

METHODS COMMITTEE REPORTS**Committee on Dietary Supplements****JAMES P. KABABICK, CHAIR**

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Committee Actions

The Study Directors of Committee K had a busy and fruitful year. Several collaborative studies have been completed and or near completion during the past year. A few SLV projects are active. The scope of the work and the involvement of all the Study Directors and participating laboratories are truly impressive. I salute and sincerely appreciate everyone's hard work and dedication. Committee K should consider to recommend the Study Directors Joseph Zhou and Mark Roman as candidates for the selection of "Study Director of the Year" to be awarded at the Annual Meeting. Last year, one of the Study Directors within Committee K was chosen to receive the honor.

(1) *Determination of Ephedrine Alkaloids in Dietary Supplements and Botanicals by LC/MS/MS*: A collaborative study was completed. Details of the study were published. A protocol entitled, "Comparison of Various Dilutions for the Determination of Ephedrine-Type Alkaloids and Internal Standard Recovery with and without SPE Cleanup in Botanical Raw Material and Ephedra Powdered Extract" was written and executed as a project resulting from the ephedra follow-up meeting held at the 2003 AOAC INTERNATIONAL meeting. A manuscript is in the process of being written to submit for publication. Continue study.

(2) *Determination of Ephedrine Alkaloids in Human Urine and Plasma by LC/MS/MS*: A collaborative study was completed. Details of the study were published and the method was adopted First Action as an Official MethodSM **2003.10**. Continue study.

(3) *Determination of Glucosamine in Raw Materials and Dietary Supplements Containing Glucosamine Sulfate and/or Glucosamine Hydrochloride by HPLC with FMOC-Su Derivatization*: A collaborative study was conducted on the method for the determination of glucosamine in raw materials and dietary supplements containing glucosamine sulfate and/or glucosamine hydrochloride by HPLC with FMOC-Su derivatization. Thirteen blind duplicates of materials consisting of various commercial products, including tablets, capsules, drink mix, and liquid products as well as raw materials, blanks, and spike recovery samples, were tested by 12 collaborating laboratories. The average determination coefficient of the calibration curves from the laboratories was 0.9995 with an RSD of 0.03%. The tests with the blank samples and the samples with glucosamine spiked showed good specificity of the method. The average spike recoveries at the spike levels of 100% and 150% were 99.0% with an RSD of 2.1% and 101% with an RSD of 2.3%, respectively. The test results between laboratories on each commercial product were reproducible with all RSDs no more than 4.4%, and the results were repeatable in the same laboratory with an average RSD of 2.7%. None of the results from the

collaborating laboratories was outlier, partly indicating the robustness of the method. It is recommended that this method be adopted as Official First Action.

(4) *HPLC-UV Determination of Ephedrine Alkaloids in Dietary Supplements and Botanicals*: The collaborative study is completed, and the results have been published in *JAOAC*. The method was approved for Official First Action for the determination of ephedrine and pseudoephedrine in botanicals and dietary supplements, with the exception of high-protein powdered drink mixes. The method was not approved for the determination of minor ephedrine alkaloids in botanicals and dietary supplements due to unacceptable recoveries in the spiked negative control. There is some evidence, however, indicating that a content uniformity problem existed with the minor alkaloids in the spiked negative controls.

A total of 11 laboratories participated in this study—5 U.S. laboratories and 6 international laboratories—however only 10 laboratories submitted their data in time for publication.

(5) *Collaborative Study of a Method for the Determination of Beta-Carotene in Supplements and Raw Materials by Reversed-Phase HPLC*: Collaborative study is in progress. Data are being collected and reviewed at the present time.

Single Laboratory Validation (SLV) Method Status

(1) *St. John's Wort: Single Lab Validation for the Determination of Components in St. John's Wort Raw and Finished Products by High-Performance Liquid Chromatography with Photodiode Array Detection*: SLV report was reviewed. The next decision for the committee is to whether move this SLV to the full study.

(2) *Aristolochic Acid in Traditional Chinese Medicines*: An expert review panel (ERP) was convened and a new SLV protocol was written. Newly authenticated validation materials may have to be acquired. Validation of the method is being conducted to enable the collaborative study protocol to be written and started. Continue study. The SLV protocol has been reviewed with comments sent to the study director in June 2004.

(3) *Saw Palmetto Berry*: Two GC methods have been selected for SLV. One is to analyze the fatty acids and the other for phytosterols in the same plant part. The SLV protocol was reviewed and sent back to AOAC with comments.

The issue was raised that the Botanicals and Plant Toxin subsections contained interest areas which were either no longer active or better suited for other committees. In response, Committee K reviewed these during a phone conference. The initial consensus was that it was not necessary to continue with all of these.

Botanicals 1: Kava Kava: It was determined that the Chair should contact the Topic Advisor. Kava is on the original "list of 17" supplements to be examined under the AOAC/FDA/NIH contract. Mark Anderson raised the issue whether the intent was to develop a marker method for kava lactones, or a method for potential contaminants in kava. NIH is interested in a marker method.

Botanicals 2: Ephedrine alkaloids in dietary supplements (LC/MS)

Botanicals 3: Ephedrine alkaloids in human serum and urine (LC/MS): These methods are those being validated under the currently funded protocols, and should be continued. Once the methods are validated, it is anticipated that these subsections could be disbanded.

Botanicals 4: Ephedra alkaloids proficiency testing

Botanicals 5: Botanical microscopy proficiency testing

Botanicals 6: Digitalis: It will be the recommendation of Committee K that these subsections be disbanded.

Botanicals 7: Mycotoxins in botanicals: It will be the recommendation of Committee K that this subsection be moved to Committee D (Natural Toxins and Food Allergens), since mycotoxins are natural toxins. It is noted that an approved method for detecting mycotoxins in food will need to be demonstrated to be efficacious in detecting mycotoxins in botanicals. Committee K Chair is to contact Committee D Chair with regard to this issue.

Botanicals 8: Hydroxycitric acid in Garcinia cambogia: The consensus of Committee K is that this subsection needs a Topic Advisor. The reported thermogenic activities of supplements containing hydroxycitric acid are causing them to be used as a replacement for ephedra-containing supplements. The volume of supplement consumption in this market segment is likely to continue to be significant. However, there is currently no funding for this, and the supplement may be a fad. Therefore, this subsection is to be continued, a Topic Advisor sought, and the topic to be reviewed at a later date.

Botanicals 9: Proanthocyanins: It will be the recommendation of Committee K that this subsection be disbanded. Extremely complex issue, both in product efficacy and in analytical chemistry. It was noted that there was not much progress regarding characterization, and that the area requires a significant amount of research.

Botanicals 10: Analysis of skullcap and germander: The consensus of Committee K is that this subsection needs to be continued, and reviewed at a later date. The Topic Advisor needs to be contacted by the Committee K Chair. This issue became a subsection due to potential adulteration of skullcap with germander. Demand for new or additional analytical methods may no longer exist.

Botanicals 11: St. John's wort: The consensus of Committee K is that the Topic Advisor needs to be contacted by the Committee K Chair. It was noted that there are valid LC methods for dianthrone, but that the inclusion of hyperforin is difficult. In any case, this subsection should be continued.

Botanicals 12: Ephedrine alkaloids in dietary supplements (LC-UV)

Botanicals 13: Ephedrine alkaloids in biologicals (LC-UV): These methods are those being validated under current study protocols, and should be continued.

Plant toxins 1: Aristolochic acid in Traditional Chinese Medicines: A method is available. The Topic Advisor should be contacted (Catharina Ang), if needed. This subsection should be continued.

Plant toxins 2: Phytoestrogens: It was noted that phytoestrogens are more appropriately considered to be botanicals, rather than plant toxins, especially if the compounds of interest in this subsection are isoflavones. It is the consensus recommendation of Committee K that this change be made. The Topic Advisor (Patricia Murphy) should be contacted.

Plant toxins 3: 997.13 Glycoalkaloids in potato tubers: It is suggested that the responsibility should be shifted to Committee D. The Chair of Committee K is to contact the Chair of Committee D with this recommendation.

Plant toxins 4: Pyrrolizidine alkaloids: This is currently a very hot topic regarding comfrey, but it is noted that the market for comfrey is currently relatively small. The Topic Advisor should be contacted for the latest information. This subsection should be continued and reviewed at a later date.

Plant toxins 5: Hypoglycine: It will be the recommendation of Committee K that this subsection be disbanded.

Plant toxins 6: Glucosinolates: It will be the recommendation of Committee K that this subsection be

disbanded, or (at best) be moved to botanicals, since glucosinolates are used as a marker compound for broccoli.

There was a discussion about how to best replace the dropped items. It was decided that the priority list from NIH/FDA would be the guideline.

Status of Ephedra Method Studies

James Kababick asked for comments regarding the Ka010 and Ka011 protocols, and received no additional comments verbally. Kababick thanked the committee for their input, and was going to provide AOAC with his compilation of all of the comments.

A potential issue regarding the availability of the d5-labelled internal standard was raised. Sigma can provide d3; the FDA not only prefers the d5-IS, it insists on the d5, since the d5 is required by the method as written.

Miscellaneous

Steven Dentali (AHPA) noted that AHPA's analytical laboratory committee is forming a methods subcommittee.