exhibited Nernstian slopes of 55.0, 57.0, and 53.0 mV/decade over the concentration ranges of $1 \times 10{-}5$ to $1 \times 10{-}3$, $1 \times 10{-}4$ to $10{-}2$, and $1 \times 10{-}4$ to $5 \times 10{-}3$ for the solid-state, PVC membrane, and carbon paste electrodes, respectively. The response of the electrodes is independent of pH in the range of 2–≤8. The electrodes showed good selectivity for DON with respect to a number of inorganic cations and amino acids. The electrodes were used for the determination of DON in pure solution and in pharmaceutical tablets with high accuracy (±2%) and precision (RSD ≤2%). The solid-state electrode is simple, economical, and rapid when compared to the PVC membrane and carbon paste electrodes.

Rapid Simultaneous Separation of Four Oral Antidiabetic Drugs and Quantitative Determination of Glibenclamide Using Conventional and Fused-Core Silica Columns

Moraes, Laura Maria Fontes Prado1; Pianetti, Gerson Antônio2; César, Isabela da Costa2; Fernandes, Christian3

ABSTRACT

Diabetes mellitus is a chronic disease with high and growing prevalence worldwide. Therefore, the development of fast and efficient methods for the QC of antidiabetic drugs is of fundamental importance. Two ultra-fast methods, using a conventional (C18 100 × 2.1 mm, 5 μm fully porous particle) column or a fused-core (C18 100 × 2.1 mm, 2.7 μm fused-core particle) column, were developed for the simultaneous determination of four antidiabetic drugs (chlorpropamide, glibenclamide, gliclazide, and glimepiride). The developed methods were compared in terms of efficiency, speed of analysis, resolution, and peak symmetry. Both methods were validated with respect to selectivity, system suitability, linearity, precision, accuracy, LOD, LOQ, and robustness, using glibenclamide as model. Conventional and fused-core methods were shown to be appropriate for the determination of glibenclamide in tablets; however, the fused-core column presented higher efficiency, detectability, and resolution. Also, it enabled faster analysis, with separation of the four drugs in less than 1 min.

Rapid and Sensitive Determination of 13 Components in a Traditional Chinese Medicine Formula of Da-Huang-Gan-Cao Decoction by High-Performance Liquid Chromatography Coupled with Triple Quadrupole Mass Spectrometry

Qu, Jialin1; Xiao, Nan2; Sui, Hua2; Liang, Lina2; Chen, Jing2; Li, Weiling3; Shang, Dong1

ABSTRACT

Da-Huang-Gan-Cao decoction (DHGCD), which is a classic formula in traditional Chinese medicine, has been clinically used for the treatment of vomiting, constipation, pancreatitis, and cholelithiasis in China and Japan. In this study, a rapid and validated method using HPLC coupled with triple quadrupole MS was developed for the simultaneous determination of 13 components in DHGCD. Separation was performed on an XBridge BEH C18 column (50 × 2.1 mm, 2.5 μm) with a gradient elution of acetonitrile and 0.1% formic acid in water. All calibration curves showed good linear regression ($r > 0.9981$) within the test range. LODs and LOQs were in the range of 1.0–20 and 2.6–69 ng/mL, respectively. The proposed method was applied for the analysis of the target compounds in 10 batches of DHGCD within a total time of 10 min. This method was conducive for the QC of DHGCD.

Comparative Evaluation of Veriflow® Salmonella Species to USDA and FDA Culture-Based Methods for the Detection of Salmonella spp. in Food and Environmental Samples

Puri, Amrita; Joelsson, Adam C.; Terkhorn, Shawn P.; Brown, Ashley S.; Gaudioso, Zara E.; Siciliano, Nicholas A.

ABSTRACT

Veriflow® Salmonella species (Veriflow SS) is a molecular-based assay for the presumptive detection of Salmonella spp. from environmental surfaces (stainless steel, sealed concrete, plastic, and ceramic tile), dairy (2% milk), raw meat (20% fat ground beef), chicken carcasses, and ready-to-eat (RTE) food (hot dogs). The assay utilizes a PCR detection method coupled with a rapid, visual, flow-based assay that develops in 3 min post-PCR amplification and requires only an 18 h enrichment for maximum sensitivity. The Veriflow SS system eliminates the need for sample purification, gel electrophoresis, or fluorophore-based detection of target amplification and does not require complex data analysis. This Performance Tested Method SM validation study demonstrated the ability of the Veriflow SS method to detect low levels of artificially inoculated or naturally occurring Salmonella spp. in eight distinct environmental and food matrixes. In each reference comparison study, probability of detection analysis indicated that there was no significant difference between the Veriflow SS method and the U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook Chapter 4.06 and the U.S. Food and Drug Administration Bacteriological Analytical Manual Chapter 5 reference methods. A total of 104 Salmonella strains were detected in the inclusivity study, and 35 nonspecific organisms went undetected in the exclusivity study. The study results show that the Veriflow SS method is a sensitive, selective, and robust assay for the presumptive detection of Salmonella spp. sampled from environmental surfaces (stainless steel, sealed concrete, plastic, and ceramic tile), dairy (2% milk), raw meat (20% fat ground beef), chicken carcasses, and RTE food (hot dogs).

Determination of Ochratoxin A in Black and White Pepper, Nutmeg, Spice Mix, Cocoa, and Drinking Chocolate by High-Performance Liquid Chromatography Coupled with Fluorescence Detection: Collaborative Study

Cubero-Leon, Elena1; Bouten, Katrien1; Senyuva, Hamide2; Stroka, Joerg1

ABSTRACT

A method validation study for the determination of ochratoxin A in black and white pepper (Piper spp.), nutmeg (Myristica fragrans), spice mix (blend of ginger, turmeric, pepper, nutmeg, and chili), cocoa powder, and drinking chocolate was conducted according to the International Harmonized Protocol of the International Union of Pure and Applied Chemistry. The method is based on the extraction of samples with aqueous methanol, followed by a cleanup of the extract with an immunoaffinity column. The determination is carried out by reversed-phase LC coupled with a fluorescence detector. The study involved 25 participants representing a cross-section of research, private, and official control laboratories from 12 European Union (EU) Member States, together with Turkey and Macedonia. Mean recoveries ranged from 71 to 85% for spices and from 85 to 88% for cocoa and drinking chocolate. The RSDr values ranged from 5.6 to 16.7% for spices and from 4.5 to 18.7% for cocoa and drinking chocolate. The RSDR values ranged from 9.5 to 22.6% for spices and from 13.7 to 30.7% for cocoa and drinking chocolate. The resulting Horwitz ratios ranged from 0.4 to 1 for spices and from 0.6 to 1.4 for cocoa and drinking chocolate according to the Horwitz function modified by Thompson. The method showed acceptable within-laboratory and between-laboratory precision for each matrix, and it conforms to requirements set by current EU legislation.

Development and Single-Laboratory Validation of a Liquid Chromatography Tandem Mass Spectrometry Method for Quantitation of Tetrodotoxin in Mussels and Oysters

Turner, Andrew D.1; Boundy, Michael J.2; Rapkova, Monika Dhanji1

ABSTRACT

In recent years, evidence has grown for the presence of tetrodotoxin (TTX) in bivalve mollusks, leading to the potential for consumers of contaminated products to be affected by Tetrodotoxin Shellfish Poisoning (TSP). A single-laboratory validation was conducted for the hydrophilic interaction LC (HILIC) tandem MS (MS/MS) analysis of TTX in common mussels and Pacific oysters—the bivalve species that have been found to contain TTXs in the United Kingdom in recent years. The method consists of a single-step dispersive extraction in 1% acetic acid, followed by a carbon SPE cleanup step before dilution and instrumental analysis. The full method was developed as a rapid tool for the quantitation of TTX, as well as for the associated analogs 4-epi-TTX; 5,6,11-trideoxy TTX; 11-nor TTX-6-ol; 5-deoxy TTX; and 4,9-anhydro TTX. The method can also be run as the acquisition of TTX together with paralytic shellfish toxins. Results demonstrated acceptable method performance characteristics for specificity, linearity, recovery, ruggedness, repeatability, matrix variability, and within-laboratory reproducibility for the analysis of TTX. The LOD and LOQ were fit-for-purpose in comparison to the current action limit for TTX enforced in The Netherlands. In addition, aspects of method performance (LOD, LOQ, and within-laboratory reproducibility) were found to be satisfactory for three other TTX analogs (11-nor TTX-6-ol, 5-deoxy TTX, and 4,9-anhydro TTX). The method was found to be practical and suitable for use in regulatory testing, providing rapid turnaround of sample analysis. Plans currently underway on a full collaborative study to validate a HILIC-MS/MS method for paralytic shellfish poisoning toxins will be extended to include TTX in order to generate international acceptance, ultimately for use as an alternative official control testing method should regulatory controls be adopted.

Simultaneous Determination of Volatile Organic Compounds in Commercial Alcoholic Beverages by Gas Chromatography with Flame Ionization Detection

Kim, Hyung Min1; Yang, Gun1; Kim, Jung Yoon1; Yoon, Sang Jun1; Shin, Byong-kyu1; Lee, Jeongmi2; Park, Jeong Hill3; Kwon, Sung Won3

ABSTRACT

A simple and fast method was developed for the determination of volatile organic compounds in alcoholic beverages. Eleven volatile organic compounds (acetaldehyde, methanol, 2-propanol, tert-butanol, 1-propanol, ethyl acetate, 2-butanol, isobutanol, 1-butanol, 3-methyl-1butanol, and 2-methyl-1-butanol) in alcoholic beverages were analyzed with a simple direct-injection method using GC with flame ionization detection. These compounds should be monitored in the QC of production processes because they are detrimental to human health. The method was validated with four types of alcoholic beverages (beers, fruit wines, rice wines, and spirits) to confirm the versatility of the method. Linearity showed r2 values from 0.9986 to 0.9995, with LODs ranging from 0.010 to 1.000 mg/L. Precision and accuracy showed acceptable results, proving the effectiveness of the method. The developed method was applied to 40 commercial samples representing the four types of alcoholic beverages, and principal component analysis was performed to determine profiles of the volatile organic compounds, depending on the type of alcoholic beverage.

Rapid Seafood Species Identification Using Chip-Based Capillary Electrophoresis and Protein Pattern Matching

Walker, Calvin C.1; Lassitter, Cheryl L.1; Lynn, Shannara N.1; Ford, Courtney B.1; Rademacher, Kevin R.2; Ruple, Angela D.1; Bell, Jon W.1

ABSTRACT

Authenticity is crucial to the seafood industry, as substitution and mislabeling have important economic, environmental, and food safety consequences. To address this problem, protein profiling and software algorithm techniques were developed to classify fish muscle samples by species. The method uses water-based protein extraction, chip-based microfluidic electrophoresis (Agilent 2100 Bioanalyzer) for the analysis of high abundance fish muscle proteins, and a novel data analysis method for species-specific protein pattern recognition. The method's performance in distinguishing commercially important fish from commonly reported substitutions was evaluated using sensitivity, specificity, and accuracy determinations with all three performance measures at >98% for common substitutions. This study demonstrates that uncooked seafood products of commercially important species of catfish, snapper, and grouper can be rapidly distinguished from commonly substituted species with a high level of confidence. A tiered testing approach to seafood species verification by sequentially applying a rapid screening method and DNA testing is proposed to more effectively ensure accurate product labeling.

Ions in Wine and Their Relation to Electrical Conductivity Under Ultrasound Irradiation

Yan, Yan-Ying1; Zhang, Qing-An1; Li, Er-Chun2; Zhang, Ya-Feng2

ABSTRACT

Change in electrical conductivity is considered a potential indicator for the on-line monitoring of wine aging accelerated by ultrasound, as determined in our previous study; however, the exact mechanism of change is currently unclear. In this study, the ion content and the total ionic strength were analyzed by ion-exchange chromatography to investigate the change mechanism of the electrical conductivity of wine under ultrasound irradiation. The results indicate that the changes in wine electrical conductivity during ultrasound treatment correlate with the changes in the cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, and NH_4^+) and in the anions from the organic acids (malic acid, citric acid, tartaric acid, oxalic acid, and formic acid) and inorganic acids (Cl^-, SO_4^{2-}, and PO_4^{3-}), especially for the ionic strength of the wine. Overall, electrical conductivity may be used to reflect the chemical reactions related to wine aging to a certain extent because the reactions can be initiated by the conversion of cations and by the degradation or auxiliary function of organic acids.

A New Dispersive Liquid–Liquid Microextraction Method for Preconcentration and Determination of Aluminum, Iron, Copper, and Lead in Real Water Samples by HPLC

Alpdoğan, Güzin; Zor, Şule Dinç

ABSTRACT

In this study, dispersive liquid–liquid microextraction coupled with HPLC with variable-wavelength detection was applied for the simultaneous determination of Al, Fe, Cu, and Pb in various water samples at trace levels. In the proposed method, all the system parameters in both the extraction and separation/determination steps, such as extraction and disperser solvent type and their volumes, complexing reagent concentration, salt addition, extraction and centrifugation times, and pH, were optimized to get not only high extraction efficiency but also lower LODs for the analytes. Hematoxylin was used as a complexing reagent, and carbon tetrachloride and methanol were chosen as the extraction and disperser solvents, respectively. Metal complexes were separated with a reversed-phase C18 column by isocratic elution, with methanol–tetrahydrofuran–water (20 + 12 + 68, v/v/v) as the mobile phase at a flow rate of 1.0 mL/min and detection at 575 nm. The accuracy of the method was checked by a Standard Reference Material of water (SRM 1643e), and the recovery values for the analytes were found in the range of 95.6–101.3%. Under the optimum conditions, the developed method was applied to tap water, bottled mineral water, lake water, and seawater for the accurate and sensitive determination of the analytes of interest.

Determination of Several Element Levels in Hardaliye Beverages Using Flame Atomic Absorption Spectrometry after Ultrasound-Probe Extraction

Bakircioglu, Dilek; Topraksever, Nukte; Kurtulus, Yasemin Bakircioglu

ABSTRACT

In the present study, concentrations of calcium (Ca), copper (Cu), magnesium (Mg), manganese (Mn), sodium (Na), and zinc (Zn) in hardaliye samples produced in Turkey were determined by flame atomic absorption spectrometry after ultrasound probe extraction (UPE), microwave-assisted extraction (MAE), and wet extraction procedures. At present, there is limited work in the literature on UPE for the determination of trace elements in beverage samples. Our single-correlation analysis showed that the elements studied with the UPE method in hardaliye were strongly correlated with the MAE procedure. The parameters affecting the UPE experimental conditions—such as ultrasound amplitude, sonication time, sample amount, extractant type, and volume—were studied. Optimal experimental conditions for the extraction of the metals with the UPE procedure were as follows: 2 min of sonication; 30% amplitude; 3 mL sample volume; 5% HNO3 extraction solution; and 1 mL extractant volume for Ca, Cu, Mg, Mn, Na, and Zn in the hardaliye samples. The results in the hardaliye samples in minimum–maximum mg/L with the UPE procedure were 33–63 for Ca, 0.10–0.27 for Cu, 3.9–14.4 for Mg, 1.0–3.2 for Mn, 32–58 for Na, and 0.39–1.1 for Zn. LODs were 0.0032, 0.012, 0.013, 0.009, 0.011, and 0.008 mg/L for Ca, Cu, Mg, Mn, Na, and Zn, respectively. The accuracy of the method was verified with a recovery test (in which recoveries between 95 and 110% were observed) and application to a NIST 1643e certified sample (trace elements in water). The UPE procedure was found to be fast, accurate, and simple, with fewer contaminants and lower concentrated reagent consumption in comparison with conventional extraction procedures.

Magnetic Graphene Oxide as an Efficient Adsorbent for the Separation and Preconcentration of Cu(II), Pb(II), and Cd(II) from Environmental Samples

Soylak, Mustafa1; Acar, Demet1; Yilmaz, Erkan1; El-Khodary, Sherif A.2; Morsy, Mohamed2; Ibrahim, Medhat3

ABSTRACT

The separation and preconcentration of copper(II), lead(II), and cadmium(II) ions on magnetic graphene oxide (MGO) by solid-phase extraction was carried out. Quantitative recovery was obtained by adsorption of analytes on MGO at pH 6 and elution of 3 M HNO3 in 10% acetone. To optimize the presented method, the effects of various parameters—including pH, eluent conditions, and vortex time—were examined. Matrix effects were also investigated. Mean recoveries of the analytes were between 95 and 105%. The proposed method was validated by applying it to certified reference materials. Addition and recovery tests were also performed. The method was applied to verify the analyte content of several water and food samples.

Simultaneous Analysis of Simple Coumarins and Furocoumarines in Cigarettes by Solid-Phase Extraction with Gas Chromatography-Mass Spectrometry

Zheng, Yang1; Xu, Xiuli1; Yuan, Fei1; Yao, Meiyi1; Ji, Shunli2; Huang, Zhiqiang3; Zhang, Feng1

ABSTRACT

A sensitive, high-throughput analytical method based on a GC-MS method was established for the simultaneous quantitative determination of two categories of harmful coumarins: simple coumarins (coumarin, 6-methylcoumarin, 7-methoxycoumarin, 3,4-dihydrocoumarin, and 7-ethoxy-4-methylcoumarin) and furocoumarines (psoralen, 8-methoxypsoralen, 5-methoxypsoralen, and trioxysalen). The nine analytes were extracted with ethyl acetate, purified with Oasis HLB solid-phase extraction (SPE) cartridges, and identified and quantitatively determined by GC-MS in selected-ion monitoring mode. The LODs and LOQs of these compounds were in the ranges of 12.5–21.2 and 41.6–70.0 μg/kg, respectively. Average recoveries for the nine analytes ranged from 72.7 to 86.6% at LOQ, 1.5× LOQ, and 2× LOQ spike levels, with RSDs that were typically lower than 5.1%. The SPE-GC-MS method developed in this study was initially applied to research coumarins in cigarette samples; it proved to be accurate, sensitive, convenient, and practical.

Near-Infrared Spectrum Detection of Wheat Gluten Protein Content Based on a Combined Filtering Method

Cai, Jian-Hua

ABSTRACT

To eliminate the random error of the derivative near-IR (NIR) spectrum and to improve model stability and the prediction accuracy of the gluten protein content, a combined method is proposed for pretreatment of the NIR spectrum based on both empirical mode decomposition and the wavelet soft-threshold method. The principle and the steps of the method are introduced and the denoising effect is evaluated. The wheat gluten protein content is calculated based on the denoised spectrum, and the results are compared with those of the nine-point smoothing method and the wavelet soft-threshold method. Experimental results show that the proposed combined method is effective in completing pretreatment of the NIR spectrum, and the proposed method improves the accuracy of detection of wheat gluten protein content from the NIR spectrum.

Establishment of a BALB/c Mouse Model for Assessing the Degree of Inebriation Induced by Luzhou-Flavor Liquors

Zhang, Meng-Yan

ABSTRACT

This investigation was carried out to determine the differences in the degree of inebriation induced by Luzhou-flavor liquors having the same ethanol content. A BALB/c mouse model was used to test the effects of two liquors on the loss of the righting reflex (LORR) and the duration (DUR) of the LORR, as indices of the degree of inebriation. The blood ethanol concentration, blood acetaldehyde concentration, acetylcholinesterase activity in the hippocampus, and concentrations of dopamine and serotonin in the striatum were also determined. The degrees of inebriation induced by the two liquors were 0.694 and 0.404, as quantified by LORR and LORR DUR. The liquor that induced the lower degree of inebriation also induced lower blood ethanol and blood acetaldehyde concentrations. Moreover, it had no significant effects on acetylcholinesterase activity in the hippocampus or on the concentrations of dopamine or serotonin in the striatum. Chinese liquors with the same ethanol content can be distinguished by the degree of inebriation they induce. A relationship was found between the internal composition of the liquor and the degree of inebriation it induced. Our data support choosing liquors with low degrees of inebriation to reduce their harmful effects.

pH-Gradient Liquid Chromatography: Fundamentals and Examples

Kubik, Łukasz; Wiczling, Paweł; Kaliszan, Roman

ABSTRACT

In this paper, we acquaint the readers with the fundamentals of gradient separation, followed by the latest innovations in this field. We describe the principles of organic modifier- and pH-gradient elution emphasizing the differences and similarities with isocratic separation. The double organic modifier-/pH-gradient is also thoroughly reviewed as a useful method for the simultaneous determination of logk w (substitute of logP) and the pk a of analytes present in complex mixtures.

Dynamic Headspace Sampling as an Initial Step for Sample Preparation in Chromatographic Analysis

Wojnowski, Wojciech1; Majchrzak, Tomasz1; Dymerski, Tomasz1; Gębicki, Jacek2; Namieśnik, Jacek1

ABSTRACT

This work represents a brief summary of the use of dynamic headspace (DHS) as a technique for sample preparation in chromatographic analysis. Despite numerous developments in the area of analyte isolation and enrichment, DHS remains one of the fundamental methods used with GC. In our opinion, interest in this technique will not diminish significantly because it conforms to stipulations of green analytical chemistry. Moreover, DHS fulfills the need for methods that facilitate detection and determination of analytes present at ultratrace levels in complex matrixes. The main focus of this work was placed on the theoretical fundamentals of this method. Also described herein were DHS development, the advantages and disadvantages of this technique compared with other headspace sampling techniques, and selected examples of its applications in food and environmental analyses.

Identification of Microorganisms by Modern Analytical Techniques

Buszewski, Bogusław; Rogowska, Agnieszka; Pomastowski, Paweł; Złoch, Michał; Railean-Plugaru, Viorica

ABSTRACT

Rapid detection and identification of microorganisms is a challenging and important aspect in a wide range of fields, from medical to industrial, affecting human lives. Unfortunately, classical methods of microorganism identification are based on time-consuming and labor-intensive approaches. Screening techniques require the rapid and cheap grouping of bacterial isolates; however, modern bioanalytics demand comprehensive bacterial studies at a molecular level. Modern approaches for the rapid identification of bacteria use molecular techniques, such as 16S ribosomal RNA gene sequencing based on polymerase chain reaction or electromigration, especially capillary zone electrophoresis and capillary isoelectric focusing. However, there are still several challenges with the analysis of microbial complexes using electromigration technology, such as uncontrolled aggregation and/or adhesion to the capillary surface. Thus, an approach using capillary electrophoresis of microbial aggregates with UV and matrix-assisted laser desorption ionization time-of-flight MS detection is presented.

Supercritical Fluid Extraction of Bioactive Compounds from Plant Materials

Wrona, Olga1; Rafińska, Katarzyna2; Możeński, Cezary3; Buszewski, Bogusław2

ABSTRACT

There has been growing interest in the application of supercritical solvents over the last several years, many of the applications industrial in nature. The purpose of plant material extraction is to obtain large amounts of extract rich in the desired active compounds in a time-sensitive and cost-effective manner. The productivity and profitability of a supercritical fluid extraction (SFE) process largely depends on the selection of process parameters, which are elaborated upon in this paper. Carbon dioxide (CO2) is the most desirable solvent for the supercritical extraction of natural products. Its near-ambient critical temperature makes it suitable for the extraction of thermolabile components without degradation. A new approach has been adopted for SFE in which the solubility of nonpolar supercritical CO2 can be enhanced by the addition of small amounts of cosolvent.

Retention Models on Core–Shell Columns

Jandera, Pavel; Hájek, Tomáš; Růžičková, Marie

ABSTRACT

A thin, active shell layer on core–shell columns provides high efficiency in HPLC at moderately high pressures. We revisited three models of mobile phase effects on retention for core–shell columns in mixed aqueous–organic mobile phases: linear solvent strength and Snyder–Soczewiński two-parameter models and a three-parameter model. For some compounds, two-parameter models show minor deviations from linearity due to neglect of possible minor retention in pure weak solvent, which is compensated for in the three-parameter model, which does not explicitly assume either the adsorption or the partition retention mechanism in normal- or reversed-phase systems. The model retention equation can be formulated as a function of solute retention factors of nonionic compounds in pure organic solvent and in pure water (or aqueous buffer) and of the volume fraction of an either aqueous or organic solvent component in a two-component mobile phase. With core–shell columns, the impervious solid core does not participate in the retention process. Hence, the thermodynamic retention factors, defined as the ratio of the mass of the analyte mass contained in the stationary phase to its mass in the mobile phase in the column, should not include the particle core volume. The values of the thermodynamic factors are lower than the retention factors determined using a convention including the inert core in the stationary phase. However, both conventions produce correct results if consistently used to predict the effects of changing mobile phase composition on retention. We compared three types of core–shell columns with C18-, phenyl-hexyl-, and biphenyl-bonded phases. The core–shell columns with phenyl-hexyl- and biphenyl-bonded ligands provided lower errors in two-parameter model predictions for alkylbenzenes, phenolic acids, and flavonoid compounds in comparison with C18-bonded ligands.

How High Pressure Unifies Solvation Processes in Liquid Chromatography

Bocian, Szymon1; Škrinjar, Tea2; Bolanca, Tomislav3; Buszewski, Bogusław1

ABSTRACT

A series of core–shell-based stationary phases of varying surface chemistry were subjected to solvent adsorption investigation under ultra-HPLC conditions. Acetonitrile and water excess isotherms were measured using a minor disturbance method. It was observed that adsorption of organic solvent is unified under high pressure. Preferential solvation due to specific interactions between the stationary phases and solvent molecules was limited. The obtained results showed that the solvation process is almost independent of surface chemistry, in contrast to HPLC conditions in which specific interactions differentiate solvation processes.

Application of Mobile Phases Containing Ionic Liquid for HPLC Analysis of Selected Isoquinoline Alkaloids

Petruczynik, Anna1; Misiurek, Justyna1; Tuzimski, Tomasz2; Waksmundzka-Hajnos, Monika

ABSTRACT

An HPLC procedure on a polar reversed-phase column with mobile phases containing ionic liquid (IL) was developed for the analysis of selected alkaloids from different chemical groups. We aimed to obtain optimal conditions for the separation of alkaloids because widely used silica-based stationary phases exhibit a silanol effect, rendering analysis of basic analytes extremely difficult. Retention, separation selectivity, peak symmetry, and system efficiency were examined in various eluent systems containing different concentrations of IL and acetonitrile. The obtained results revealed substantial influence from the concentrations of IL, the organic modifier, and temperature on the retention behavior of the investigated alkaloids. The most selective and efficient chromatographic systems were applied for the analysis of several alkaloids in a plant extract.

Evaluation of Gas Chromatography Stationary Phases Based on Morpholinium Ionic Liquids by McReynolds Constants and Activity Coefficients at Infinite Dilution

Yavir, Kateryna1; Marcinkowski, Łukasz2; Kloskowski, Adam2; Namieśnik, Jacek1

ABSTRACT

In this work, four ionic liquids (ILs) based on the N-alkyl-N-methylmorpholinium cation ([Mor1,R], in which R = 2, 4, 8, or 10) and bis(trifluoromethanesulfonyl)imide anion were synthesized. Using GLC, a number of parameters describing the sorption properties of the investigated ILs were determined. The values of Kovats indices, McReynolds constants, and activity coefficients at infinite dilution were the basis for the evaluation of intermolecular interactions. The effect of the chain length of the alkyl substituent in the cation, which was used to modify their polarity, has been discussed. Comparison of the characteristics of the investigated IL-based stationary phases with commercially available ones allowed for the statement that the investigated ILs were more polar. The tested ILs had a relatively high polarity. Increasing the length of the alkyl chain in the morpholinium ring reduced polarity. ILs based on the morpholinium cation were tunable in a wide range of their polarity.

A QuEChERS-Based Sample Preparation Method for the Analysis of 5-Nitroimidazoles in Bovine Milk by HPLC–DAD

Tuzimski, Tomasz; Rejczak, Tomasz

ABSTRACT

In this study, a simple, cost-effective, and sensitive HPLC diode-array detection method was developed for the simultaneous determination of six different 5-nitroimidazoles [metronidazole, 2-hydroxymethyl-1-methyl-5-nitro-1H-imidazole, dimetridazole (DMZ), ronidazole, ornidazole, and ipronidazole] in bovine milk samples. A QuEChERS-based sample preparation procedure was optimized by evaluating different cleanup sorbents, including zirconium-based sorbents (Z-Sep and Z-Sep+), C18, and primary–secondary amine (PSA), as well as EMR-Lipid cleanup solution. Acceptable analytical performance for all analytes was observed with recoveries in the range of 45–93% and RSDs of less than 15%. Negligible matrix interference was observed for most of the analytes due to application of PSA sorbent in a dispersive solid-phase extraction cleanup step. Method LOQs (mLOQs) for five of the six investigated analytes were set at a satisfactory low food product value of 2.5 ng/mL. For DMZ only, the mLOQ was set at 10 ng/mL. The procedure was evaluated through the analysis of 10 different natural samples.

Comparison of Various Extraction Techniques of Medicago sativa: Yield, Antioxidant Activity, and Content of Phytochemical Constituents

Krakowska, Aneta1; Rafińska, Katarzyna2; Walczak, Justyna1; Kowalkowski, Tomasz1; Buszewski, Bogusław2

ABSTRACT

Medicago sativa L. (M. sativa) is a source of many valuable secondary metabolites. Extraction yield and the concentration of phenolics, flavonoids, and saponins, as well as antioxidant potential were determined in extracts from different parts of M. sativa obtained using extraction methods such as maceration, ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE). The concentrations of the listed groups of compounds were spectrophotometrically determined and confirmed by HPLC-MS. The results showed that ASE of flowers with 70% ethanol (EtOH) provided the highest yield of extraction (47.5 ± 4.0%), whereas the lowest yield was obtained in stems (4.0 ± 0.2%). The 70% EtOH extract from flowers showed the highest phenolic content [48.4 ± 4.6 mg gallic acid equivalents/g dry matter (DM)], as well as the highest antioxidant activity. The highest total flavonoid content (139.0 ± 7.1 mg rutin equivalents/g DM) was observed in the extract from leaves obtained through SFE. This extract was also especially rich in saponins [622.2 ± 30.3 mg oleanolic acid equivalents (OAE)/g DM]. However, the lowest compound content was observed in maceration extracts from stems (54.6 ± 27.0 mg OAE/g DM). The results suggest that EtOH extracts from alfalfa flowers and SFE extracts from M. satvia leaves, especially, may serve as potential sources of natural antioxidants for nutraceuticals, food additives, and cosmetic ingredients.

Catechin Composition and Antioxidant Activity of Black Teas in Relation to Brewing Time

Koch, Wojciech1; Kukula-Koch, Wirginia2; Głowniak, Kazimierz3

ABSTRACT

Black tea infusions are one of the most popular beverages across the world. Their extract composition depends on several factors, brewing time being one of the most important determinants. The aim of the present study was to determine the catechin composition of different black tea infusions using a validated LC electrospray ionization time-of-flight MS method. Additionally, total phenolic content (TPC) and antioxidant activity of infusions were evaluated using Folin–Ciocalteu reagent and stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). An optimized LC-MS method enabled the precise identification of the studied catechins [epicatechin (EC), EC gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG)] and gallic acid (GA). The major catechin in all investigated teas was EGC (25.6 mg/100 cm3 after 4 min of brewing). EC was present at the lowest concentration in all extracts. TPC and antiradical scavenging activity were in a good agreement with catechins and GA content. In general, the longer the brewing time, the higher the concentration of catechin, TPC, and antioxidant activity values. However, it should be noted that after 2 min brewing, most phenolics had already been extracted, and extract composition did not significantly change at a prolonged extraction time.

Metabolic Profile of and Antimicrobial Activity in the Aerial Part of Leonurus turkestanicus V.I. Krecz. et Kuprian. from Kazakhstan

Sermukhamedova, Olga1; Wojtanowski, Krzysztof Kamil2; Widelski, Jarosław2; Korona-Głowniak, Izabela3; Elansary, Hosam O.4; Sakipova, Zuriyadda1; Malm, Anna5; Głowniak, Kazimierz6; Skalicka-Woźniak, Krystyna2

ABSTRACT

An HPLC quadrupole time-of-flight (Q-TOF) MS method was developed for the identification of secondary metabolites in Leonurus turkestanicus V.I. Krecz. et Kuprian. Ethanolic and chloroform extracts from the plant's aerial parts were tested. A total of 16 compounds (iridoid glycosides, phenylpropanoids, flavonoids, and nitrogen-containing compounds, as well as diterpene acetate derivatives) were identified and tentatively characterized based on (or using) their retention times and UV and Q-TOF-MS data. Previously reported aucubin (1), 6-deoxy-8-acetylharpagid (2) , and stachydrine (13, 15) and homostachydrine isomers (14, 16) were identified, along with a lavandulifolioside isomer (3), verbascoside (4), rutin (5), 3-O-kaempferol rutinoside (6), and an unknown diterpene acetate (8). Compounds 3–6 were detected for the first time in this plant. Additionally. antimicrobial activity was evaluated. No significant differences were found between ethanolic and chloroform extracts of L. turkestanicus; however, the alcoholic extract showed stronger antifungal activity [minimal inhibitory concentration (MIC) of 2.5–5 mg/mL], whereas the chloroform extract showed stronger activity against the tested spore-forming Bacillus species (MIC 1.25–2.5 mg/mL).

Characterization of Low-Molecular-Weight Heparins by Strong Anion-Exchange Chromatography

Sadowski, Radosław1; Gadzała-Kopciuch, Renata1; Kowalkowski, Tomasz1; Widomski, Paweł2; Jujeczka, Ludwik2; Buszewski, Bogusław1

ABSTRACT

Currently, detailed structural characterization of low-molecular-weight heparin (LMWH) products is an analytical subject of great interest. In this work, we carried out a comprehensive structural analysis of LMWHs and applied a modified pharmacopeial method, as well as methods developed by other researchers, to the analysis of novel biosimilar LMWH products; and, for the first time, compared the qualitative and quantitative composition of commercially available drugs (enoxaparin, nadroparin, and dalteparin). For this purpose, we used strong anion-exchange (SAX) chromatography with spectrophotometric detection because this method is more helpful, easier, and faster than other separation techniques for the detailed disaccharide analysis of new LMWH drugs. In addition, we subjected the obtained results to statistical analysis (factor analysis, t-test, and Newman–Keuls post hoc test).

Determination of Adsorption Equations for Chloro Derivatives of Aniline on Halloysite Adsorbents Using Inverse Liquid Chromatography

Słomkiewicz, Piotr M.1; Szczepanik, Beata1; Garnuszek, Magdalena1; Rogala, Paweł1; Witkiewicz, Zygfryd2

ABSTRACT

Chloro derivatives of aniline are commonly used in the production of dyes, pharmaceuticals, and agricultural agents. They are toxic compounds with a large accumulation ability and low natural biodegradability. Halloysite is known as an efficient adsorbent of toxic compounds, such as phenols or herbicides, from wastewater. Inverse LC was applied to measure the adsorption of aniline and 2-chloroaniline (2-CA), 3-chloroaniline (3-CA), and 4-chloroaniline (4-CA) on halloysite adsorbents. A peak division (PD) method was used to determine a Langmuir equation in accordance with the adsorption measurement results. The values of adsorption equilibrium constants and enthalpy were determined and compared by breakthrough curve and PD methods. The physical sense of the calculated adsorption enthalpy values was checked by applying Boudart's entropy criteria. Of note, adsorption enthalpy values for halloysite adsorbents decreased in the following order: aniline > 4-CA > 2-CA > 3-CA.

Development and Validation of a Stability-Indicating HPLC Method for Imidapril and Its Degradation Products Using a Design of Experiment (DoE) Approach

Arumugam, Abiramasundari1; Joshi, Amita2; Vasu, Kamala K.3

ABSTRACT

The present work focused on the application of design of experiment (DoE) principles to the development and optimization of a stability-indicating method (SIM) for the drug imidapril hydrochloride and its degradation products (DPs). The resolution of peaks for the DPs and their drug in a SIM can be influenced by many factors. The factors studied here were pH, gradient time, organic modifier, flow rate, molar concentration of the buffer, and wavelength, with the aid of a Plackett–Burman design. Results from the Plackett–Burman study conspicuously showed influence of two factors, pH and gradient time, on the analyzed response, particularly, the resolution of the closely eluting DPs (DP-5 and DP-6) and the retention time of the last peak. Optimization of the multiresponse processes was achieved through Derringer's desirability function with the assistance of a full factorial design. Separation was achieved using a C18 Phenomenex Luna column (250 × 4.6 mm id, 5 µm particle size) at a flow rate of 0.8 mL/min at 210 nm. The optimized mobile phase composition was ammonium–acetate buffer (pH 5) in pump A and acetonitrile–methanol (in equal ratio) in pump B with a run time of 40 min using a gradient method.

Comparative Study Between Zero-Order Spectra-Processing and Ratio Spectra-Manipulating Methods Applied for the Determination of Isoxsuprine Hydrochloride in the Presence of Its Oxidative Degradation Product

Attia, Khalid A.M.; El-Abassawi, Nasr M.A.; Said, Ragab A.M.; El-Olemy, Ahmed; Ramzy, Sherif

ABSTRACT

Four accurate, precise, and validated stability-indicating spectrophotometric methods handling either zero-order spectra or ratio spectra have been developed and compared for the analysis of isoxsuprine hydrochloride (ISX) in the presence of its oxidative degradation product. The first two methods processed zero-order spectra, namely graphical absorbance ratio or Q-Analysis and area under the curve, whereas the third and fourth methods manipulated ratio spectra, namely the ratio difference spectrophotometric method and derivative ratio. The proposed methods showed good linearity in the range of 2–23 µg/mL. The methods were tested for specificity using laboratory-prepared mixtures containing the drug and its degradation product. The proposed methods were applied for the determination of ISX in Vascular tablets and the obtained results were acceptable, with small percentage RSD values. The validity of the proposed procedures was further assessed by applying the standard addition technique, which showed no interference from excipients. The obtained results were statistically compared with those obtained by the reported method, showing no significant differences when t- and F-tests were applied.

Pinaverium Bromide: Development and Validation of Spectrophotometric Methods for Assay and Dissolution Studies

Martins, Danielly da Fonte Carvalho; Florindo, Lorena Coimbra; Machado, Anna Karolina Mouzer da Silva; Todeschini, Vítor; Sangoi, Maximiliano da Silva

ABSTRACT

This study presents the development and validation of UV spectrophotometric methods for the determination of pinaverium bromide (PB) in tablet assay and dissolution studies. The methods were satisfactorily validated according to International Conference on Harmonization guidelines. The response was linear ($r2 > 0.99$) in the concentration ranges of 2–14 μg/mL at 213 nm and 10–70 μg/mL at 243 nm. The LOD and LOQ were 0.39 and 1.31 μg/mL, respectively, at 213 nm. For the 243 nm method, the LOD and LOQ were 2.93 and 9.77 μg/mL, respectively. Precision was evaluated by RSD, and the obtained results were lower than 2%. Adequate accuracy was also obtained. The methods proved to be robust using a full factorial design evaluation. For PB dissolution studies, the best conditions were achieved using a United States Pharmacopeia Dissolution Apparatus 2 (paddle) at 50 rpm and with 900 mL 0.1 M hydrochloric acid as the dissolution medium, presenting satisfactory results during the validation tests. In addition, the kinetic parameters of drug release were investigated using model-dependent methods, and the dissolution profiles were best described by the first-order model. Therefore, the proposed methods were successfully applied for the assay and dissolution analysis of PB in commercial tablets.

Design of a Sensitive and Selective Electrochemical Aptasensor for the Determination of the Complementary cDNA of miRNA-145 Based on the Intercalation and Electrochemical Reduction of Doxorubicin

Mohamadi, Maryam1; Mostafavi, Ali2; Torkzadeh-Mahani, Masoud3

ABSTRACT

The aim of this research was the determination of a microRNA (miRNA) using a DNA electrochemical aptasensor. In this biosensor, the complementary complementary DNA (cDNA) of miRNA-145 (a sense RNA transcript) was the target strand and the cDNA of miRNA-145 was the probe strand. Both cDNAs can be the product of the reverse transcriptase-polymerase chain reaction of miRNA. The proposed aptasensor's function was based on the hybridization of target strands with probes immobilized on the surface of a working electrode and the subsequent intercalation of doxorubicin (DOX) molecules functioning as the electroactive indicators of any double strands that formed. Electrochemical transduction was performed by measuring the cathodic current resulting from the electrochemical reduction of the intercalated molecules at the electrode surface. In the experiment, because many DOX molecules accumulated on each target strand on the electrode surface, amplification was inherently easy, without a need for enzymatic or complicated amplification strategies. The proposed aptasensor also had the excellent ability to regenerate as a result of the melting of the DNA duplex. Moreover, the use of DNA probe strands obviated the challenges of working with an RNA probe, such as sensitivity to RNase enzyme. In addition to the linear relationship between the electrochemical signal and the concentration of the target strands that ranged from 2.0 to 80.0 nM with an LOD of 0.27 nM, the proposed biosensor was clearly capable of distinguishing between complementary (target strand) and noncomplementary sequences. The presented biosensor was successfully applied for the quantification of DNA strands corresponding to miRNA-145 in human serum samples.

Development and Validation of Eco-Friendly Liquid Chromatographic and Spectrophotometric Methods for Simultaneous Determination of Coformulated Drugs: Phenylephrine Hydrochloride and Prednisolone Acetate

Mostafa, Nadia M.; Elsayed, Ghada M.; Hassan, Nagiba Y.; El Mously, Dina A.

ABSTRACT

Five simple, sensitive, and eco-friendly LC and UV spectrophotometric methods have been developed for the simultaneous determination of phenylephrine hydrochloride (PHE) and prednisolone acetate (PRD) in their combined dosage form. The first method was reversed-phase (RP) LC using methanol–water–heptane-1-sulfonic acid sodium salt (75 + 25 + 0.1, v/v/w) as a mobile phase. Separation was achieved using an XSelect HSS reversed-phase C18 analytical column (250 × 4.6 mm, 5µm). The flow rate was 1.0 mL/min and UV detection was done at 230 nm. Quantification was achieved over the concentration ranges of 5–50 µg/mL for PHE and 2–90 µg/mL for PRD. Four spectrophotometric methods were proposed, namely dual wavelength, first derivative of ratio spectra, ratio difference, and mean-centering of ratio spectra. Linearity was observed in the concentration ranges of 10–120 and 5–35 µg/mL for PHE and PRD, respectively, for the spectrophotometric methods. Green solvents were used in the proposed methods because they play a vital role in the analytical methods' influence on the environment. The suggested methods were validated regarding linearity, accuracy, and precision according to the International Conference on Harmonization guidelines, with satisfactory results. These methods could be used as harmless substitutes for routine analysis of the mentioned drugs, with no interference from excipients.

Development and Validation of a Rapid and Sensitive LC-MS/MS Method for the Pharmacokinetic Study of Osimertinib in Rats

Xiong, Shan1; Deng, Zhipeng1; Sun, Peilu1; Mu, Yanling1; Xue, Mingxing2

ABSTRACT

Osimertinib is a new-generation epidermal growth factor inhibitor for the treatment of non-small cell lung cancer. In the present study, a rapid and sensitive LC with tandem MS method was developed and validated for the determination of osimertinib in rat plasma. Chromatographic separation was carried out on a C18 column using acetonitrile and water containing 0.1% formic acid. The assay was validated over a concentration range of 1.0–1000 ng/mL for osimertinib, with a lower LOQ of 1.0 ng/mL. The intra- and interday accuracy values for osimertinib ranged from 92.66 to 101.50% and from 97.08 to 99.15%, respectively, and the intra- and interday precision values for osimertinib ranged from 6.25 to 10.34% and from 3.43 to 10.44%, respectively. The method was successfully applied in a pharmacokinetic study of osimertinib after oral administration of osimertinib (4.5 mg/kg) to rats.

Optimization of a Decoction Process for an Herbal Formula Using a Response Surface Methodology

Zhang, Xian-Fei1; Yang, Jun-Li2; Chen, Juan3; Shi, Yan-Ping2

ABSTRACT

An herbal formula, Huang-Qi-Liu-Yi Tang, is a prescription medicine that has been commonly used for the treatment of prostatitis and condyloma acuminatum over the last thousand years. In this study, response surface methodology, with a Box–Behnken design (BBD), was used to optimize the decoction conditions of an herbal formula. Astragaloside, calycosin-7-glucoside, glycyrrhizic acid, liquiritin, polysaccharide, and extractum were used as multiple evaluation markers. Soak time, water-to-medicinal herb ratio, and extraction time were determined as the three main variables by single-factor experiments and further optimized to obtain the maximum yields for the six marker compounds. Data from well-designed experiments were fitted to obtain second-order polynomial equations using multiple regression analysis, and the accuracies of these equations were evaluated and verified using statistical methods. By solving the regression equations and analyzing the three-dimensional response surfaces, optimum conditions were obtained and are summarized as follows: soak time of 57 min, water-to-medicinal herb ratio of 9:1 (mL/g), and extraction time of 48 min. Under optimized conditions, the experimental yields of astragaloside, calycosin-7-glucoside, glycyrrhizic acid, liquiritin, polysaccharide, and extractum were 0.13, 0.085, 1.64, 1.56, 72.19, and 25.64%, respectively, which was in good agreement with the values predicted by the BBD.

Determination of Sulfite in Food by Liquid Chromatography Tandem Mass Spectrometry: Collaborative Study

Carlos, Katherine S.; de Jager, Lowri S.

ABSTRACT

Sulfites are added to a wide range of food and beverage products to prevent browning or oxidation. Although most of the population do not experience side effects from consuming sulfites, a small subset has been shown to experience an “allergic-like” response. For this reason, the U.S. Food and Drug Administration requires that sulfites be labeled on all products that contain more than 10 mg/kg (parts per million) sulfur dioxide. The current regulatory method, optimized Monier–Williams (OMW) Official Method 990.28, has been successful in quantifying sulfites in most matrixes, but is time-consuming and has a method detection limit at the regulatory-labeling threshold. Recently, an LC-tandem MS (MS/MS) method was published that was applicable to a wide range of sulfite-containing matrixes. This method converts free and reversibly bound sulfite to the formaldehyde adduct hydroxymethylsulfonate, which could then be separated from matrix constituents using a hydrophilic interaction LC analytical column and subsequently be detected with tandem MS (MS/MS). In this study, multilaboratory validation was conducted with 11 laboratories in the United States and Canada. Four matrixes were spiked at varying concentrations and three additional commercially sulfited matrixes were included. An abbreviated comparison study between the LC-MS/MS and OMW methods was conducted for select samples. Average recoveries for all matrixes ranged from 86 to 114% with RSDr and RSDR values of 4.5–17.5 and 8.6–22.5%, respectively. Further comparisons will be necessary to draw comparisons between the two methods. This method proved to be a faster and more sensitive way to determine sulfites in food and beverages, showing promise for the continuing improvement of enforcement of sulfite labeling requirements to protect individuals who have sulfite sensitivity.

Validation of Spectrophotometric Methods for the Determination of Total Polyphenol and Total Flavonoid Content

Matić, Petra; Sabljić, Marija; Jakobek, Lidija

ABSTRACT

The aim of this study was to validate spectrophotometric methods for the measurement of total polyphenol (TP; via the Folin–Ciocalteu method) and total flavonoid (TF) content [via the aluminum chloride ($AlCl_3$) method]. Validation parameters of these methods were determined, including linearity, sensitivity, precision (intra-assay and intermediate), accuracy, LOD, and LOQ. For the validation process, groups of polyphenol standards were used, including phenolic acids (gallic, p-coumaric, caffeic, and chlorogenic acids), flavan-3-ols [(+)-catechin and procyanidins B1 and B2], flavonols (quercetin and quercetin-3-rutinoside), and dihydrochalcones (phloretin and phloretin-2-glucoside). Obtained validation parameters were within acceptable ranges with high determination coefficients, reasonably low LODs and LOQs, and high slopes in the calibration curves for both methods, except for phloretin and phloretin-2-glucoside, for which there were low slopes in the calibration curves for the $AlCl_3$ method. To evaluate differences in polyphenol content, the validated spectrophotometric methods were used to determine TP and TF content in wines (Plavac, Graševina, and Vranac) and juices (blueberry, strawberry, and blackcurrant juice) according to the polyphenol calibration curves. Polyphenol contents were different for both methods in all wines and juices.

A Multivariate Analysis of the Composition and Properties of Extra Virgin Olive Oils Produced from Different Cultivars Grown in Iran

Shirzad, Habib1; Niknam, Vahid1; Taheri, Mehdi2; Ebrahimzadeh, Hassan3

ABSTRACT

The present study investigated variations in extra virgin olive oils in relation to fatty acid (FA) composition and the characteristics of 10 olive cultivars. The findings demonstrated that their oil yield properties, including refractive index, acid value, peroxide value, saponification value, iodine value, and composition, were significantly different. Moreover, based on GC-MS analysis, the presence of oleic acid [C18:1(9)] was identified as one of the major components. The highest amount of 18:1(9) was found in four major varieties of cultivars, namely Zard, Roghani, Karidolia, and Korfolia. Hierarchical cluster analysis of principal component analysis revealed two distinct categories of cultivars based on their FAs. The first category (cluster I), consisted of Arbequina, Karydolia, Roghani, and Zard cultivars, which can be considered cultivars with good commercial cultivation potential due to their high contents of unsaturated FAs and oil quantities produced.

Validation of a Method for the Determination of Balenine/Ophidine in Whale

Valdersnes, Stig; Birkenes, Anita; Froyland, Livar

ABSTRACT

The aim of this study was to develop and validate a method for the determination of balenine/ophidine (hereafter referred to as "balenine") in whale extracts and muscle samples from Balaenoptera acutorostrata. Further, the goal was to evaluate the method's applicability for the determination of other histidine-containing dipeptides (HCDs): anserine and carnosine and their amino acids π-methylhistidine, τ-methylhistidine, histidine, and β-alanine. For balenine, the LOD and LOQ were found to be 0.03 and 0.1 mg/g, respectively, and the linear range was validated up to 160 mg/g. Trueness was evaluated by spiking experiments with balenine, and the recovery was found to be 88–90%. A comparison of the results showed that most of the other analytes were within 80–120% of the value found with the previously developed and validated method. Precision and internal reproducibility for balenine was around 0.9 and 2%, respectively, with measurement uncertainties of 2–4%. Therefore, the method was found to be fit for purpose for the determination of balenine and other HCDs and their constituent amino acids in whale meat and extracts.

Interlaboratory Comparison Test as an Evaluation of Applicability of an Alternative Edible Oil Analysis by 1H NMR Spectroscopy

Zailer, Elina1; Holzgrabe, Ulrike2; Diehl, Bernd W.K.3

ABSTRACT

A proton (1H) NMR spectroscopic method was established for the quality assessment of vegetable oils. To date, several research studies have been published demonstrating the high potential of the NMR technique in lipid analysis. An interlaboratory comparison was organized with the following main objectives: (1) to evaluate an alternative analysis of edible oils by using 1H NMR spectroscopy; and (2) to determine the robustness and reproducibility of the method. Five different edible oil samples were analyzed by evaluating 15 signals (free fatty acids, peroxides, aldehydes, double bonds, and linoleic and linolenic acids) in each spectrum. A total of 21 NMR data sets were obtained from 17 international participant laboratories. The performance of each laboratory was assessed by their z-scores. The test was successfully passed by 90.5% of the participants. Results showed that NMR spectroscopy is a robust alternative method for edible oil analysis.

Determination of Nitrite in Milk- and Soy-Based Nutritional Ingredients by Derivatization with 2, 3-Diaminonaphthalene and Fluorescence Spectrometry

Ehling, Stefan; Reddy, Todime M.

ABSTRACT

Nitrite (NO_2^-) is an inorganic anion that can be found in various powdered milk- and soy-based nutritional ingredients as an incidental contaminant. Reliable determination of NO_2^- in nutritional ingredients is of paramount importance to ensure the safety of finished products. The derivatization reaction of NO_2^- with 2,3-diaminonaphthalene with the formation of fluorescent 2,3-naphtotriazole has been adapted to milk- and soy-based nutritional ingredients. The sample preparation consisted of protein precipitation with Carrez solution, simple pass-through cleanup of extracts utilizing a carbon black-based cartridge and derivatization, followed by batch fluorometry. The method was validated in six representative ingredient matrixes—i.e., whole-milk powder, nonfat dry milk, milk protein concentrate, whey protein concentrate, sodium caseinate, and soy protein isolate. Recovery values were 82–109%, whereas within-day and intermediate precision were 0.6–5.2 and 3.6–11% (RSDs), respectively. The method LOQ was 0.1 or 0.2 μg/g sodium nitrite ($NaNO_2$), depending on the ingredient matrix. Surveyed NO_2^- concentration levels in 25 lots of 10 types of nutritional ingredients ranged from between less than 0.1 to 29 μg/g $NaNO_2$. This method is proposed as a more sensitive and rugged alternative to the widely used ion chromatographic and colorimetric approaches.

Simple and Rapid Dual-Dispersive Liquid–Liquid Microextraction as an Innovative Extraction Method for Uranium in Real Water Samples Prior to the Determination of Uranium by a Spectrophotometric Technique

Khan, Naeemullah1; Tuzen, Mustafa2; Kazi, Tasneem Gul3

ABSTRACT

An innovative, rapid, and simple dual-dispersive liquid–liquid microextraction (DDLL-ME) approach was used to extract uranium from real samples for the first time. The main objective of this study was to disperse extraction solvent by using an air-agitated syringe system to overcome matrix effects and avoid dispersion of hazardous dispersive organic solvents by using heat. The DDLL-ME method consisted of two dispersive liquid–liquid extraction steps with chloroform as the extracting solvent. Uranium formed complexes with 4-(2-thiazolylazo) resorcinol in the aqueous phase and was extracted in extracting solvent (chloroform) after the first dispersive liquid–liquid process. Uranium was then back-extracted in the acidic aqueous phase in a second dispersive liquid–liquid process. Finally, uranium was determined by a spectrophotometric detection technique. The variables that played a key role in the proposed method were studied and optimized. The LOD and sensitivity enhancement factor for uranium were found to be 0.60 μg/L and 45, respectively, under optimized conditions. Calibration graphs were found to be linear in the range of 5.0–600 μg/L. The RSD was 2.5%. Reliability of the proposed method was verified by analyzing certified reference material TM-28.3.

A New Turn-On Fluorometric Detection Method for the Determination of Ag(I) in Some Food and Water Samples

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ABSTRACT

A new sensitive and selective turn-on fluorometry procedure for the determination of silver using a laboratory-built fluorometry system is described herein. After synthesis and characterization, a [2-((E)-{[3′,6′-bis(ethylamino)-2′,7′-dimethyl-3-oxospiro[isoindole-1,9′-xanthen]-2(3H)-yl]imino}methyl) cyclopenta-2,4-dien-1-yl] (cyclopenta-2,4-dien-1-yl)iron (DKMS) fluorescent reagent was used for the first time. Ag(I) was complexed with the new fluorescent reagent, and direct measurements were made using fluorometry without any separation/preconcentration. The fluorescence intensity of the Ag(I)–DKMS complex remained unchanged for over 18 h at room temperature and was a linear function of the concentration of Ag(I) in the 0–2.5 mg/L range. The optimum determination conditions were established by testing different reagent quantities, the acidity and pH of the sample, and the effect of temperature and interfering ions. The LOD of the method was 1.00 μg/L whereas the RSD was 0.1% for 0.1 mg/L Ag(I) concentration. "The developed method was applied successfully for the Ag(I) determination of silver in burn cream, anode slime, some food and water samples". Results of the analysis of Certified Reference Materials (NCS DC73349 Bush Branches and Leaves – Trace Elements, and CWW-TM-D Certified Wastewater and Trace Metals) are in good agreement with the certified values.

Simultaneous Determination of 11 Aminoglycoside Residues in Honey, Milk, and Pork by Liquid Chromatography with Tandem Mass Spectrometry and Molecularly Imprinted Polymer Solid Phase Extraction

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ABSTRACT

An analytical method was developed for the simultaneous determination of 11 aminoglycoside (AG) antibiotics, including amikacin, paromomycin, dihydrostreptomycin, gentamicin C1a, hygromycin, kanamycin, netilmicin, spectinomycin, sisomicin, streptomycin, and tobramycin in honey, milk, and pork samples by LC with tandem MS and molecularly imprinted polymer (MIP) SPE. The AG antibiotics in milk and homogenated meat samples were extracted with a solution composed of 10 mmol/L potassium dihydrogen phosphate, 0.4 mmol/L EDTA-Na2, and 2% trichloroacetic acid. For honey samples, the extractant was 50 mmol/L potassium dihydrogen phosphate. The extracts were cleaned up with MIP SPE cartridges. The separation was performed on a zwitter ionic-HILIC column (50 × 2.1 mm, 3.5 μm), with the mobile phase consisting of methanol, 0.3% formic acid, and 175 mmol/L ammonium formate at 0.50 mL/min in gradient elution. A triple-quadrupole mass spectrometer equipped with an electrospray ionization source, which was operated in positive mode, was used for detection. The quantification was based on matrix-matched calibration curves. The method was applied to real samples with three different matrixes. The LODs of the method were 2–30 μg/kg and the LOQs were 7–100 μg/kg; the average recovery ranged from 78.2 to 94.8%; intraday RSDs and interday RSDs were ≤15 and ≤18%, respectively; and the absolute values of matrix effect for all AGs were RSDs ≤23%.

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