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MFDS's national laboratory system of food and nutrient: Focus on quality control

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The Ministry of Food and Drug Safety (MFDS)'s National Laboratory System (NLS) is originated to establish reliable national database of nutrients on actually consumed food in Korea. The fundamental purpose of the NLS is securing database for promoting national nutrition policies such as reducing sodium intake, expansion of nutrient labeling in restaurant foods and improvement of national health care. (MFDS)'s nutrient database contains data for about 1,600 foods for 53-79 compounds including vitamins, minerals, fatty acids, amino acids and sugars since 2009. The overall system of the NLS to produce data is as follows. First, target foods are selected among the National Health and Nutrition Examination Survey. Second, the selected food is collected according to the standardized principle. Third, for the food the analytical institution carries out quality control to ensure the reliability of the data. At here describes more about data quality control. Quality control includes: (1) Participation in FAPAS that international quality management programs, (2) analysis of CRMs (Certified Reference Materials) and (3) in house control analysis. The strength of the NLS was a rigorous quality control program that enabled independent verification of the accuracy and precision of analytical results. Data for materials were used to monitor analytical work, to identify end result suspected analytical problems, to ensure the accuracy and precision of results for the food samples.

Biography

Soo-Jung Hu has completed her PhD majored in Analytical Chemistry from Sungshin Women's University. She is a Senior Scientific Officer in the Ministry of Food and Drug Safety (MFDS) since 1997. Currently, she is responsible for functional health food and nutrition related work in MFDS.

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A study on the influence of water on the L-isoleucine fragmentation

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Low-energy electrons are produced due to the interaction of X/gamma rays with biomolecules. These low-energy electrons Locause damage of molecules, including amino acids, e.g. isoleucine. L-isoleucine is important for blood sugar regulation, muscle development and repair. Nevertheless, information on L-isoleucine fragmentation of amino acids in water are scarce, although they are relevant to the analysis of processes in organisms. Our research is performed aiming to determine the influence of water to the process of fragmentation of isoleucine. We used density functional theory B3LYP method and ccpVTZ basis to evaluate the energy of appearance of the positively charged fragments. Polarizable continuum model is used to evaluate the presence of water. We applied Gaussian 03 Rev D.01 program. Cations for study were selected on the basis of the experimental data of mass spectrometry published in the NIST database. The most intensive peaks in the mass spectrum of L-isoleucine correspond to m=86 a.m.u., 75 a.m.u. and 30 a.m.u. We determined that fragment with mass 86 a.m.u. is $C_5H_{12}N+$, m=75 a.m.u. $C_2H_5NO_2+$, m=30 a.m.u. CH_4N+ . Energy of appearance of $C_5H_{12}N+$ is equal to 8.85 eV, of $C_2H_5NO_2+$ -9.48 eV, of CH_4N+ - 10.01 eV in vacuum, while in water- of $C_5H_{12}N+$ - 9.76 eV, of $C_2H_5NO_2+$ - 10.32 eV, of CH_4N+ - 10.86 eV. In conclusion, more energy is needed for the formation of identical fragments in the water.

Biography

Laura Baliulyte is a PhD student in the Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania. Her field of research includes theoretical modeling of amino acids fragmentation, biochemical reactions and biophysical processes.

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MASS SPECTROMETRY AND ANALYTICAL TECHNIQUES September 19-20, 2018 Singapore

Study of the microchip device's geometry based on the distribution constant of the analytes

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۲ The chip geometry is one of the most important parameters to optimize since the length, width and depth will determine L the efficiency of the extraction. On the other hand, the efficiency of the liquid membrane extraction depends on several different parameters. These include flow rate, dimensions of the channels of the membrane holder, the membrane porosity and thickness, chemical composition of the phases, kinetic and thermodynamic properties. Basically, two main sets of conditions for SLM extraction can be distinguished. With donor-controlled conditions the rate of extraction is controlled by the mass transfer in the donor phase. This is the case when the distribution coefficient K₄ between the organic membrane phase and the donor phase is relatively large (K_d >10) for the analyte molecules. Diffusion coefficients in the phases also play a role here when liquid phase micro extraction is the technique used for the extraction. With donor-controlled conditions, the extraction efficiency should increase with the flow rate of the donor buffer. Also, if $K_{\rm d}$ is small, or the mass transfer in the membrane is unusually low, the mass transfer in the membrane phase controls the rate of extraction (membrane-controlled conditions). In that case, the donor flow rate per seconds is not important and the extraction efficiency is determined by the total extraction time. Then, the dimensions of the channel have a large influence on the recovery. The depth of the donor channel should be as low as possible so that a large part of the sample is in contact with the membrane. This is particularly important when the mass transfer in the donor phase is the limiting factor (that is, K_{4} is high). On the other hand, a very shallow channel (<0.1 mm) is hard to machine and can cause stoppage, especially with viscous samples such as plasma. To achieve a high recovery, a large exposed membrane area is preferable, but a too wide channel can cause bulging of the membrane, while the length is limited by the back pressure arising in the channel. The exposed membrane area is also determined by the maximum acceptable volume and possible depth of the acceptor channel. Based on this discussion, we propose the most suitable channels dimension for a microchip device based on the distribution constant of each analyte and the time required to be extracted through the support liquid membrane by passive diffusion.

Biography

Maria Ramos Payan has pursued her PhD from University of Seville, Spain and Postdoctoral studies from University of Copenhagen, Denmark, University of North Carolina, USA and Microelectronic National Center of Barcelona, Spain. She is the Leader of the microfluidic research line. She has published more than 30 papers in reputed journals and has been serving as an Editorial Board Member of repute.

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MASS SPECTROMETRY AND ANALYTICAL TECHNIQUES September 19-20, 2018 Singapore

Development, optimization and validation of a method for the determination of veterinary drugs from different therapeutic classes in animal urine by high resolution liquid chromatography

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A short-time, precise and robust HPLC separation procedure has been developed and optimized for the determination of a series of drugs of different therapeutic classes: chlortetracycline, oxitetracycline, cefoperazone, diclofenac, tiamphenicol, marbofloxacin, ciprofloxacin, danofloxacin, enrofloxacin and flumequine. The chromatographic method used a monolithic C_{18} column and both diode array and fluorescence detection. This procedure was validated for the analysis of drugs in cow urine, using a simple and fast extraction procedure with methanol/acetonitrile, allowing the simultaneous and efficient extraction of most of the studied drugs. The proposed method was successfully applied to the determination of enrofloxacin (and its metabolite ciprofloxacin) in cow urine, collected after the administration of this antibiotic. A robust and simple HPLC method has been developed to the separation of 11 veterinary drugs of different therapeutic classes. The employed monolithic C_{18} column (with high porosity and permeability) allowed the application of high volume flows with resulting tolerable precision flow levels and achieved an efficient separation within a short time (12 minutes). The DAD and FLD systems allowed the quantification of the studied drugs with low LOD and LQD and good repeatability. Also, a simple and fast extraction procedure has been developed and applied to the determination of drugs in cow urine. The procedure allowed the extraction of most of the studied drugs, achieving high recoveries with low sample volume consumption. The procedure was applied to the analysis of cow urine after an enrofloxacin treatment allowing its quantification.

Biography

Maria Ramos Payan has pursued her PhD from University of Seville, Spain and Postdoctoral studies from University of Copenhagen, Denmark, University of North Carolina, USA and Microelectronic National Center of Barcelona, Spain. She is the Leader of the microfluidic research line. She has published more than 30 papers in reputed journals and has been serving as an Editorial Board Member of repute.

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Correlation of serum ferritin level with left ventricular function in β -thalassemia major patients with increased transfusion dependence

Amna Imtiaz

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Aim of study was to correlate serum ferritin with left ventricular function in beta thalassemia major patients with increased transfusion dependence and to find out whether echocardiography can be used to assess pre-clinical cardiac disease in these patients. 60 patients of beta thalassemia major with increased transfusion dependence were enrolled in this study. Serum ferritin levels of all patients were measured by using indirect Enzyme Linked Immunosorbent Assay (ELISA). Echocardiography was performed on all patients by a consultant cardiologist by linking conventional echocardiography with tissue Doppler imaging. Based on serum ferritin level, patients were divided in to three groups. Group-1 consisted of patients having serum ferritin level equal to or less than 2500 ng/ml. A total of 25 patients were placed in this group. Group-2 included patients having serum ferritin level between 2500 to 5000 ng/ml. A total of 22 patients were placed in this group. Group-3 included patients having serum ferritin level more than 5000 ng/ml. This group consisted of 13 patients. All patients having serum ferritin below 2500 ng/ml had normal systolic function and only 16% of the patients in this group had diastolic dysfunction as reflected by abnormal E/A ratio. In group-2, 27% of the patients had systolic dysfunction reflected by subnormal ejection fraction while 40% of the patients had diastolic dysfunction. In group-3, 62% of the patients had abnormal systolic and diastolic function. Pearson correlation was used to find correlation between serum ferritin and left ventricular function. A strong negative correlation was found which is reflected by a p value of less than 0.05 which is significant. Chi square test is used to correlate serum ferritin with E/A ratio. P value came out to be less than 0.05 which is significant.

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Quantitative assay of metal impurities and pharmaceutical quality evaluation of multisource Levofloxacin tablets registered in Nigeria

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evofloxacin is a broad-spectrum antibiotic of the class fluoroquinolone. There are many generics of levofloxacin tablets vavailable within the drug delivery system globally. This has placed health practitioners in dilemma of generic substitution. The serious toxic effect of arsenic and mercury has made the quantification in dosage forms necessary today. The post market surveillance studied on 15 brands of levofloxacin 500 mg tablets registered in Nigeria was aimed at assessment of the physicochemical quality of the brands and determination of the quantity of selected metal impurities. To determine the brands that could be substituted for the innovator brand using similarity factor. The concentration of selected metal (Hg and As) impurities in all the brands of levofloxacin 500 mg (LEV01 to LEV15) tablets were carried out using ICP-OES. The physicochemical parameters of evaluated using both official (USP) and unofficial standards including uniformity of weight test, hardness test, friability test, disintegration test, dissolution profiles and quantitative assay was carried out using high performance liquid chromatography. The concentration of mercury and arsenic in all the brands analyzed is below the Permissible Daily Exposure (PDE). The results showed that all the brands passed the physical tests except Lev 11, Lev 10 and Lev 01, 02, 09, 11 that failed the uniformity of weight test, disintegration test and hardness test respectively. Lev 09 failed the quantitative assay test. Only Lev 3, 6, 7, 8, 11, 13 and 15 can be substituted for the innovator brand Lev 01. All the brands manufactured in Nigeria (Lev 6, 11, 13) passed the similarity factor, f., test and therefore could be substituted for the innovator brand. The study showed that the locally manufactured levofloxacin tablets could be substituted for the innovator brand. The post market surveillance study of medicinal agents is a source of very important information for health practitioners.

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Diagnostic accuracy of cannabinoid testing by liquid chromatography-tandem mass spectrometry in human hair

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Objective: To determine the diagnostic accuracy of cannabinoids testing by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS) in human hair and to compare it with urine for detection of cannabis use in civil heavy vehicle drivers.

Methodology & Theoretical Orientation: This diagnostic accuracy (validation) study was carried out at Department of Forensic Medical Sciences Laboratory (FMSL), Forensic Toxicology Section, Armed Forces Institute of Pathology Rawalpindi, Pakistan. Hair and urine samples of about 151 civil heavy vehicle drivers were collected from various urban and rural areas of Punjab. Sampling technique was non-probability convenient. About 10 ml of urine volume was collected and stored at -20 °C. Hair strands about the thickness of a pencil shaft, were collected from the posterior vertex of scalp. It was cut as close to the root as possible and kept at room temperature till further analysis separation of compounds was done on agilent poroshell 120 EC-C18 column (2.1×7.5 mm, 7 micron) and analyzed on a 6460 triple quadrapole LC-MS along with software mass hunter.

Result: All the 151 male civil heavy vehicle drivers, who were included in the study, were categorized into three main groups. There were 69 (71.5%) truck drivers, 43 (28.5%) were 20-wheeler drivers, whereas bus drivers were 39 (25.8%). Mean age was 36 ± 10.82 years. Subjects were stratified according to the age into four main groups: 20-25 years: 28 (18.5%); 26-40 years: 73 (48.3%), 41-60 years: 47 (31.1%) and >60 years: 3 (2%). Paired t test was applied to check significance of study at 95% confidence interval which was significant at p<0.05 (p=0.00). Various parameters of diagnostic accuracy in hair and urine samples were sensitivity (97% and 77%), specificity (92% and 93%), positive likelihood ratio (13% and 12%), negative likelihood ratio (0.04% and 0.24%), positive predictive value (89% and 83%) and negative predictive value (98% and 91%) respectively. Overall diagnostic accuracy of cannabinoids in hair was 94.04% while in urine it was 88.67%. Receiving Operating Characteristics (ROC) curve was plotted which showed area under curve of 0.967 and 0.793 for hair and urine respectively, therefore signifying a better diagnostic accuracy of hair as compared to urine for cannabis detection.

Conclusion & Significance: This study highlights the importance of hair as an alternative biological matrix due to its good diagnostic yield, easy non-invasive specimen collection and distinctive potential of analyte stability, as well as wider period of detection as compared to urine.

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Analytical characteristics of nanoelectrospray operated under super-atmospheric pressure

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High-pressure nanoelectrospray ionization (nanoESI) source is a recently developed technique in which the electrospray ionization is generated inside an enclosed chamber with gas pressure higher than the atmospheric pressure. In this paper, the performance of nanoESI under different gas pressures, emitter position, ion inlet temperature, additive for desalination are presented. Under a pressure of 2 bars, the nanoESI is almost eased from the electrical discharge problem and that offers a wider tuning window for the emitter potential to produces a higher and more stable ion signal. With optimized ion inlet temperature, the high-pressure operation facilitates the generation of ion species of higher charge-state from the highly aqueous solution and produced less sodium adducts. A preparation method for the high-throughput analysis of raw biological samples using disposable plastic nanoESI emitter could be described.

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Analysis of catechins in some samples of Indian brand tea by high performance liquid chromatography

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The beneficial effects of tea (*Camellia sinensis*) leaves have already been studied in detail. However, the researchers are quite interested to explore the exact scientific reasons for these benefits. The biochemical analysis of tea samples has indicated that they contain mostly catechins besides other phenolic acids. Catechins are present in food and beverages of plant origin. Keeping this in mind, high performance liquid chromatographic analysis was performed to estimate catechins in 14 samples of tea in different kind of extraction, 7 types of Indian market brand such as Janhit Green Tea[™] (JGT), Tata Tea premium[™] (TT), Brooke Bond Taaza[™] (BBTZ), Lipton Darjeeling Tea[™] (LDT), Tea City[™] (TCT), Taj Mahal Tea[™] (TAJM), Tetley Tea[™] (TET).

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Recent advances in tandem mass spectrometry, rational application workflows for research and

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development of pharmaceuticals and understanding future needs

Mass Spectrometry (MS) is an analytical technique/tool can identify and quantify verity of organic, inorganic and biological compounds. In pharmaceutical research and development mass spectrometry plays a key role from initial to late development phases for all type of drug molecules; small organic molecules, peptides, monoclonal antibodies (mAbs), Antibody Drug Conjugates (ADC) and Biosimilar (BS). Use of advanced mass spectrometry instruments is continuously increasing in analytical research laboratories, especially tandem mass spectrometry with high resolution mass analyzers, ion mobility and advancement in fragmentation techniques. These recent advances features along with rational workflows allow researchers for in-depth research with minimum experiments. Use of rationally designed workflows further helps to increase the productivity and quality of the research data. So the present study focused on the recent advances, rational analysis workflows and for utilization of technological advancement to fulfill research needs for various research arenas. Recent advances covered during study are; Ion Mobility (IM) feature used to drive the Collision Cross Section (CCS) of molecules under pre-set gas and temperature conditions, low and high energy dissociations, some other rational lab workflows with case studies using wide verity of drug molecules. For better understanding of workflows and latest technological advancement some of basic interpretation rules and other rational interpretation tools also discussed during the study. Discussed recent advances, rational workflow and case studies will surely guide researcher for better utilization of existing technology and to predict technology enhancement needed for future.

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September 19-20, 2018 Singapore

Determination of trace amounts of copper in the wastewater samples of Sarcheshmeh Copper Mine using dispersive liquid-liquid micro extraction based on the solidification of floating organic droplets prior to FAAS

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In this article, Dispersive Liquid-Liquid Micro Extraction based on the Solidification of Floating Organic drop (DLLME-SFO) was successfully used as a sample preparation method prior to flame atomic absorption determination of trace amount of copper in standard and wastewater samples. Several factors that may be affected on the extraction process, such as extraction and disperser solvent, the volume of extraction and disperser solvent, effect of salt, pH of the aqueous solution and extraction time were optimized. Under the best experimental conditions, the calibration curve was linear in the range of 0.8 ng mL⁻¹ - 0.5 μ g mL⁻¹ of copper and detection limit was 0.2 ng mL⁻¹ in the original solution (3 Sb/m). The relative standard deviation seven replicate determination of 0.1 μ g mL⁻¹ copper was ±1.9%. The high efficiency of DLLME-SFO to carry out the determination of copper in complex matrices was demonstrated. Finally, the proposed method has been applied for determination of trace amount of copper in standard and wastewater samples with satisfactory results.

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Analysis of organo-chlorine residues in River Benue at Makurdi using gas chromatography coupled with electron capture detector

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Organo-Chlorine Pesticides (OCPs) concentrations along the Benue River at NASME and at Benue Brewery were studied during both wet and dry seasons. A total of 12 water samples were collected and determinations were made. Gas Chromatography/Electron Capture Detector was used for OCPs analysis. In these analyses, aldrin, α -BHC, β -BHC, δ -BHC, chlorothalonil, dieldrin, endosulphan I, endosulphan II, endrin, endosulphan sulfate, heptachlor B, heptachlor epoxide, lambda cyhalothrin, lindane, permethrin, P'P-DDT, P,P' DDD were widely distributed at the various locations. α -BHC has the least average concentration of 0.00643 ppm while permethrin has the highest average concentration of 2.2506 ppm. The result portrayed by this research suggests some intolerable levels of pesticides concentrations at some locations.

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On-line pre-concentration for determination of glycosaminoglycans in cosmetic samples

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Capillary Zone Electrophoresis (CZE) was developed for separation and determination of Chondroitin Sulfate (CS), Dermatan Sulfate (DS) and Hyaluronic Acid (HA). Large-volume sample stacking using an EOF pump (LVSEP) was coupled with CZE for sensitivity improvement. A Background Electrolyte (BGE) for the separation consisted of 200 mM NaH₂PO₄, pH 4.0 and 200 mM butylamine. In addition, 0.5% w/v Polyethylene Glycol (PEG) was also added to the BGE to reduce sample adsorption on the capillary, thus improving precision of the system. The separation was performed using an applied voltage of -16 kV. For quantitative analysis of CS, DS and HA, the standard addition method was used to construct calibration curves, which were in the ranges of 100-300, 100-500 and 50-250 mg L⁻¹, respectively. Limits of detection and quantitation of the proposed method for determination of CS, DS and HA were 3.0, 5.0, 1.0 mg L⁻¹ and 10.0, 15.0 and 3.0 mg L⁻¹, respectively. In addition, intra-day (n=5) and inter-day (n=3) precisions were investigated and found that the percentages of relative standard deviation of the migration time and corrected peak area were about 0.4% and 5.9% (for intra-day) and 0.5% and 5.4% (for inter-day), respectively. The developed method was first applied for determination of HA in cosmetic samples and found that the recovery percentages of HA were in the range of 95.3-104.4%, indicating high accuracy of the method. Therefore, the developed method was reliable and simple without complicated sample preparation. Moreover, this method will be further applied for determination of the HA in human cerebrospinal fluid for checking and monitoring the HA levels in patients with brain injury diseases.

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Determination of organic acid preservatives in shrimp paste by headspace solid-phase microextraction combined with gas chromatography

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Organic acids, which are benzoic acid, sorbic acid and propionic acid, are the most common preservatives used in foods because of their low cost, high efficiency and having antimicrobial property. However, these preservatives at very high level can cause health problems. Hence, the analytical method for determination of benzoic acid, sorbic acid and propionic acid in food is necessary. In Thailand, shrimp paste (Kapi) is an integral ingredient in many Thai dishes because it gives a depth of flavor in curry pastes. Owing to complex matrix in shrimp paste, sample preparation is required. In this work, Headspace Solid-Phase Micro-Extraction (HS-SPME) which is a green method analysis was developed for extraction of organic acid preservatives in shrimp pastes and analyzed by Gas Chromatography with Flame Ionization Detector (GC-FID). The Polyacrylate coated fiber (PA) was used to extract the analytes from headspace and were desorbed by thermal desorption at 250°C in the GC injection port. The optimum condition for HS-SPME was extraction temperature of 60°C, extraction time of 30 min and the addition of 0.5 mol L⁻¹ sulfuric acid and 4% w/v of anhydrous sodium sulfate. Under the optimum condition, high accuracy and precision of the determination of three organic acid preservatives in shrimp paste were achieved.

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Steroid analysis in LCMS/MS era and the paradigm shift in clinical laboratories

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The accurate measurements of steroids have been a pivot to many clinical laboratories for better patient care in endocrinology, metabolic disorder, reproductive health and sports doping. Most commonly used immunoassays have their limitations in specificity and sensitivity, this has led to the popularity and need to develop steroid assays by LCMS/MS. Currently, many laboratories have adopted this technique and much more to follow the trend, but analyzing steroids by LCMS/MS has set of challenges making it very difficult. Our laboratory has developed various multi-analyte methods for steroid analysis casing its metabolism pathway in a single run without any derivatization and making use of the Atmospheric Pressure Photo-Ionization source (APPI) as part of our routine assays. Not only does the availability of MS-based steroid assays resolve the problems of direct steroid immunoassays, but multi-analyte profiling also ushers in a new era of snapshot profiling of the overlooked importance of steroid pathway fluxes that will broaden the interpretation of clinical studies beyond the standard restricted single analyte-by-analyte analysis. We have methods for serum, urine and dried blood spot detecting steroids of interest by LCMS/MS in human and mice samples which helps better understanding for research and clinical trials. It is time that clinical research and practice operates under equally high standards that have now become feasible. Ultimately, the need for the highest standards in clinical research and practice will drive us to this paradigm shift.

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Heavy metals contamination in highly consumed small indigenous fish species and possible human health risk implications in Bangladesh

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F ish is the most important single source of high-quality protein which contributes about 55% of the animal protein of Bangladeshi people. However, due to the rapid urbanization and unplanned industrialization, aquatic environments are being polluted with various types of chemical contaminants especially with heavy metals that are non-biodegradable and carcinogenic. Consequently, it is emergent to evaluate the level of contamination in fish species as they are one of the most important ingredients among different types of biota. Indigenous small fishes are supposed to be contained high quality protein and people usually take them with high rate. Therefore, the research was aimed to determine the toxic elements in highly consumed indigenous small fish with a good number of varieties with a view to assess the possible human health. The concentrations of five heavy metals (Pb, Cd, Cr, As and Hg) in 14 fish species collected from local markets of Dhaka city, Bangladesh were measured using Atomic Absorption Spectrometry (AAS) technique. The estimated values for Pb, Cd, Cr and Hg were found to be 0.284-1.554, 0.010-0.014, 0.055-0.431 and 0.027-0.252 mg/kg, respectively. However, the concentration of As in the investigated fish species was not detected (method quantification limit, 0.08 mg/kg). The Estimated Daily Intake (EDI) of heavy metals was calculated based on mean fish consumption of 49.5 g/person/day by Bangladeshi households and indicated that no risk to people's health due to consumption of indigenous small fish. Moreover, from the estimation of non-carcinogenic risk, the intake of individual heavy metals as well as combined heavy metals through the consumption of selected fish species were found safe for human health.

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Quantification of selected heavy metals in chewable part of Chata edulis from Gurage zone, Ethiopia

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Fresh chewable part of khat leaf samples were collected from three woredas of the zone namely 'Cheha', 'Ezha' and 'Ene'. All the collected samples were transported to Wolkite University chemistry laboratory using PE plastics. The sample was air dried and grinded to powder size. Then the powdered *Chata edulis* sample was digested using the optimized microwave digestion method. The concentration of five heavy metals (Mn, Zn, Pb, Cd and Cr) in the digested and diluted solutions of khat was analyzed with FAAS and GFAAS. Among those metals, Mn and Zn were the most abundant heavy metals. Moreover, the concentrations of toxic metals Cu, Pb and Cd in the studied *Chata edulis* leaves were too low to be detected. The order of metal mean concentration in mg/kg is Mn>Zn>Cu=Cr=Cd. Thus, the concentration of Mn (21.55 to 23.78 mg/kg), Zn (19.93 to 323.26 mg/kg), Cd, Cr and Pd were not detected. Finally, the overall results of this study implies that, there were no significant variations in the level of Mn between the *Chata edulis* samples, but there was significance difference between the khat samples of the three district for Zn metal. The variation may be attributed due to deferent factors such as age of the harvested khat, geographical and climatic variation, deference in physicochemical nature of the soil and deferent agricultural practices among khat cultivars.

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Recent advances in sample preparation methods

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Cample preparation is a crucial bottleneck in the whole analytical process. Moreover, samples such as environmental and Diological samples are complex in which the concentration of the target analytes is very low. Sample preparation methods have aroused increasing interest in research on analytical the bioanalytical techniques field, as they have the key roles in obtaining high clean-up and enrichment efficiency in the analysis of trace targets presented in the complex matrices. Recently, except the traditional sample preparation methods (i.e. SPE and LLE), more and more new sample preparation methods are applied prior to chromatography, spectrometry and electrochemistry analysis, for example, Dispersive Liquid-Liquid Micro Extraction (DLLME), Salting-out Assisted Liquid-Liquid Extraction (SALLE), cloud point extraction (CPE), etc. In DLLME, an appropriate mixture of extraction and disperser solvents is rapidly injected into an aqueous sample by a syringe. The fine particle of extracting solvent that is dispersed into aqueous phase forms a cloudy solution and allows its interaction with the analytes. The analytes are extracted from the sample to the fine droplets of extraction solvents. After centrifugation, phase separation is achieved and the enriched analytes is analyzed by some instrumental methods. SALLE is based on the formation of a biphasic system of mutual miscibility of two liquids by addition of additives, i.e. salts and sucrose. Considering the organic solvents as extraction and protein precipitation agents, SALLE is an excellent alternative used for biological sample. CPE is based primarily on the hydrophobic interaction between solutes and surfactants. It has become an alternative to conventional solvent extraction due to several possible advantages such as low cost, environmental safety, high capacity to concentrate a wide variety of analytes of widely varying nature with high recoveries and high enrichment folds.

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