

**Nutri-Net Project: Canadian Product Quality Initiative**

# **Final Report**

***IMPROVING PRODUCT QUALITY THROUGH DEVELOPMENT OF  
VALIDATED ANALYTICAL METHODS, INCREASING TESTING  
PROFICIENCY AND CAPACITY WITHIN CANADA***

---

## 1.0 Project Executive Summary

This project was designed to assist industry and regulators address critical issues for this sector; an assessment of current microbial load testing demands; the paucity of validated analytical testing methods; concerns over the variability in analytical test performance and the need to increase industry capacity and improve perceptions of NHP quality. Three subprojects were conducted:

### A. Development of Microbial Load Criteria Recommendations

Microbial load limits for natural health products (NHPs) are required because microbial contamination can pose a health risk. Currently, the regulatory criteria for finished or formulated NHPs require meeting purity specifications. This is used to show adherence to Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP), whether the product is suitable for the intended purpose, and/or stability of the product (shelf-life), and when import of a product into Canada is undertaken and the product's production parameters are unspecified. Control of the supply chain via compliance with GAP and GMP is the top priority to mitigate risk of microbial contamination of NHPs.

A comprehensive literature review to determine the state of the science was compiled and dissemination of this to industry is ongoing. The review results guided the design of the microbial sampling plan and testing strategy used. Baseline data collection entailed generation of new data using Canadian samples and analyses of this data and data provided by the American Herbal Products Association permitting evaluations for three botanicals – ginseng, ginger and licorice. Based on current industry practice, five specific microbial tests were chosen to cover the range of microbial limit purposes, including shelf-life and contamination from local environmental reservoirs,

Several issues with meeting the current microbial criteria as set by the Natural Health Products Directorate were identified. One is the lack of specified sampling units required for statistical confidence in establishing an acceptable measurement for an NHP lot. Levels set at zero or “absent” means that, depending on the sampling plan and the associated sensitivity factor, a “pass” material may still pose a risk. In this project, the International Commission on Microbiological Specifications for Food was used as a guide to create an appropriate sampling plan and it is recommended that this approach be utilized, especially if the supply chain of a NHP is not well understood or unknown. However, the second issue identified is a major challenge. This entails the technical limitations in interpreting data for counts of total aerobic bacteria and yeasts and moulds which stymies setting appropriate microbial limits for NHPs. This is a consequence of the fact that many botanicals naturally have bacteriostatic or antimicrobial properties and consequently inhibit assays based on microbial growth.

To address these issues, several recommendations are made. In short term, i.e. immediately, increased emphasis on control of the supply chain and use of appropriate sampling plans. Ongoing acquisition of baseline data on microbial load to build the needed information base for a range of botanicals is needed. Over the longer term, thorough investigation of whether current microbial testing methods can be validated for use with NHPs using a secondary testing method such as microbial DNA-based assays, a mycotoxin testing survey to determine whether this is an area of concern for common NHPs, and longer term hazard characterization.

### B. Initiation of a National Lab Proficiency Program

Successful validation of appropriate methods to meet international standards, as well as a fair and equitable approach to laboratory proficiency determinations have been developed. Methods validation for three Natural Health Products of interest to Canadian manufacturers was undertaken - for North American ginseng (*Panax quinquefolius*), goldenseal (*Hydrastis canadensis*) and Echinacea spp. To do this, existing methods were collected and assessed. The most robust and cost-effective method then underwent a Single Laboratory Validation (SLV), a Youden Ruggedness Trial (YRT), and each will undergo a full inter-collaborative study (IS) which provides multi-laboratory method validation. According

to AOAC International, multi-laboratory validation is required to archive the highest degree of confidence in performance as required to generate credible, defensible, and reproducible results. Only AOAC® Official Methods are recognized worldwide as authoritative, because of their thorough and rigorous testing and characterization and are cited in the U.S. Code of Federal regulations. According to AOAC International the Collaborative Study process takes 12 months minimum, and requires participation of 8-10 independent laboratories. Upon successful completion of the study, the results are published in the *Journal of AOAC INTERNATIONAL* and may go through the process to becoming an *Official Methods of Analysis (OMA)*.

**For the Ginseng Quality Assurance Program**, analysis of the preliminary validation studies and YRT data, determined that the High Performance Liquid Chromatography method selected by AOAC International was not adequately rugged to pass an SLV, let alone an inter-collaborative study. Consequently, an additional, exhaustive series of method optimization experiments was conducted. A full SLV and YRT were conducted on the optimized method. These demonstrated that the validated method was fit for its intended purpose and that small deviations in conditions would not have significant impact on analytical results, respectively. The SLV and the YRT results were completed in January 2008 and a draft manuscript submitted to the Journal of the AOAC. This is the maximum level of certainty that can be achieved within a single laboratory and is considered to be the first step on the path to becoming an Official Method of Analysis.

While undertaking the SLV, BCIT prepared an Inter-collaborative Study (IS) protocol template for Ginseng because it is a requirement that any IS protocol be accepted through AOAC's peer-review process *prior* to initiating the study or the method cannot proceed through the process to become an Official Method of Analysis. By February 2008, the protocol was approved in full and a total of 13 out of 14 recruited laboratories participated. The fair and equitable approach to laboratory proficiency determinations developed in this project included providing laboratories with practice samples to complete and support with addressing minor problems following protocol until results were deemed acceptable. This caused another delay in the proposed timeline but resulted in very high level of participation with 12 laboratories continuing in the project. As most of the recruited laboratories provide professional analytical contract services in support of environmental, pharmaceutical and food safety industries, all were engaged with other projects and should be commended for their commitment to this project. To date 11 out of 12 laboratories have submitted their test sample results and the statistical analysis is in the final stages. The majority of the data submitted from the participating laboratories has been compiled and statistically analyzed for potential outliers. Once the IS is completed, an official manuscript will be submitted to the AOAC International Dietary Supplement Method Committee for consideration as Official First Action. The anticipated completion date for the study is June 2008.

**For the Goldenseal Quality Assurance Program**, a validated method for a method that includes a measure to ensure the common economic adulterant is not present and SLV were prepared as a manuscript and published in January 2008 in *Pharmaceutical Biology*. From this work, an existing protocol with modifications to maximize the use of collaborative data from participating labs was employed. Extensive revision and statistical evaluation of laboratory data resulted in the development of an IS manuscript. Working with the AOAC International Subject matter expert and statistical advisor, the results were approved to be accepted as Official First Action and assigned *Official Methods SM number 2008.04*. A notice of the adopted method was published in the AOAC magazine, *Inside Laboratory Management* April 2008 and will be published in "For Your Information" in the *AOAC Journal*. The method will be published as part of the collaborative study and will be included in the *Official Methods of Analysis* online and in the next print edition.

It was noted during this study that the method preparation was not ideal for raw goldenseal. The SLV and YRT did not demonstrate any significant variation due to extraction parameters; however, when moved to a multi-laboratory scenario, the variation became significant. Despite acceptance as Official First Action by AOAC International, it was a marginal pass for raw materials. Thus, while awaiting AOAC deliberations over the statistical data of the IS, BCIT initiated an optimization study and a matrix-specific SLV to address this.

For the **Echinacea Quality Assurance Program**, a HPLC method for ensuring identity and potency by quantification of phenolic marker compounds was chosen by an AOAC International Expert Review Panel as the most promising to undergo further investigation. Sample and reference materials were obtained with some challenges. Sample materials should reflect the common products available on the marketplace and sufficient quantity of identical lots of each sample obtained so both a SLV and an IS with a maximum of 15 participating labs could be completed. Further verification of the reference materials was undertaken to determine their purity was conducted.

Significant shortcomings of the original method were identified. The original method utilized only a single standard from which total phenolics were quantified and the quantification of each phenolic calculated through the use of response factors. Analyses revealed that this yielded neither precise nor accurate results, each phenolic compound needed to be quantified using its own standard to ensure precise and accurate results. Consequently, additional standards were obtained to allow for a full complement of reference materials to be provided to IS participants. Poor accuracy and precision of the method could be attributed to sub-optimal extraction procedures and less than ideal chromatography. To address these issues, an extraction study and a chromatography optimization study were designed and performed. This additional experimentation resulted in important revisions to the method.

The YRT performed on the revised method demonstrated that the method was robust and suitable for validation studies. An SLV study on the optimized method was performed for analysis of raw materials (root and aerial parts) and extracts as per AOAC International guidelines. The SLV data demonstrated the method was fit for its intended purpose. Results from the optimization study, SLV and YRT were combined into a manuscript for submission to Analytical & Bioanalytical Chemistry. Following completion of the SLV, BCIT intended to conduct the IS and potential participants were recruited; however, there was resounding consensus from laboratories that only one IS could/should be pursued at a time, as the effort and contribution from the participants is significant.

During recruitment of laboratories, a potential confounding analytical issue was raised, the stability of one of the phenolic compounds in extracts. Further investigations indicated an experiment to examine the extent of degradation and potential methods to circumvent degradation, if present, was necessary. This was initiated and the information will be incorporated into the SLV protocol developed to evaluate product formulations, by including a set of additional raw materials. This SLV protocol has been submitted to AOAC International. Depending on the findings of the degradation experiment, changes to the method may be necessary and thus minor changes to the SLV protocol will occur. Until such time as the method has been finalized and the requisite laboratories are recruited and confirmed, test samples and standards will not be prepared and distributed. All of the materials have been obtained and can be prepared and distributed upon completion of the degradation study and SLV. Once the protocol is finalized with the details of the method that will be employed for raw materials affected by enzymatic degradation of cichoric acid, an Echinacea IS will be scheduled.

All materials and a preliminary study designs for Ginseng Lab Proficiency Determinations, Goldenseal Lab Proficiency Determinations and Echinacea Lab Proficiency Determinations have been developed. With NNC support for a Lab Proficiency Study Coordinator, these can be initiated and completed in 2008/2009.

### **C. Development of a Product Quality Program Business Plan**

This component of the project encompassed a market assessment to evaluate industry interest in product quality, evaluation of previous and existing product quality programs for this industry, identification of a preliminary business model for the Program, a Product Quality Workshop to stakeholders to present information collected and to identify next steps for the business case. The draft business plan was prepared.