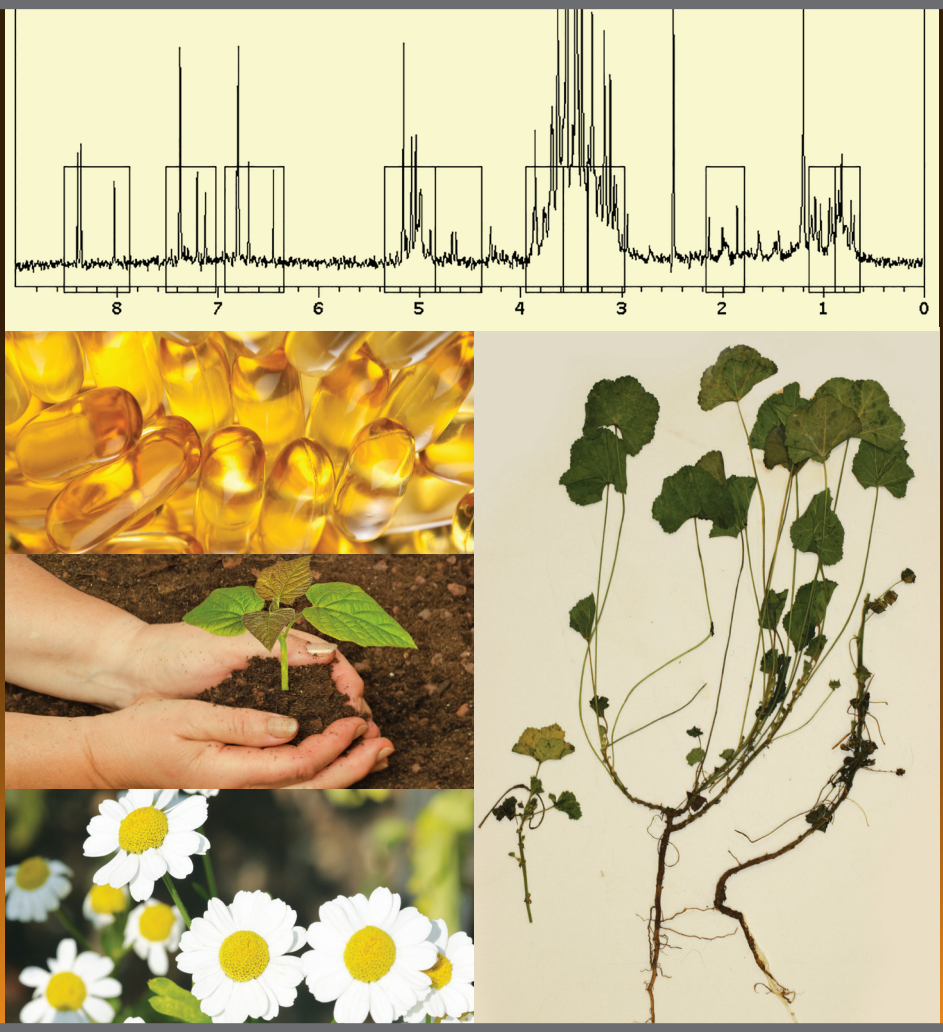
BOTANICALS

Methods and Techniques for Quality & Authenticity

EDITED BY KURT A. REYNERTSON • KHALID MAHMOOD



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Preface

Anyone who works with botanicals has at one time or another been confronted with concerns about the quality and authenticity of their materials. The news media fan the flames of these issues; propagating stories of contamination, adulteration, and poor quality; driving apprehension; and seeding doubt in the minds of consumers.

Whether working in academia, government, or industry, at some point in the life cycle of botanical materials, we relinquish some aspect of certainty to others. The concerns are universal and must be addressed by experts, researchers, sourcing managers, and consumers alike.

To that end, the purpose of this book is to compile methods and techniques that can be used to help guide quality and authenticity determinations. Assembling the most current information in one place provides guideposts for method applications and helps point to gaps in this important area of botanical quality assurance.

With the natural trend growing in popularity annually for food, dietary supplements, cosmetics, and personal care products, it was our hope that this volume would serve as a focal point and reference for identifying appropriate quality and authenticity methods specific to the need of the reader.

As new technologies are developed, software and computing power have advanced rapidly to assure that this is an area that will continue to modernize and adapt. The ability to statistically analyze big data sets is emerging as an important aspect, even when applied to more *traditional* chemometric analyses. This edition contains methods which are currently in use as well as methods which are still being developed, with the promise of becoming more routine soon. We deliberately chose to have both the new technologies and more traditional techniques. The authors represent industry, government, and academia. And while we realize that there are as many omissions as inclusions in the topics covered, we have tried to make this first edition as comprehensive as possible. Gaps remain, which future editions may rectify. For that reason, we hope that the readers and users of this book will reach out with their feedback and constructive suggestions.

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I thank and acknowledge my brother Tariq Mahmood. Without him, I would not have been able to initiate or complete this project.

Khalid Mahmood

The original idea for this book sprang out of a conversation with Hilary Rowe following a scientific session on the topic at the International Congress on Natural Products Research in New York City in 2012. It has been a long journey to assemble, and I appreciate the support of my family and management in allowing me the time to dedicate to the project.

Kurt A. Reynertson

Editors

Kurt A. Reynertson has worked in natural products chemistry for over 15 years and published numerous research articles in international journals. He completed his PhD in phytochemistry at the City University of New York and worked as a post-doctoral researcher in cancer pharmacology at Weill Cornell Medical College before joining the Naturals Platform team at Johnson & Johnson Consumer Products, Inc. His work is primarily focused on early discovery R&D, which puts him on the front lines of botanical quality and authenticity issues.

Khalid Mahmood graduated with a PhD degree in organic synthesis chemistry from a Natural Products Institute in Pakistan. Pursuing a synthesis-based graduate program at the center of excellence for natural products chemistry provided an opportunity to learn both disciplines. This turned out to be the basis upon which he would build his professional career. Since his arrival to the United States as a post-doctoral scholar, Dr. Mahmood has accumulated significant exposure in the areas of brain receptor research at University of Pittsburgh and consumer products research working for small-sized companies in Illinois and California. For the past eight years, Dr. Mahmood has been the Naturals Platform leader at Johnson & Johnson Consumer Products, Inc. This is where combined experience from various sectors has helped him obtain inventorships on eight patents and multiple patent applications claiming natural technologies useful for personal care products. Dr. Mahmood has won multiple leadership awards for his initiatives at Johnson & Johnson to innovate and to manage discovery programs. He is recognized by Johnson & Johnson Consumer Products, Inc. for his research and outstanding record of collaborations earning him a Johnson & Johnson Consumer Fellow designation.

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1 The Importance of Quality and Authenticity for Botanical R&D

Kurt A. Reynertson and Khalid Mahmood

The international trade in plants is growing steadily as the worldwide demand for natural and botanical raw materials increases. In 2012, herbal dietary supplement sales were the highest ever, at approximately $5.6 billion [1]. However, this doesn't include cosmetics and teas. One recent estimate of the global market in herbal personal care and cosmetics sets the value at $12 billion in 2005 [2].

Increasingly, customers value natural products and botanicals as *green* alternatives; herbs and naturally derived ingredients are perceived as safer for both human use and environmentally and socially responsible choices. They are increasingly distrustful of what is perceived as *unnatural* chemical ingredients. Mainstream consumers expect that the companies who make these products are leaders in this respect.

Although it may be true that plants with a long history of traditional use are generally regarded as safe for human consumption, when considering the production of raw materials, the life of a plant from seed to shelf involves many complicated issues. Botanicals are different from traditional chemical raw materials and require a specific type of quality assurance and quality control. Botanical ingredients for herbal supplements and personal care products are partially manufactured as living organisms. In addition, they also typically undergo processing to become crude extracts or highly processed ingredients. Regardless of the level of final processing, quality considerations fall into three general categories: safety, authenticity, and sustainability.

There have been many well-publicized accidental and intentional contamination of botanicals with pharmaceuticals and/or incorrect plant species. In the absence of good manufacturing practices (GMPs) for herbals in 1997, several lots of plantain (*Plantago* spp.) were found to be adulterated with *Digitalis lanata* [3]. Lack of proper quality measures resulted in the mislabeling of over 3000 pounds of a relatively innocuous herb with one containing potent cardiac glycosides. Over 15 years later, we still face the need to develop new quality control methods for botanical materials. Unintentional contamination and economically motivated adulteration are issues that must be considered when dealing with plant materials or extracts that have been purchased from traders and other consolidators, or when the provenance of the raw materials is unclear or unknown. In order to insure that botanical raw materials are safe and effective, the supply chain must be tightly monitored and

carefully considered. It is also necessary to have testing methods that are accurate, appropriate, and cost-effective in order to test and analyze materials.

Many countries have regulations governing the safety and labeling of botanicals for food, medicine, and supplements. In the United States, the Dietary Supplement Health and Education Act of 1994 (DSHEA) defines the regulatory environment for supplements; 21 CFR 111 states that a *scientifically valid* method of analysis must be used to ascertain the purity, authenticity, and quality of a dietary supplement. However, the onus of rigorously applying safety standards comes from peer review, consumer pressure, and industry alignment.

Generally, plants should be grown in accordance with good agricultural and collection practices and should be stored and processed in accordance with GMPs. In addition, consideration must be given to international treaties such as the Convention on International Trade in Endangered Species, the Convention on Biodiversity, and the Endangered Species Act. This insures a level of quality that affects all levels of safety, authenticity, and sustainability; issues of access and benefit-sharing related to ethnobotanically sourced material are also fundamental to ethical business practice.

Quality and authenticity assurance for natural materials may have its roots in classical taxonomic methods, but there are limitations. The shifting natural landscapes calls for a need to develop appropriate technologies in addition to traditional tools. The development of new and sensitive methodologies that can answer specific questions is partly due to the increased popularity of natural ingredients and partly due to technological advances that have been applied in new ways to answer those questions. Most of the publications detailing the use of new methodologies for the authentication of natural materials tend to focus on high-value commodities like flavors and fragrances [4,5], spices [6,7], and honey [8,9]. The quality of botanical products, however, is more than an economic issue; regardless of whether the raw materials are used for food, supplements, or medicine; the identification and authentication issue is also a public health and safety concern. In a post-DSHEA United States, the quality issue as it relates to botanicals has been brought to the forefront of consumer awareness.

Today natural ingredients are used in cosmetics, dietary supplements, herbal medicines, personal care, and household products. These industries deploy natural ingredients in slightly different ways requiring differing needs of quality assurance. We saw a need to consolidate various methodologies in one place for the user to consider how to apply appropriate methods to achieve authentication of new and existing natural ingredients, thereby enhancing quality and safety of the ingredients. This knowledge should also help to mitigate economically motivated adulteration or incidental contaminations. This volume is an attempt to bring together several different tools for botanical quality and authentication in one place, including traditional, taxonomic, and newer analytical tools. It is not perfect to the satisfaction of every need but a good start. We hope that future revisions of the volume, if taken up, continue to capture most recent advances of this area. This book may not provide how-to instructions, but should be able to point the reader in the right direction.

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2 The Importance of Proper Selection of Product Quality Specifications and Methods of Analysis for Botanical Product Evaluation

Paula N. Brown, Michael Chan, and Joseph M. Betz

CONTENTS

INTRODUCTION

Recently, there has been a significant public outcry over the presence of adulterated food products in the marketplace. The most highly publicized of these incidents resulted in human deaths or injuries in China and was caused by adulteration of wheat gluten and other protein-based ingredients with melamine and cyanuric acid [1]. The public reaction focused on the quality of imported food and the inadequacy of import monitoring systems, but a deeper issue is the adequacy of test methods used for the evaluation of product and ingredient quality. The problem is not unique to conventional plant-derived food products. Many tests that are used for the evaluation and quality assurance of botanical products, regardless of whether they are considered drugs or dietary supplements, can give misleading answers if not selected

and interpreted correctly. The proper selection and implementation of analytical methods is essential for the assurance of product quality, safety, and regulatory compliance.

In 2007, the US Food and Drug Administration (FDA) issued a final rule on current good manufacturing practice (CGMP) in manufacturing, packaging, labeling, or holding operations for dietary supplements [2]. This is only the most recent of many regulations that have been introduced in the United States to address product quality. The first such legislation was the Drug Importation Act of 1848 [3–5]. This particular legislation was introduced in response to adulterated botanical drug products entering the United States [3–5]. Among its provisions, the legislation required that imported drug products be inspected and tested prior to acceptance into the country [3]. The products had to conform to standards set in the pharmacopoeias and dispensatories of the United States and Europe [3].

Passage of the Act resulted in a reduction in the number of adulterated products entering the US market [4–6]. Large quantities of *greatly adulterated* botanical products, some *deteriorated by age and other causes* and others having had their *active properties removed*, were seized and condemned by customs authorities. Among the notable seizures were opium and scammony that already had their alkaloids and purgative glycosides, respectively, removed; jalap root of "spurious or bastard varieties, mixed with a small proportion of the genuine root"; and yellow bark and cinchona bark that was of a "bastard variet[y] that afford[ed] no quinine and very little if any cinchonine" and was considered "worthless for medicinal purposes" [6]. Yet, despite these successes, the Act could not solve all the adulteration problems associated with botanicals as several technical barriers that hindered its enforcement and limited its impact became readily apparent [4,5]. For instance, although the different pharmacopeias and dispensatories served as guides, they did not always agree on quality standards, leading to confusion among both regulators and suppliers over exactly what constituted a quality product [4,5]. Furthermore, there was a paucity of analytical methods for testing against many of the standards, and several of the methods that were available were complicated, were time consuming, and required specific training and expertise to perform [4,5].

Over 150 years later, the aforementioned final rule for CGMPs was published in 2007 [2]. The CGMP regulations require that manufacturers identify steps requiring control and establish specifications for identity, purity, strength, and composition, including limits on those types of contaminants that may adulterate or lead to adulteration of the finished product [2]. Furthermore, Section 111.75 of the CGMP final rule compels manufacturers to verify that these specifications have been met through appropriate, scientifically valid testing or examination [2]. In the century and a half between the two sets of requirements, there have been significant advances in technology and science, yet the issues faced in compliance and enforcement of the dietary supplement CGMP regulations are similar to those that plagued the Importation Act of 1848.

The current regulations specify that manufacturers "ensure that the test and examinations that you use to determine whether the specifications are met are appropriate, scientifically valid methods" and state that "a scientifically valid method is one that is accurate, precise, and specific for its intended purpose" [2]. These three requirements cannot be addressed independently, and it is not possible to say how precise and accurate a given measure is without first knowing the nature of the measurand.

The determination of what is measured and how it is measured is the core of the method's intended purpose. In turn the intended purpose of a method is very much dependent on the matrix in which measurements will be made, the quality standards it is meant to measure against, and what these standards ultimately reveal about the product and/or ingredient.

Many factors need to be considered when selecting and/or developing a method for evaluation against specifications and there exist numerous approaches to evaluating method performance characteristics [7–10]. This chapter will discuss some of these topics but will focus primarily on the importance of the measurand or property being analyzed when selecting and evaluating a method of analysis. These measurands or properties become the measureable components of the product specification through which product quality is judged. A thorough understanding of what each specification represents in terms of its characteristics, liabilities, and limitations is essential to ensure proper selection and evaluation of the methods underpinning the specifications.

Ideally, a product would be easily recognizable and would always conform to its specifications. Unfortunately, products can become adulterated through accidental, negligent, or even intentional means, and proper specifications will shield consumers against these mishaps.

Proper specifications that include scientifically valid methods suitable for the uses detailed therein are essential to product and ingredient characterization. When used as aids in development and maintenance of quality assurance programs, complying with regulations, and planning and interpreting scientific studies; this characterization is a critical element in assuring public safety and effectively documenting salubrious and adverse outcomes [2].

Quality specifications, including levels and profiles of desirable and undesirable constituents, are created for a variety of reasons. Specific chemical constituents, parameters, and/or profiles can be set to define product properties such as permissible natural toxin and pesticide levels and microbial load (and by proxy, safety), phytochemical marker levels (and by proxy, efficacy), ingredient identity, and provenance. It is important to keep in mind that the existence of a specification for an efficacy surrogate does not necessarily establish a causal relationship between the surrogate and the biological endpoint. Associations between markers and biological effects have been sometimes inferred by observing positive clinical or other biological endpoints and correlating these with the composition of the product used, but unless studies designed to establish causality have been performed, only associations can be made, and the identity of the specific active agent may be unknown. For that reason, measuring the definitive bioactive agent or property may be difficult or not possible. In fact, for most herbal products, the identity of these compounds is not known. In other cases, the identity of the putative active chemical(s) may be known, but they are unstable or not accessible to current analytical techniques, making them extremely difficult and/or costly to analyze directly. In these cases, it may be more feasible and economically attractive to measure other compounds or properties that are in some way associated with the causative agents or properties. When utilizing such surrogate methods however, it is important to be aware of and be able to account for their limitations. Failure to do so can have significant consequences, a notable example of which can be seen with the aforementioned protein adulteration.

LIMITATIONS OF INDIRECT METHODS—PROTEIN PRODUCTS ADULTERATED WITH MELAMINE

One of the most publicized series of incidents of product adulteration was the recent reports of adulterated protein products from China. Although the products associated with the incident are not strictly botanicals, the issues, limitations, and consequences associated with the analytical methods at the center of the incident are very similar to the ones that are faced by the botanical industry. Starting in early 2007, severe adverse event reports led to the discovery that *high protein* pet foods, wheat gluten, baby formula, and other dairy products imported from China were adulterated with melamine and cyanuric acid [1]. For these products, protein content is a measure of their quality [11]. The two common analytical methods used to measure protein content, the Kjeldahl method and the Dumas method, do not actually measure protein [12]. Instead, they measure total nitrogen content as a surrogate, assuming that the source of any nitrogen in the product is from protein. The methods cannot differentiate between protein and nonprotein nitrogen. Total nitrogen values obtained using the method(s) are converted to an estimated protein content by using a response factor. The addition of nonprotein nitrogen-containing compounds to products is thus one way to fool these tests and increase their apparent protein content.

The adulteration of these products meant product quality could no longer be assured simply through the determination of the product's nitrogen level. Specifications for these products were expanded to include a test for the detection of certain known adulterants. To ensure safety and quality, several regulatory agencies now require that certain protein-containing products be analyzed for melamine and other nitrogen compounds [1,12]. The FDA published two methods for detecting these substances in 2008, a gas chromatography-mass spectrometry (GC-MS) screening method for dry protein materials [13] and an liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for infant formula [14].

Although these methods have been effective at detecting many adulterated products, it is still important to keep in mind that they too possess a very limited scope. They are intended to test against only one aspect of the product's specification, namely, the absence of melamine and certain other nitrogen-containing compounds. These methods do not provide any information on protein content, and there is no guarantee that they could detect other nitrogen-containing compounds.

The addition of nonprotein nitrogen compounds to these products appears to be predominantly motivated by a desire to increase the products' apparent protein level and mask other adulteration activities [1]. As such, perhaps the most effective method for product quality would be one that would provide a definitive protein level that does not rely on estimation via nitrogen content. Near-infrared methods that measure peptide bonds [15] and methods that measure amino acids [16] are available but were previously considered more expensive and difficult to perform than the nitrogen methods. Concerns about adulteration expressed by the regulatory, scientific, and industrial community have affected this view and driven further development of more specific methods that have shown promise in at least some matrices [12,15,16].

LIMITATIONS OF INDIRECT METHODS—AMARANTH DYE ADULTERATION IN BILBERRY

Another example of overreliance on a nonspecific quality test that has led to adulteration involved bilberry extracts. Bilberry, *Vaccinium myrtillus* L., is a popular dietary supplement and phytomedicine used for vascular and vision conditions [17]. Several clinical trials lend support to claims made about the fruit's therapeutic action [17]. The majority of these trials utilized bilberry fruit extracts standardized to a total anthocyanin content of 25% [17]. Although anthocyanins have not been definitively demonstrated to be responsible for the positive clinical outcomes, the association of 25% bilberry anthocyanin in products with positive outcomes has led to the adoption of this content as a quality specification for the dark red to purple bilberry extracts [17].

For a number of years, a spectrophotometric assay that measured absorbance at 528 nm (a wavelength characteristic of bilberry anthocyanins) was widely used to estimate anthocyanin content in bilberry extracts [18,19]. The method, published in the 2004 British Pharmacopeia, is simple, rapid, and effective, provided the material analyzed is genuine bilberry, an assumption that cannot always be made. Unfortunately, the method is not specific to bilberry extract, as it will respond to any substance that is more or less the same color as bilberry extract. In 2006, Penman et al. analyzed commercial bilberry extracts using two different analytical methods [20]. When the accepted spectrometric method was used, the measured anthocyanin content was 24%; however, when the investigators used a liquid chromatography (LC)/photodiode array detection method, the measured anthocyanin content was only 9% [20]. Further mass spectrometry (MS) and nuclear magnetic resonance (NMR) analyses revealed that the extract contained amaranth dye, a banned synthetic dark red to purple azo-dye commonly referred to as Red Dye No. 2 [20]. As in the protein case described above, an adulterant within the product was used to fool the indirect method used to measure one of the product specifications.

To address the adulteration issues, additional tests that could assess a quality standard other than absorbance at 528 nm had to be added to product specifications. The American Herbal Products Association published several tools and methods to detect the adulteration of bilberry powdered materials [21]. The first is a simple procedure that involves raising the pH of a dilute solution of bilberry extract and observing the presence or absence of a color change. At elevated pH, very dilute anthocyanin-containing solutions turn blue while dilute solutions containing amaranth dye do not. A high-performance thin-layer chromatography (HPTLC) method provides a visual image of separated individual anthocyanins from any dye present [21]. The latter method not only detects the presence of the dye but also provides for the visualization of an anthocyanin profile characteristic of bilberry providing a higher level of confidence that the extract has not been adulterated with other anthocyanin-containing berries [21].

Neither of these methods is intended to replace the original spectrometric method, which allows determination of whether or not the amount of anthocyanins present in the extract is equivalent to the amount found in the product used in the successful clinical trials. To achieve confidence in identity and compositional quality, both the HPTLC and spectrophotometric methods must be used.

To accomplish both qualitative and quantitative evaluation in a single test, more sophisticated methods such as the high performance liquid chromatography (HPLC) method utilized by Penman et al. are required [20]. In 2007, the botanical extract supplier Indena SpA published a validated liquid chromatographic method for bilberry extract, noting in their publication that if used as directed, only 6 of 40 marketed products they evaluated (15%) would deliver a quantity of anthocyanins that was equivalent to the standard of quality established in the previous clinical trials [22]. Furthermore, 10% of the products analyzed lacked anthocyanins and 25% of those analyzed exhibited an LC profile different from typical bilberry extract [22]. This method was subsequently adopted by the European Pharmacopoeia as the official analytical method for bilberry extract [23].

METHOD PRECISION, ACCURACY, AND INTENDED PURPOSE IN RELATION TO PRODUCT SPECIFICATIONS

Even though the use of indirect methods for quantitative determinations in both the melamine and bilberry examples led to significant problems, the use of these methods, or indirect methods in general, may in some cases be appropriate. As with all methods, the context in which they are used and the assumptions associated with their use must be considered when assessing their applicability. The UV method for bilberry extracts as described in the British Pharmacopeia is appropriate for the particular quality specification for which it was designed. Clinical trials had shown that bilberry extracts exhibiting a prescribed value of absorbance at 528 nm was of sufficient quality to be considered effective. The specific purpose of the method was to test this specification. The assumption underlying the utility of this test is that the extract being tested is a bilberry extract. An additional specification accompanied by a test or tests would be needed to determine that the extract is bilberry and does not contain other substances such as dyes or other anthocyanin-containing fruits. The single spectrophotometric specification is insufficient to ensure the quality of the product. Additional tests for the presence of dyes or nontarget anthocyanins are required. Once these specifications are determined, test methods whose purposes are specific for those specifications can be developed and selected. Note that a working knowledge of potential confounders (dyes, other berries) would be very useful in establishing meaningful specifications.

Additional considerations in the creation or selection of quality specifications involve the capabilities of the analytical methods required to test whether or not specifications are met. For dietary supplements, 21 CFR Part 111.75 requires manufacturers to "ensure that the tests and examinations that you use to determine whether the specifications are met are appropriate, scientifically valid methods," and notes that "a scientifically valid method is one that is accurate, precise," as previously discussed, the method must also be "specific for its intended purpose" [2]. Accuracy can be simply defined as the closeness of the value obtained from an analysis to the true value in the sample, whereas precision is defined as the measure of how close individual measurements are to each other [24]. Detailed definitions of these two parameters, as well as the procedures through which they are determined and assessed, are available in guidelines published by several organizations including the AOAC International, FDA, the International Union of Pure and Applied Chemistry,

and the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use [7–10]. By following these guidelines, it is possible to determine if a method is accurate and/or precise when used to measure particular properties. However, it is still imperative that the properties being measured are the ones that give the desired information about quality. The Kjeldahl and Dumas methods demonstrated high accuracy and precision in the analysis of nitrogen [11], and the bilberry spectrophotometric method provides precise and accurate absorbance values [20]. Yet, despite their high accuracy and precision, these methods are not useful for the identification of ingredients or the detection of the adulterants associated with the respective products.

Typically, highly precise and accurate methodologies are desirable; however, these traits can be dictated by the quality specification being examined. The most direct example of this can be observed in a phenomenon referred to as the *Horwitz Trumpet* [25]. While reviewing collaborative study data, Dr. William Horwitz discovered a striking relationship between analyte concentration in a matrix and standard deviation [25]. Horwitz observed that in general, as concentration of analyte decreased across two orders of magnitude, the relative standard deviation of reproducibility ($rRSD_r$) increased by a factor of 2 [25]. This relationship was observed regardless of the nature of the analyte or the type of methodology used [25], and thus, the achievable accuracy and precision of a measurement of a selected quality parameter are related to the concentration of the analyte in the test article.

The relationship between $rRSD_r$ and analyte concentration has since been used as a means to evaluate analytical method performance [7,26]. Horwitz developed a formula through which a predicted relative standard deviation of reproducibility ($pRSD_r$) could be determined based on the concentration of analyte [26]. Methods that have $rRSD_r$ that is substantially different from the $pRSD_r$ can be considered flawed [26]. Such methods may benefit from further optimization or development. In certain cases, a reevaluation of the quality standard under scrutiny could be warranted.

IMPACT OF SPECIFICATION SELECTION ON ACCURACY AND PRECISION—DETERMINATION OF GINSENOSIDES IN GINSENG

The main constituents of interest in North American and Asian ginseng (*Panax quinquefolius* L. and *P. ginseng* C.A. Mey., respectively) are the triterpene saponin ginsenosides, which are present in both neutral and acidic malonyl forms [27]. The malonyl ginsenosides are more polar and water soluble than their corresponding neutral counterparts and are not measured by most ginsenoside assays [27]. They are, however, susceptible to hydrolysis and can be readily converted to their neutral counterparts during analytical extraction [28,29]. Uncontrolled hydrolysis during sample preparation can result in inconsistent values when quantifying the neutral ginsenosides [29,30]. A recent interlaboratory study evaluating a HPLC method for ginsenoside quantification demonstrated the poor precision that can result under uncontrolled circumstances [30]. The analysis of two separate ginseng finished product samples following a controlled alkaline hydrolysis step designed to quantitatively convert the acidic malonyl ginsenosides to their neutral counterparts prior to HPLC determination demonstrated greatly improved precision compared to analyses

performed without forced hydrolysis [30]. Subsequent analyses included the controlled hydrolysis step and the improved method was found to be precise enough to meet AOAC International requirements [30].

An alternative approach to achieving precision in the ginsenoside analysis could have been to simply remove the malonyl ginsenosides by some other technique, such as solid-phase extraction, and measure only the native neutral compounds. However, although the malonyl ginsenosides are apparently not as biologically active as the neutral forms, they can exert a pharmacological effect in the body after they are converted to their neutral counterparts in the gut [27]. As in the bilberry example, a specification designed to assure the quality of a ginseng product in terms of its ability to elicit a biological effect *in vivo* must be expanded. In this case, the method is required to take the malonyl ginsenosides into account.

CONCLUSION

Concerns about product quality and the means through which it is assessed have existed since the earliest development of commerce and trade. Both Pliny the Elder's (23–79 CE) *Naturalis Historia* [31] and Dioscorides' (40–90 CE) *Materia Medica* [32] described herbal products based on their predominant characteristics as well as methods through which adulteration of these products could be detected. Although there have been significant advances in science and technology since the original publications of these books, the same fundamental questions and challenges remain, namely, what specifications should be selected to represent the quality of a product and how those specifications can be evaluated. A firm understanding of specifications and what they are intended to represent is essential to ensuring product quality and consistency. This understanding is incomplete unless the properties of the methods used to evaluate against specifications are also known. These properties include the nature of the measurand, the nature of the product matrix, a grasp of what the measurement results represent, the inherent limitations of the method, and any potential confounders. Even with this knowledge, specifications (and tests) should be continuously evolving as new confounders and technologies come into existence.

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