AOAC Analytical Methods Week Delivers Official Methods[™] for PAC, BPA, and Flavanols

> fter a week of virtual AOAC expert review panel (ERP) meetings on July 8-12, 2019, AOAC announces two new *Official MethodsSM* for proanthocyanidins (PAC) and one for bisphenol A (BPA). In addition, AOAC *Official MethodSM* **2012.24** (flavanol and procyanidin) was approved as a new method as a result of a major modification. >>

"The series of virtual meetings were highly focused, leading to good discussion and consensus by stakeholders from all over the world," said **Deborah McKenzie**, senior director of Standards and *Official MethodsSM*. "The AOAC Analytical Methods Week (AMW) consolidated and streamlined ongoing ERP method reviews, keeping these important AOAC activities moving forward."

ERPs focused on methods in the areas of food, infant formula, and microbiology. The week featured traditional ERP meetings for:

- (1) BPA Methods
- (2) Flavanol Methods
- (3) PAC in Cranberries Methods
- (4) Sugars and Fructans Methods

(5) Microbiology Methods for Foods and Environmental Surfaces

The First Action methods for PAC and BPA adopted during AMW were all previously submitted for review. At the time, the respective ERPs agreed that the methods showed promise but that more performance information and clarity were needed to demonstrate requirements of the respective AOAC *Standard Method Performance Requirements* (SMPRs[®]). Method authors addressed the ERPs' feedback and recommendations, and the methods were resubmitted for review.

ERP members, who are thoroughly vetted by the AOAC Official Methods Board (OMB), provided in-depth reviews of candidate methods. Methods were reviewed by a primary and secondary expert reviewer. Panel members summarized their findings, and advantages and disadvantages of each method were then thoroughly discussed by the entire panel.

The ERPs agreed that the methods acceptably meet SMPRs developed and approved through voluntary stakeholder consensus, as well as any ERP requirements.

BPA

On July 9, 2019, the AOAC ERP for BPA Methods, chaired by **Melissa Phillips** of the U.S. National Institute of Standards and Technology, adopted a First Action method for BPA in carbonated/noncarbonated and nonalcoholic beverages by ultra-performance liquid chromatography (UPLC). The method was evaluated against AOAC SMPR 2017.018, which detailed analysis standards consistent with the increasingly conservative regulatory requirements for BPA such as those enacted in California.

This Official MethodSM, the second approved for BPA, expands testing options by using liquid chromatography, a common analytical tool in many laboratories. Liquid chromatography identifies and quantifies components in a mixture by separating them according to their molecular weight and polarity.

"Approval of this method means more labs can test for BPA, which will greatly facilitate the efforts of beverage manufacturers to produce products that are safe, fulfill consumer expectations, and comply with regulations," said **Darryl Sullivan**, chief scientific officer at Eurofins Food Integrity and Innovation.

Growing public concern has led beverage manufacturers to source reduced-BPA or BPA-free containers and seek more accurate ways to measure its presence in their products. The AOAC project that produced the new method was launched in response to a need identified by the American Beverage Association.

In the paper, "Determination of Free Bisphenol A in Commercially Packaged Ready-to-Consume Carbonated/ Noncarbonated and Nonalcoholic Beverages with Immunoaffinity Column (IAC) Purification and UPLC with Fluorescence Detector," submitted by Abbott Nutrition, the authors describe a study for an IAC-facilitated method for BPA analysis based on a UPLC/FLD system. The study evaluated method performance characteristics, including sensitivity, accuracy, and repeatability.

The authors addressed the ERP's concerns and recommendations from the initial review and conducted a limit of detection/quantitation (LOD/LOQ) study using a lower concentration blank beverage sample. Also based on ERP recommendations, the authors included information on system suitability requirements, lower level calibrant preparation, safety, and BPA column capacity/size parameters, among other clarifications.

The method was shown to be applicable to the determination of free BPA in commercially packaged, ready-toconsume carbonated/noncarbonated and nonalcoholic beverages, including regular carbonated soft drink with sugar and caffeine, 100% orange juice with pulp, and whole milk. Based on its collective expertise and judgment, the ERP reached consensus that the method acceptably meets AOAC SMPR 2017.018, and that method authors have satisfactorily addressed the ERP's comments.

PAC

On July 10, 2019, the AOAC ERP for Proanthocyanidins in Cranberries Methods, chaired by **Holly Johnson** of American Herbal Products Association (AHPA), reviewed candidate methods evaluated against AOAC SMPRs 2017.003 Quantitation of Proanthocyanidin Content in Cranberry Products and/or AOAC SMPR 2017.004 Identification of Type-A Proanthocyanidins in Cranberry Products.

In "Identification of A-Type Proanthocyanidins in Cranberry-Based Foods and Dietary Supplements by Matrix-Assisted Laser Desorption/ Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)," submitted by Complete Phytochemical Solutions, LLC, a study is described showing that deconvolution of MALDI-TOF MS spectra distinguishes the A-type PAC in cranberry fruit and cranberry products from other botanical sources containing mostly B-type interflavan bonds with a probability greater than 90% and a confidence of 95%. Results also demonstrated that the deconvoluted MALDI-TOF MS spectra data in combination with principal component analysis (PCA) allows a better understanding of the chemical profile of PAC, and that MALDI-TOF MS can be a powerful tool for structural characterization and identification of PAC.

The method can identify the presence of type-A PAC in cranberry *(Vaccinium macrocarpon)* in one or more of the following: fruit, juice, beverage, dried cranberry fruit, cranberry sauce, ingredients (concentrates, extracts, powders, and presscake), or dietary supplements.

Based on ERP recommendations, method authors provided additional information on the specificity of the MALDI for the determination of type-A (Continued on page 30) and type-B PACs, LOQ, and recovery. The ERP agreed that the method is well-described and meets requirements.

Also submitted by Complete Phytochemical Solutions, "Multilaboratory Validation of 4-(Dimethylamino)cinnamaldehyde (DMAC) Assay Using Cranberry Proanthocyanidin Standard for Quantification of Soluble **Proanthocyanidins in Cranberry Foods** and Dietary Supplements," describes a multilaboratory study to validate DMAC colorimetric assay using a 96-well plate spectrophotometer for the accurate quantification of PAC in cranberry products. The study also evaluated the comparison of the procyanidin A2 (ProA2) dimer and cranberry PAC (c-PAC) reference standards. Four test materials were analyzed in the study: cranberry fiber powder, cranberry extract powder, concentrated cranberry juice, and a purified cranberry PAC juice.

The study evaluated parameters of repeatability and intermediate precision, accuracy, and mean recovery. The method shows advantages of using a 96-well plate spectrophotometer for the accurate quantification of PAC. The method is able to quantify total PAC content as the sum of all extractable oligomers (>DP2) and polymers present in cranberry (*Vaccinium macrocarpon*) in one or more of the following: fruit, juice, beverage, dried cranberry fruit, cranberry sauce, ingredients (concentrates, extracts, powders, and presscake), or dietary supplements. The ERP agreed that the DMAC method is precise and, when used with the appropriate reference standard, is also an accurate method for the quantification of cranberry PAC.

Flavanois

On July 12, 2019, the AOAC ERP for Flavanol Methods, chaired by **James Harnly** of the U.S. Department of Agriculture-Agricultural Research Service, reached consensus that the method described in, "Method Evaluation of Revised AOAC **2012.24**: Determination of Total CF (1–7) by HPLC-FLD for the Analysis of Total Cocoa Flavanols (CF) in Cocoa Extract." submitted by Mars Symbioscience, meets SMPRs. The ERP approved the method modification as a new First Action *Official Method*SM (separate and distinct from **2012.24**).

A single-laboratory validation of the update to AOAC **2012.24** evaluated

More AMW Highlights

n addition to expert review panel (ERP) meetings to evaluate candidate methods, the AOAC Analytical Methods Week (AMW) featured virtual orientations, working group meetings, and the first AOAC public comment sessions.

Virtual ERP orientations were held at the beginning of the week to familiarize panel members with AOAC policies and procedures, standards development process, and roles and responsibilities.

AMW provided a forum for a joint meeting of the AOAC ERP for Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) Nutrient Methods and working group chairs for the first six nutrients (vitamins A, D, B₁₂, E, inositol, and folic acid). Stakeholders examined a strategy on a review process for these *Official Methods*SM, which were adopted First Action by the respective ERPs, but are not slated for multilaboratory testing or for Final Action status. However, these methods may still be in use and have utility in the broader infant formula community.

During AMW, AOAC held for the first time virtual public comment sessions for three draft documents developed by working groups for the AOAC Stakeholder Panel on Agent Detection Assays (SPADA). These sessions, moderated by **Darryl Sullivan** of Eurofins Food Integrity and Innovation, focused on seeking feedback from the biothreat detection community on voluntary consensus guidance documents for *in silico* analysis, bacterial strain verification, and soil testing (*see* accompanying story on page 31 of this issue).

relevant matrixes: cocoa cake, cocoa extract, dark chocolate, chocolate liquor, and a dietary supplement capsule. Modifications included changes to the analytical separation column and gradient conditions used in the HPLC system, and replacement of the epicatechin standard with a cocoa extract calibrant standard consisting of DP1-7 species at known concentrations. This commercially available calibrant allows for the direct determination of each DP without the need for relative response factors. As a consequence of the change to column used, sample preparations were adjusted to ensure all analytes remained within the linear range of the method.

The ERP agreed that the modifications are well-documented and improve the method. The rationale for the change in HPLC column and the resulting change in the gradient program are thoroughly explained.

Conclusions

Methods adopted as AOAC First Action *Official Methods of Analysis* will be published by AOAC INTERNATIONAL.

Over the next 2 years, the ERPs will monitor each method, and AOAC INTERNATIONAL will solicit feedback on real-world experience with the methods. After 2 years of the methods' adoption, the ERPs will assess feedback on method performance and will use this information to make a recommendation to the OMB regarding Final Action status.

AOAC invites method users to provide feedback on the use and performance of First Action *Official Methods*SM. This information is critical in determining whether these methods should advance to Final Action status after the 2-year period from adoption.

It is anticipated that the next AMW will be held in December 2019, with more virtual ERP meetings and additional activities to advance the important work of AOAC.

"Building on the success of AMW, planning is underway for more of these virtual meetings to seek consensus on and advance the important work of AOAC," McKenzie said.

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