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November 14, 2008

Dr. Siu-kay Wong
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Dear Dr. Wong:

We are pleased to inform you that upon favorable reviews, members of the Methods Committee on Dietary Supplements have voted unanimously to approve your method, "Determination of Aconitum Alkaloids in Dietary Supplements and Raw Botanical Materials Using LC-UV with Confirmation by LC/MS: Collaborative Study" as a First Action *Official Method*[SM]. This method will be designated as method **2008.11.**

A notice of the adopted method will be published in the AOAC magazine, *Inside Laboratory Management*, and in "For Your Information" in the AOAC *Journal*. AOAC staff editors will prepare the manuscript for publication in the *Journal of AOAC INTERNATIONAL*. The modification will be included in an upcoming revision of the *Official Methods of Analysis*.

AOAC would like to take this opportunity to thank you for all your contributions in the development of this method.

Sincerely,

Robert Rathbone
Director, OMA Program and Publications

CC: Chair: Richard Myers
General Referee: Steve Lunetta
Program Manager, Methods Validation: Dawn Dowell

Determination of *Aconitum* Alkaloids in Dietary Supplements and Raw Botanical Materials by Liquid Chromatography/UV Detection with Confirmation by Liquid Chromatography/Tandem Mass Spectrometry: Collaborative Study

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An interlaboratory study was conducted to evaluate a method for the determination of 3 *Aconitum* alkaloids, viz., aconitine, mesaconitine, and hypaconitine, in raw botanical material and dietary supplements. The alkaloids were extracted with diethyl ether in the presence of ammonia. After cleanup by solid-phase extraction to remove matrix interferences, the alkaloids were determined by reversed-phase liquid chromatography (LC)/UV detection at 235 nm with confirmation by LC/tandem mass spectrometry (MS/MS). A total of 14 blind duplicates were successfully analyzed by 12 collaborators. For repeatability, the relative standard deviation (RSD_r) values ranged from 1.9 to 16.7%, and for reproducibility, the RSD_R values ranged from 6.5 to 33%. The HorRat values were all <2 with only one exception at 2.3. All collaborating laboratories had calibration curves with correlation coefficients of >0.998. In addition, 6 collaborators performed the confirmation and were able to verify the identities of the alkaloids by using LC/MS/MS.

The diester-diterpene *Aconitum* alkaloids, viz., aconitine, hypaconitine, and mesaconitine, are highly toxic compounds commonly present in aconite roots such as *R. aconiti* (Chuanwu), *R. aconiti kusnezoffii* (Caowu), and *R. aconiti lateralis* (Fuzi). Many dietary supplements for enhancing sexual ability and circulation, restoring health, and relieving pain contain processed aconite roots and thus the *Aconitum* alkaloids. Proper processing by heating, steaming, and soaking the aconite roots can hydrolyze the highly toxic diester-diterpene *Aconitum* alkaloids to compounds of much lower toxicity, e.g., benzoylaconine, benzoylmesaconine, and benzoylhypaconine (1). Pharmacological studies indicated that the diester-diterpene *Aconitum* alkaloids have the same or similar anti-inflammatory and analgesic actions as their hydrolyzed analogs (2). The absence of standardized methods for processing aconite roots has resulted in drastic variation in the alkaloid content, and thus in the safety, of the supplement products containing aconite roots. Intoxication cases arising from consumption of improperly processed aconite roots have been reported in many countries.

Because of this significant public health impact, a single-laboratory validation (SLV) study was conducted for the determination of the 3 *Aconitum* alkaloids in 6 representative matrixes of aconite root products, including processed raw material (Fuzi), single-ingredient dry powder extract, multi-ingredient dry powder extract, pills, and capsules found in the marketplace (3). This method will facilitate the accurate determination of the quality of botanicals and dietary supplements with respect to the 3 *Aconitum* alkaloids. In addition, the use of this method may allow dietary supplement manufacturers to set quality standards, and regulatory agencies to monitor safety in the use of dietary supplements containing aconite roots.

Collaborative Study

Study Design

The liquid chromatography/UV detection (LC/UV) phase of this study was conducted with 7 materials as blind duplicates. Some of the materials contained known concentrations of naturally occurring or purposely added (formulated or fortified) aconitine, mesaconitine, and hypaconitine. Two of the materials were negative controls. In addition, each participant was supplied with sufficient quantities of reference standards to conduct the study. Random identification numbers were assigned to each of the blind duplicate materials. A practice sample of dietary supplement material of known concentration was provided for participants. Also, a blank of botanical raw material was provided for the recovery study. These samples were used to

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The recommendation was approved by the Methods Committee on Dietary Supplements as First Action. *See* "Official Methods Program Actions," (2008) *Inside Laboratory Management*, November/December issue.

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control replicates. These results might be due to contamination or to carry-over peaks in the LC/UV analysis.

Table 2008.11A presents statistical summaries of the results from Laboratories A–K. Statistical analysis to determine repeatability and reproducibility was performed by using the AOAC INTERNATIONAL Statistical Program (Version 2.0) for Blind Replicates. The values for repeatability standard deviation (S_r), reproducibility standard deviation (S_R), RSD_r, reproducibility relative standard deviation (RSD_R), and number of statistical outliers are presented. HorRat values are also presented and are calculated as RSD_R (observed)/RSD_R(predicted), where the RSD_R(predicted) is calculated by using the following equation:

$$RSD_R = 2C^{-0.1505}$$

where C is the measured analyte concentration in decimal mass units (4). The Cochran, Grubbs, and Double Grubbs tests were used to remove statistical outliers where appropriate. Data from laboratories reporting values for individual alkaloids as greater-than or less-than values were not included in the statistical analysis.

Collaborators' Comments

The collaborators were able to follow the method with very few difficulties. Five laboratories reported difficulties in mixing the entire sample inside the centrifuge tube with the 1 mL 10% ammonium hydroxide solution before the extraction. One collaborator suggested using 2 mL 5% ammonium hydroxide solution instead. Another laboratory performed the extraction by using a 50 mL conical flask instead of the centrifuge tube.

Performance Characteristics of the Method

The method performed well in the collaborative study for both detection and confirmation of the 3 *Aconitum* alkaloids. For repeatability, the RSD_r values ranged from 1.9 to 16.7% and for reproducibility, the RSD_R values ranged from 6.5 to 33%. Five materials had acceptable HorRat values except for the aconitine determined in the processed *R. aconiti*, for which the HorRat value was 2.37. For the 2 negative control materials, the HorRat values were not applicable. The number of results identified as outliers and disregarded was ≤4 out of 21 or 22 for all samples except for hypaconitine determined in the spiked dietary supplement negative control for which 2 pairs of Cochran and 1 pair of Grubbs outliers were identified. The number of outlier laboratories identified, excluding those suspected of having results mixed up with those of other samples, was <2 out of 11, except for the determination of hypaconitine in the spiked dietary supplement negative control, for which 3 laboratories were identified as outliers.

In addition, all collaborating laboratories had correlation coefficients of >0.998 for the calibration curves generated. Among the participating laboratories, 6 verified the identity of the *Aconitum* alkaloids found in the samples by using LC/MS/MS. The reason that the other laboratories failed to do so might be the unavailability of the required equipment.

Recommendations

On the basis of the results of this collaborative study, it is recommended that the method be adopted Official First Action for the determination of *Aconitum* alkaloids in dietary supplements and raw botanical materials.

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