Oral Presentations

1- **Protect Public Health Through Improving Test Methodology and Modernizing the FSIS ALP Program.** Food Safety and Inspection Service Laboratory Quality Assurance Staff (USDA). **Oliver Ou, Ph.D.**

2- **Method Development/Validation of the Direct Determination of Gyphosate, Glyfosinate, and AMPA in Food by LC/MS.** FDA Southeast Regional Laboratory. **Narong Chamkasem, Ph.D.**

3- **Method Development for Evaluation of Pesticides Residue in Drinking Water Using Disposable Pipette Extraction (DPX) and Gas Chromatography–Mass spectrometry (GC-MS).** School of Science and Technology, Georgia Gwinnett College. **Hongxia Guan, Ph.D.**

4- **Identification of Penicillin G Metabolites under Various Environmental Conditions using UHPLC-MS/MS.** Florida Department of Agriculture and Consumer Services. **Fadi Aldeek, Ph.D.**

5- **Comparison of Dumas and Kjeldahl Methods in Diverse Standard Reference Materials for the Accurate Determination of Protein Content.** FDA Southeast Regional Laboratory. **Eric Clevenger, Ph.D.**

6- **Update to the Changes in Chemical Residue Testing in the USDA/FSIS/OPHS Testing Program and Methods**. Food Safety and Inspection Service Laboratory Quality Assurance Staff (USDA). **Chilton Ng**

7- **Analysis of Proteins by Mass Spectrometry-Based Method,** Centers for Disease Control and Prevention. **John Barr, Ph.D.**

8- **Profiling of Medium-to-Long Chain Acyl-CoAs Converted from Dietary Fatty Acids in Mammalian Cells using LC-MS/MS,** Department of Pharmaceutical and Biomedical Sciences University of Georgia**. Xiangkun Yang**

9- **Detection of Trace Levels of Toxins in Drinking Water on X500R QTOF,** SCIEX. **Farzad Pakdel, Ph.D.**

10- **Toxicity of Hexahydriosohumulone in an Vitro Canine Model as an Example of Analytical Challenges and Opportunities in the Pet Food Industry**, Mars, **Denise Mitchell**

Poster Presentations

11- **Rapid Quantitation of Biotin in Infant Formula, Medical Foods, and Dietary Supplements by Isotope Dilution LC-MS/MS.** FDA Southeast Regional Laboratory. **Aubrey Shahriari, Ph.D.**

12- **A Validated LC-UV Method for the Analysis of Synthetic Thiamine Analogs, Benfotiamine and Sulbutiamine, in Biological Samples.** Department of Pharmaceutical and Biomedical Sciences University of Georgia. **Jaeah Kim**

13- **How Repeatable is your Laboratory's Sample Processing? An Estimation of Measurement Uncertainty Including Laboratory Sample Processing.** Florida Department of Agriculture and Consumer Services. **Amy Brown**

14- **Finding and Identifying Unsuspected Contaminants in Food.** Florida Department of Agriculture and Consumer Services. **Mark Crosswhite, Ph.D.**

15- **Direct Determination of Glyphosate, Glufosinate, and AMPA in honey by liquid chromatography/tandem mass spectrometer**. FDA Southeast Regional Laboratory. **Narong Chamkasem, Ph.D.**

Abstracts

Protect Public Health Through Improving Test Methodology and Modernizing the FSIS ALP Program.

Oliver Ou,* Jenny Scifres, Louis Bluhm, and Charles Pixley
US Department of Agriculture, Food Safety and Inspection Service, Laboratory Quality Assurance Staff, Athens, GA, USA
**LT, US Public Health Service*

Americans enjoy the safest food supply in the world. This is due in part to efforts of the Food Safety and Inspection Service (FSIS), the public health agency in the US Department of Agriculture. The FSIS's mission is to ensure that the nation's commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged. It follows a scientific approach in administering its food safety programs. Office of Public Health Science (OPHS) within the FSIS provides expert scientific analysis and advice to support these science-based food safety programs. Under OPHS there are three field service laboratories and laboratory quality assurance staff, who work together and conduct analysis to detect and identify potential hazards to public health.

The FSIS laboratory system constantly adopts modern and more efficient methods to ensure the timely testing of food products before they will be allowed into commerce, and to effectively monitor for the increasing number of chemical residues and to protect public health. Most recently, FSIS implemented a new multi-residue method (MRM) among other modern, high-efficiency methods. MRM produces reliable results and dramatically reduces cost, while enabling FSIS to analyze more chemical compounds per sample than was previously possible. As of today, FSIS' multi-residue method has the capability to analyze as many as 108 pesticides and 89 veterinary drugs in meat and poultry. An overview of this method's capabilities will be provided. FSIS also administers the Accredited Laboratory Program (ALP), which is authorized by the Code of Federal Regulations (CFR) Section 9, Parts 439 and 391. This program was originally designed to qualify non-federal analytical laboratories to conduct analyses of official meat and poultry samples. To keep this program current and relevant, ALP staff is redesigning and modernizing this program by emphasizing proficiency testing and allowing flexibility to test present-day food safety concerns. Currently, the ALP provides PT service to approximately 45 private laboratories and 15 state labs for Food Chemistry Analysis (Moisture, Protein, Fat and Salt) and Pesticide Residue Analysis (Chlorinated Hydrocarbons (CHC) and Polychlorinated Biphenyls (PCB)). Just recently, after two years of intense preparation, the ALP gained accreditation as a proficiency testing (PT) provider under the ISO/IEC 17043 international standard. The ALP is one of very few accredited PT providers in the federal government, perhaps the only one for food chemistry and chemical residues in meat and poultry products. This presentation will provide background information about the ALP, discuss improvements made, and touch upon future goals.

Method Development/Validation of the Direct Determination of Glyphosate, Glyfosinate, and AMPA in Food by LC/MS.

Narong Chamkasem
Southeast Regional Laboratory, U.S. Food and Drug Administration, Atlanta, GA

Glyphosate and glufosinate are non-selective post emergence herbicides used for the control of a broad spectrum of grasses and broad-leaf weed species in agricultural and industrial fields is the most used pesticide in the world. AMPA is the major metabolite of glyphosate and also classified as a toxicologically significant compound. The use of glyphosate in agriculture has increased significantly with the introduction of transgenic crops such as Roundup-Ready® soybeans and corn, which enable farmers to directly apply low cost broad spectrum herbicide products to their fields without harming crops. Glyphosate analysis in environmental and biological matrices is problematic because it is highly soluble in water, thereby making its extraction with solvents difficult and matrix effects highly prevalent. As a result, glyphosate isolation and quantification has posed a challenge to the analytical chemist. A simple high-throughput liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed for the determination of glyphosate, glufosinate and aminomethylphosphonic acid (AMPA) in different food matrices including soybean, corn, milk, egg, and honey using a reversed-phase with weak anion-exchange and cation-exchange mixed-mode Acclaim™ Trinity™ Q1 column. Several challenges in sample extraction and HPLC conditions will be discussed. These methods will be used to screen these samples for the 2016 FDA food survey program.

Method Development for Evaluation of Pesticides Residue in Drinking Water Using Disposable Pipette Extraction (DPX) and Gas Chromatography-Mass spectrometry (GC-MS).

Hongxia Guan,[1] Qingsong Cai,[2]
[1]*Geogia Gwinnett College, 1000 Lawrenceville, GA 30043*
[2] *University of Georgia, 315 Riverbend Road, Athens, GA 30602*

Pesticides have become ubiquitous environmental and human health hazards. There is clear evidence that long term exposure to pesticides can cause serious diseases such as neurological disorders, endocrine disruption, birth defects, and cancer. Regulatory and public concern over pesticide residues in water supplies has been increasing.

Monitoring of pesticide exposure requires rapid and selective sample preparation so that preventive and treatment protocols can be initiated promptly. Conventional solid-phase extraction (SPE) methods generally require multiple steps due to the need for conditioning and wash steps. In this study, disposable pipette extraction (DPX) method used reversed phase (RP) mechanism has been found to be a rapid and reliable SPE method for pesticides extraction from water. Sample solutions are mixed with the DPX sorbent to provide efficient extractions without concerns of channeling or solution flow rates. Recoveries and relative standard deviation (%RSDs) for the target pesticides are greater than 80% and less than 10%, respectively.

Identification of Penicillin G Metabolites under Various Environmental Conditions using UHPLC-MS/MS.

Fadi Aldeek,* Daniele Canzani, Matthew Standland, Mark R. Crosswhite, Walter Hammack, Ghislain Gerard, Jo-Marie Cook
Florida Department of Agriculture and Consumer Services

In this work, we investigate the stability of penicillin G in various conditions including acidic, alkaline, natural acidic matrix, and after treatment of citrus trees that are infected with the citrus greening disease. The identification, confirmation, and quantitation of penicillin G and its various metabolites were evaluated using two UHPLC-MS/MS systems with variable capabilities (i.e. Thermo Q Exactive Orbitrap and Sciex 6500 Qtrap). Our data show that under acidic and alkaline conditions, penicillin G at 100 ng/mL degrades fast, with a determined half-life time of approximately 2 hours. Penillic acid, penicilloic acid, and penilloic acid are found to be the most abundant metabolites of penicillin G. These major metabolites, along with isopenillic acid, are found when penicillin G is used for treatment of the citrus greening infected trees. The findings of this study will provide insight regarding penicillin G residues for food safety purposes, in agricultural and biological applications.

Comparison of Dumas and Kjeldahl Methods in Diverse Standard Reference Materials for the Accurate Determination of Protein Content.

Eric S. Clevenger,* Soo-Kwang Lee, Brian M. Hawes, Jessie Oliver, Amy E. Irwin, James M. Farrow, Anjum Waqar, Edwin Phifer, and Khalil Kerdahi
Atlanta Center for Nutrient Analysis, Southeast Regional Laboratory, U.S. Food and Drug Administration

The growth of public health awareness has led to a dramatic increase in the number of protein supplements available on the market, with the industry valued at approximately $16 billion.
To protect consumers from unscrupulous suppliers, the U.S. FDA tests for protein content in foods and supplements. Procedures for quantifying protein within food and supplement sources have historically favored crude protein analysis due to the complex macromolecular structure of the analytes. The two most widely used crude protein analytical methods are the Kjeldahl method-via acid digestion followed by titration-and the Dumas method-a combustion based technique. Using AOAC Official Methods 2001.11 and 990.03 as a basis, the Dumas method showed more accurate results (13.12 ± 0.06 g/100g) against the certificate value (13.225 ± 0.056 g/100g) of SRM1849a, and statistically significant differences from those by Kjeldahl (12.75 ± 0.17 g/100g). This report provides further comparative results from the two methods on a variety of traceable food and supplement matrices, with consideration of the advantages of greater speed, cleanliness, and precision of the Dumas method.

Update to the Changes in Chemical Residue Testing in the USDA/FSIS/OPHS Testing Program and Methods.

Chilton Ng, Sam Zipperer, and Louis Bluhm
Food Safety and Inspection Service Laboratory Quality Assurance Staff (USDA)

In recent years, the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) has made improvements to the way the Agency screens meat for chemical residues. Historically, FSIS completed screening of one sample with one method, with each method analyzing from one to 36 analytes. FSIS has transitioned to using larger multi-residue methods, and each sample is now analyzed by multiple methods. Primary methods of analysis are for pesticides, veterinary drugs, and heavy metals. Each method is available for public access in the FSIS Chemistry Laboratory Guidebook.

Profiling of Medium-to-Long Chain acyl-CoAs Converted From Dietary Fatty Acids in Mammalian Cells using LC-MS/MS.

Xiangkun Yang
Department of Pharmaceutical and Biomedical Sciences University of Georgia

Acyl-Coenzyme As (acyl-CoAs) are a group of endogenous molecules involved in multiple cellular pathways including autophagy, lipid oxidation and synthesis, and post-translational modifications. Acyl-CoAs are the activated form of fatty acids when a CoA attaches to a fatty acid inside living cells. There is evidence that exogenous saturated fatty acids can accelerate tumor progression, and the process is mediated via biosynthesis of acyl-CoAs and acylation of key proteins in tumorigenesis. Detecting acyl-CoAs is challenging due to their instability in aqueous solution. Our goal was to develop a sensitive analytical method to profile a series of acyl-CoAs in mammalian cells with high performance liquid chromatography (HPLC) and tandem mass spectrometry (MS/MS), to quantitate acyl-CoAs converted from exogenous fatty acids, and to compare the synthesis acyl-CoAs between normal and cancer cells lines. Hep G2, Hep 3B, PNT2 and Du 145 cells were cultured with different dietary fatty acids (C10-C20) for overnight. Cell culture media were removed when cells reached 80% confluence. Cells were washed with phosphate-buffered saline (PBS), incubated with odd-numbered acyl CoAs as internal standards, and extracted with methanol. After evaporation and reconstitution of extract, analytes were separated at 32 °C on a Luna C18 column (100 × 2.0 mm i.d., 3 µm; Phenomenex) coupled with a Phenomenex Security Guard C-18 guard column (4.0 mm × 2.0 mm). Multiple reaction monitoring (MRM) function in positive ion mode was used for quantification of various acyl CoAs (C10-C22). After validation of this analytical assay, the method was applied to investigate metabolism of fatty acids in mammalian cells. Absolute amount of acyl-CoAs were determined in control and fatty acid-treated mammalian cells. The amount of intracellular acyl-CoA was markedly increased after addition of the corresponding fatty acid. Exogenous fatty acids were mainly converted into the corresponding acyl-CoAs, but a small portion underwent metabolic conversion to shorter or longer chain acyl-CoAs. Acyl-CoA

profiling was accomplished after different fatty acid treatments to present the conversion from each fatty acid to various acyl-CoAs. This is the first LC-MS/MS method developed to profile acyl-CoAs in mammalian cells after metabolism of various exogenous fatty acids. Data are valuable to allow a better understanding of molecular pathways in diet-related diseases.

Toxicity of Hexahydriosohumulone in an Vitro Canine Model as an Example of Analytical Challenges and Opportunities in the Pet Food Industry

Denise Mitchell
Mars

As consumers demand pet foods with ingredients that mimic human diets, the need to verify the safety of common human ingredients for pets becomes increasingly important. Identification of compounds in food with toxic potential for pets creates the need for new and better analytical methods to quantitate these compounds to control their inclusion in pet foods and treats by either addition or cross contamination. For example, ingestion of hops by dogs has been reported to cause fatal intoxication consistent with malignant hyperthermia syndrome (MHS). Susceptibility to MHS is an autosomal dominant trait in dogs and has been reported in a variety of dog breeds. Administration of hexahydroisohumulone (HEX) - a component of hops has been linked to MHS. Recent *in vitro* work provides confirmation of the toxicity of hops in four canine cell systems. This work also demonstrates a species specific method by which toxic compounds can be identified. Analytical methods with low limits of detection in a variety of matrices including finished pet food is a critical factor for applying this knowledge practically.

Detection of Trace Levels of Toxins in Drinking Water on X500R QTOF

Farzad Pakdel, Ph.D.
SCIEX

Sciex X500R QTOF was used to detect trace levels of toxins in drinking water. 460 compounds (pesticides, herbicides, pharmaceuticals etc.) were analysed in a single high volume sample injection of surface waters and clean waters. The method included a TOF-MS scan followed by a High Resolution MRM, using Information Dependent Acquisition (TOF-IDA-MRM^{HR}). Calibration curves as low as 10 PPT were achieved

Finding and Identifying Unsuspected Contaminants in Food.

Mark Crosswhite
Florida Department of Agriculture and Consumer Services

Non-traditional chemical contaminates in food are hard to find and identify. This is because most chemical screens are developed based on what is legally allowable in food. Extraction and analysis protocols are highly tailored and very specific for various classes of chemical, e.g. antibiotics, herbicides, fungicides, insecticides. When a non-traditional chemical is in food, a non-classical extraction and analysis must be applied. These non-classical analyses require advanced chemical interpretation. Some of those techniques will be discussed here.

Rapid Quantitation of Biotin in Infant formula, Medical Foods, and Dietary Supplements by Isotope Dilution LC-MS/MS.

Aubrey L. Smith Shahriari, Sookwang Lee, Amy Irwin, Anjum Waqar, Edwin C. Phifer and Khalil F. Kerdahi
Atlanta Center for Nutrient Analysis, Southeast Regional Laboratory, U.S. Food and Drug Administration

Biotin (Vitamin B7) is currently measured using a microbiological assay that is non-specific, matrix dependent, labor intensive and very time consuming. Biotin occurs in food in the free form of D-(+)-biotin or bound to peptides. Quantitation of naturally occurring biotin in food is dependent on extraction and liberation of the molecule using enzyme-mediated digestion or acid hydrolysis. However, for dietary supplements and fortified foods in which the biotin is added in its free form during manufacturing, a simple “dilute-and-shoot” extracting can be sufficient. Another challenge to the quantitation of biotin lies in difficult detection of the target analyte that is not UV, fluorescent, or electrochemically active. Presented herein is an isotope dilution liquid tandem mass spectrometry (ID-LC-MS/MS) method used for the rapid quantitation of biotin. Isotopically labeled d4-biotin was used as an internal standard to ensure against degradation of the material during extraction or analysis. Chromatographic separation was achieved with reverse phase chromatography. Several unique transitions for the biotin were measured by multiple reaction monitoring (MRM) by positive mode electrospray ionization (+ESI). This method was shown to be successful in a variety of fortified samples including infant formula, medical foods, and dietary supplements.

A Validated LC-UV Method for the Analysis of Synthetic Thiamine Analogs, Benfotiamine and Sulbutiamine, in Biological Samples.

Jaeah Kim, Jason Zastre, Michael G. Bartlett
Sciences University of Georgia

Benfotiamine and sulbutiamine, lipid-soluble thiamine analogs, have been thought to increase cellular levels of thiamine. The impact of high-dose thiamine in reducing cancer cell proliferation has been reported. As a result, quantification of benfotiamine and sulbutiamine in biological samples is essential for the study of the utilization of thiamine analogs for potentially weakening cancer cell proliferation. There are no validated methods for the determination of benfotiamine and sulbutiamine in cell and media samples. Therefore, a sensitive and robust method to quantitate and verify these thiamine analogs in cells and media is required. Cell pellets and media were treated with 15 % trichloroacetic acid (TCA) solution to precipitate proteins in the samples, and then different concentrations of benfotiamine and sulbutiamine were added to both cells and media. Following addition of the internal standard, the samples were treated with di-isopropyl ether to remove the TCA. Samples were filtered and analyzed by liquid chromatography by utilizing a Shim-pack MAqC-ODS I (4.6 x 150mm, 5µm) column. A mobile phase system containing of 15mM sodium phosphate buffer (pH 3.6) and 100 % acetonitrile were used. This method was validated in accordance with the current FDA guidance for bioanalytical method validation. The method provided a linear ranged of 50-50000nM for benfotiamine in cells and media, and 300-30000nM for sulbutiamine in cells and media. The method was validated for accuracy within 15% relative error (RE) and precision within 15% relative standard deviation (RSD). Benfotiamine and sulbutiamine were used as internal standards against each other to achieve good reproducibility. A sensitive and robust bioanalytical method was developed and validated. This method can be applied in studies to determine the impact of benfotiamine and sulbutiamine as novel thiamine analogs on cancer cell proliferation.

How Repeatable is your Laboratory's Sample Processing? An Estimation of Measurement Uncertainty Including Laboratory Sample Processing.

Amy Brown
Florida Department of Agriculture and Consumer Services

How repeatable is your laboratory's Sample Processing? **An Estimation of Measurement Uncertainty including Laboratory Sample Processing**
Laboratories frequently estimate their measurement uncertainty based on an analytical method's quality control (QC) spike repeatability. This poster presents the Florida Department of Agriculture, Chemical Residue Laboratories (CRL) quality control procedure to estimate measurement uncertainty which includes sample processing (e.g. room temperature and cryogenic blending) and residue analysis. The CRL receives and processes over 3,000 samples annually for pesticide and antibiotic residue analysis. Samples include fresh fruits, vegetables and honey for regulatory enforcement of maximum residue limits (MRLs). If a sample is found

violative, regulatory actions could result in destruction of valuable food product. Tremendous resources are spent on the development and validation of new analytical instrumentation and extraction methods for chemical residues, but the important step of sample processing may be overlooked. As every matrix is different, it is important to ensure all parts of the sample handling are checked including cleaning equipment; chopping, blending and storage of samples. The laboratory participates in over six proficiency test samples annually which checks the extraction and analysis of samples, but this does not check sample processing as proficiency test samples do not require processing. This presentation focuses on the laboratory's ability to reproduce results for samples with incurred residues by repeat analysis from the comminuted portion. Results from initial repeatability studies will be presented. It is hoped that, over time, many matrix / analyte combinations will be screened and we will have a better understanding of the uncertainty in laboratory processing. While not measuring the repeatability of the actual sampling step, this is one more routine QC measure that may be included in the laboratory's overall estimation of analytical measurement uncertainty. This presentation will demonstrate how this QC procedure can capture this source of error and check the processing step which is an important part of a sample's journey through a laboratory.