ABC AHP NCNPR
Botanical Adulterants Prevention Program
American Botanical Council • the American Herbal Pharmacopoeia • the University of Mississippi's National Center for Natural Products Research

Grape Seed Extract Laboratory Guidance Document

By Steve A. Kupina,[a] Mark A. Kelm, PhD,[a] Maria J. Monagas, PhD,[b] and Stefan Gafner, PhD[c]
[a] Polyphenolics, Madera, CA 93637
[b] United States Pharmacopeia, Rockville, MD 20852
[c] American Botanical Council, PO Box 144345, Austin, TX 78714
Corresponding author: email

Vitis vinifera
Photo ©2019 Steven Foster

Keywords: *Vitis vinifera*, grape seed extract, adulterant, adulteration

Citation (JAMA style): Kupina SA, Kelm MA, Monagas MJ, Gafner S. Grape seed extract laboratory guidance document. Austin, TX: ABC-AHP-NCNPR Botanical Adulterants Prevention Program. 2019.

1. Purpose

Grape Seed Extract (GSE)* has received acceptance almost globally as an ingredient for human consumption. It is one of the more widely used botanical extracts, due to increasing scientific findings supporting health benefits.[1,2] However, it remains a specialty item relative to global commodities. In the United States, GSE has ranked among the top 20 best-selling dietary supplements in the Food, Drug and Mass Market channel. The motivation behind purposeful adulteration in commercial products is financial gain (also known as economically motivated adulteration) and to increase the concentration in proanthocyanidins (PACs) as primary marker compounds, as a means to contribute to the misperception of quality. Adulterants include other PAC-rich materials (Table 1), which are available at lower cost. Thus, a bulk distributor of GSE or another manufacturer along the value chain can take advantage of the chemical similarity between GSE and peanut skin extract since the spectrophotometric assays typically used in industry are not specific enough to discriminate between grape seed PACs and PACs from other plant extracts. Due to reliance on non-specific proximate assays across the value-chain, adulteration can go undetected downstream in the commodity chain, such as those involved in distribution, packaging, wholesale, and retail sales. This laboratory guidance document presents a review of the various analytical technologies and methods used to differentiate between grape seed extracts and potential adulterants.

2. Scope

The analysis of PACs by chromatographic methods such as high-performance liquid chromatography (HPLC) or high-performance thin-layer chromatography (HPTLC), which are commonly used to determine the identity of botanical ingredients, is challenging as current stationary phases have limited capacity to separate the PACs due to the structural similarity of the many PACs in GSEs and their polymeric structure. Other tools to authenticate GSEs are available, but instrumentation to perform the analysis may not be available in many quality control laboratories or the expertise to perform the necessary work may be lacking.

This laboratory guidance document intends to assess analytical methods for GSE analysis, and to determine the suitability of each of these methods with regards to its ability to authenticate GSEs and to detect adulteration with PAC-rich extracts from other plant sources. In addition, existing methods are evaluated for their ease-of-use in a quality control laboratory. A specific method for testing GSEs in this Laboratory Guidance Document does not remove the responsibility of quality control and laboratory personnel to demonstrate adequate method performance in their own laboratory (and/or in a qualified third-party contract laboratory) using accepted protocols outlined in the Good Manufacturing Practices for dietary supplements in the United States (21 CFR Part 111) and/or by AOAC International, International Organization for Standardization (ISO), the World Health Organization (WHO), and the International Conference on Harmonization (ICH).

3. Common and Scientific Names

3.1 Common name: Grape

3.2 Other common names
English: European grape, wine grape[3]
Chinese: *Pu tao* (葡萄)†
French: Raisin
German: Traube, Weintraube

* The acronym GSE should not be confused with the acronym GFSE, referring to Grapefruit Seed Extract, which is an entirely different material. In some original publications on GFSE adulteration, the authors use "GSE" to refer to grapefruit seed extract.

† Grape seed in Chinese is known as *pu tao zi* (葡萄籽).

Italian: Uva
Spanish: Uva

3.3 Accepted Latin binomial: *Vitis vinifera* L.[3]
3.4 Synonyms: *Cissus vinifera* (L.) Kuntze[4,5]

3.5 Botanical family: Vitaceae

4. Botanical Description

Grapes, the fruit of the grape vine, have been used as a source for food and beverages for thousands of years, and are easily distinguished from adulterating species when present in the whole form. The seeds of grapes, obtained as a by-product from the juice or wine industry, are used fresh, or more commonly dried to produce a liquid extract using a solvent (e.g., water, or mixtures of water with ethanol, ethyl acetate, or acetone) which is filtered, and may be subjected to further processing before it is typically spray-dried to obtain a dry extract containing high levels of naturally occurring grape seed phenolic compounds.

Peanut skin extract, which is a high-volume byproduct of the peanut industry, is less expensive and typically available at a much greater volume than GSE. In the United States, a typical peanut mill may produce up to 17 tons of peanut skins per week, and the material was sold for as little as US $0.02/kg in 2009.[8]K.A. Costs for processed materials are still low, e.g., in China, in 2015, the price for peanut skin

Table 1. Scientific names, family, and common names of potential grape seed extract adulterants

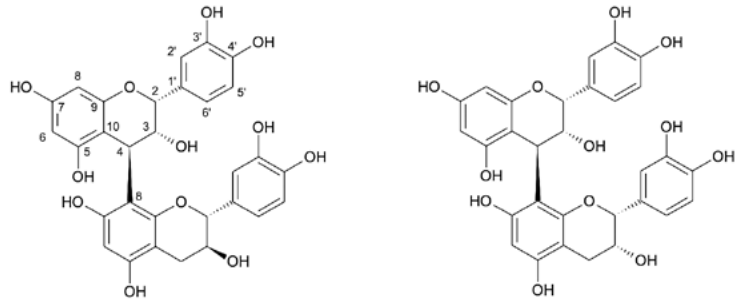
Species	Synonym(s)	Family	Common name[a]	Other common names	Plant Part
Arachis hypogaea L.	*Arachis nambyquarae* (Hoehne) Burkart	Fabaceae	Peanut	Arachis	Skin
Camellia sinensis (L.) Kuntze	*Thea sinensis* L.	Theaceae	Tea	Black tea, Chinese tea, green tea	Leaf
Malus domestica (Suckow) Borkh.	*M. pumila* auct. *M. pumila* var *domestica* (Borkh.) C.K. Schneid *M. sylvestris* auct. *M. sylvestris* var. *domestica* (Borkh.) Mansf. *Pyrus malus* L.	Rosaceae	Apple	Cultivated apple	Fruit
Pinus massoniana Lamb	*P. massoniana* (Lamb.) Opiz *P. argyi* Lemée & H.Lév. *P. canaliculata* Miq. *P. cavaleriei* Lemée & H.Lév. *P. crassicorticea* Y.C.Zhong & K.X.Huang *P. nepalensis* J.Forbes *P. sinensis* D.Don	Pinaceae	Masson pine	Chinese red pine, southern red pine	Bark
Pinus pinaster Aiton	*P. lemoniana* Benth. *P. nigrescens* Ten. *P. syrtica* Thore	Pinaceae	Maritime pine	Cluster pine, pinaster pine	Bark
Prunus dulcis (Mill.) D.A. Webb[b]	*P. amygdalus* Batsch *P. amygdalus* var. *dulcis* (Borkh. ex DC.) Koehne	Rosaceae	Almond	Bitter almond, sweet almond	Hull
Sorghum bicolor (L.) Moench	*Andropogon sorghum* (L.) Brot. *S. basiplicatum* Chiov. *S. caudatum* (Hack.) Stapf *S. centroplicatum* Chiov. *S. dochna* (Forssk.) Snowden *S. durra* (Forssk.) Trab *S. eplicatum* Chiov. *S. roxburghii* Stapf *S. saccharatum* (L.) Moench *S. vulgare* var. *vulgare*	Poaceae	Sorghum	Columbus grass, Johnsongrass, common wild sorghum, grain sorghum, Sudan-grass	Skin

[a]*Herbs of Commerce*, 2nd ed.[3]
[b]Propelargonidin containing extracts from non-grape seed sources.[6,7]

extract was at US $10-13/kg, compared to pine bark extract at US $20-22/kg, and GSE at US $30-35/kg, although proprietary GSEs may be sold for up to US $110/kg.

Sections 5-8 of this document discuss macroscopic, microscopic, genetic, and phytochemical authentication methods for grape seed. A comparison among the various approaches is presented in Table 3 at the end of section 9.

Figure 1: Chemical structures of the two predominant procyanidins in GSE[6]



Procyanidin B1 Procyanidin B2

5. Identification and Distinction using Macroanatomical Characteristics

Macroscopic identification criteria of grape seeds[9,10] can be helpful to manufacturers of GSE that purchase dried grape seeds, to avoid confusion with other dried seeds. Macroscopic evaluation of GSEs may help to determine the absence of exogenous anthocyanin-containing materials based on variations in the typical brown color of GSEs. However, macroscopic identification is inadequate to authenticate GSEs or detect adulteration.

6. Identification and Distinction using Microanatomical Characteristics

There are some publicly available data on the microscopic features of grape seeds.[10,11] However, microscopic distinction among GSEs and extracts of its adulterants has not been done. It is not possible to identify these adulterants by microscopy because the extraction process removes any characteristic cellular structures that may be used to determine the identity of the material. Therefore, botanical microscopy is not an appropriate means to authenticate grape seed extracts.

7. Genetic Identification and Distinction

There are no published genetic methods to authenticate GSE, or to detect adulteration with extracts from other plant sources. While some DNA-based methods have successfully been able to authenticate certain dried extracts,[12-15] the limitations in determining the identity and composition of highly processed materials are well-known,[14,16,17] and, as such, genetic methods are of limited use at this time for GSE authentication or detection of adulterants.

8. Chemical Identification and Distinction

While there is an abundance of published analytical methods for grape juice and red and white wines, the number of methods for authentication of GSEs, and the detection of GSE adulteration is limited. Distinction between GSEs and their adulterants by chemical analysis requires knowledge of the composition of the ingredients, in particular the structures of the PACs found in grape seeds and their adulterating species. The composition of GSEs, and extracts of their main adulterants, is indicated below. The data are based on published literature; however, the composition of these extracts depends on many factors, including the geographic origin of the source material, the cultivar, and the extract manufacturing process. Review of additional PAC types occurring in various food and non-food ingredients has been published by Hellström et al, and Monagas et al.[18,19] The main characteristics of PACs from grape seed and other low-cost food sources are listed in Table 2.

8.1 Chemistry of GSE and the potential adulterants

Vitis vinifera: GSE is almost exclusively supplied to dietary supplement manufacturers in the form of a dry extract. The extract contains phenolic compound concentrations ranging from ca. 50 – 90% of the extract. The main phenolic compounds are flavan-3-ol monomers and polymers and their gallic acid esters. Grape seeds contain predominantly B-type PACs, which are flavan-3-ol polymers where the units are linked by a single bond (Figure 1). Appeldoorn et al. isolated procyanidin B1‡, B2, B3, and B4 from a commercial GSE, accounting for 3.2%, 7.1%, 1.5%, and 1.2%, respectively, of the extract.[20] Similar results were reported by Weseler and Bast,[21] with concentrations of 7.7%, 8.3%, 2.8% and 1.6% of procyanidins B1, B2, B3, and B4, respectively. The presence of B-type dimers, trimers, tetramers, and polymers of up to the size of a dodecamer trigallate was described by Weber et al.[22] The authors analyzed four commercial GSEs, and found that the molecular weight distribution varied substantially depending on the product. Average degrees of polymerization (DP) for commercial GSEs were reportedly between 3-11,[23,24] although depending on processing, the DP may deviate substantially from these values. Other authors have described two different types of GSE on the market, with polyphenol contents depending on the treatment with the enzyme tannase, which cleaves gallic acid units from molecules such as flavan-3-ols, converting e.g., epicatechin-3-*O*-gallate into epicatechin and gallic acid. While untreated GSEs generally have gallic acid concentrations

‡The terms proanthocyanidin and procyanidin seem to be used interchangeably in the literature. However, proanthocyanidin is a generic term for a family of structurally related polyphenolic compounds comprised of the procyanidins, prodelphinidins, propelargonidins, etc. The different proanthocyanidin classes are distinguished by the specific flavan-3-ol hydroxylation pattern, e.g., 3,3′,4′,5,7-pentahydroxyflavan-3-ol in case of the procyanidins, or 3,4′,5,7-tetrahydroxyflavan-3-ol for the propelargonidins. The name "proanthocyanidin" is derived from the fact that these compounds produce anthocyanidins when treated with a mineral acid. Specifically, a procyanidin will produce the anthocyanidin cyanidin, a prodelphinidin will yield the anthocyanidin delphinidin, a propelargonidin will be converted into pelargonidin, etc.

of less than 0.5%, tannase-treated extracts have gallic acid concentrations increased to levels between 1.75 – 4.8%, but only trace amounts of galloylated procyanidins.[7,25-28]

Arachis hypogaea: Peanut skin extracts contain both A-type and B-type PACs.[29,30] Appeldoorn isolated a number of PACs from peanut skin, with A-type dimer procyanidin A1 and A2 as most abundant (6.9% and 2.1%, respectively).[20] Procyanidin B7 was present at 0.2%.[20] Dudek et al. confirmed the presence of procyanidins A1 and A2, and isolated four trimers and two tetramers, named peanut procyanidins A-F. Besides procyanidin A1, peanut procyanidin E was the most abundant in a 70% aqueous acetone extract of the skins.[31] Other phenolic compounds in peanut skin include flavonols (quercetin, kaempferol, isorhamnetin and their glycosides), isoflavones (genistein, hesperetin), anthocyanins (cyanidin, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-sophoroside, peonidin-3-*O*-galactoside, and petunidin-3-*O*-galactoside), and the stilbene resveratrol.[32]

Camellia sinensis: Green tea extracts are well characterized and dominated by flavan-3-ol monomers and their gallates. The main flavan-3-ol in green tea leaves is epigallocatechin gallate (EGCG) at 0.7-14.4%, followed by epigallocatechin (0-4.7%), epicatechin gallate (0.2-4.2%), epicatechin (0-1.5%), catechin (0-1.2%) and gallocatechin (0.3-0.8%).[33-36] Highly purified extracts containing up to 95% of epigallocatechin gallate (EGCG) are marketed, but concentrations in extracts used in commercial dietary supplements are generally between 40-70%. Green tea leaf also contains 1-3% caffeine, 0.01-0.9% theobromine, and small amounts (< 0.1%) of theophylline.[33,34,37] In addition, the leaf contains flavonoids, predominantly glycosides of quercetin, kaempferol, and myricetin as 3-*O*-glucosides, 3-*O*-galactosides, 3-*O*-rutinosides, 3-*O*-galactosylrutinosides, and 3-*O*-glucosylrutinosides.[35,38,39] The chemical profile of green tea extract and GSE is altogether different and allows an easy distinction between the two ingredients.

Malus domestica: The phenolics in apple peel are composed of flavonol glycosides (e.g., hyperoside, quercitrin, aviculanin), flavan-3-ol monomers (epicatechin, catechin) and polymers, hydroxycinnamic acids (chlorogenic acid, caffeic acid), and the dihydrochalcone phloridzin.[40,41] Red apple peels also contain anthocyanins, mainly cyanidin-3-*O*-galactoside and other cyanidin glycosides.[42] The PACs have the highest concentration of phenolics in whole apples with > 70% of all phenolic compounds followed by the hydroxycinnamic acids (4-18%), flavonols (1-11%), dihydrochalcones (2-6%) and anthocyanins (1-3%).[42-44] Apple peels were found to contain between 3-28.4 times more phenolic compounds than the flesh. Flavonol glycosides are absent in the flesh, which contains mainly PACs and phenolic acids. Epicatechin and procyanidin B2 were the main flavan-3-ols according to the investigation by Kschonsek et al.[41] The apple PACs consist mainly of linear B-type procyanidins with epicatechin as the predominant unit.[45] According to Feliciano et al., 88.3% of the flavan-3-ol polymers in apples are made of these B-type PACs.[46] While the adulteration of grape seed extract with apple peel PACs does not make economic sense, it is a good source of procyanidin B2, one of the main procyanidins in GSE. (J. Xin [Skyherb] email communication to S. Gafner, November 25, 2018).

Pinus spp.: Weber et al.[22] investigated the PAC type and size in extracts from maritime pine and Masson pine. From an economic perspective, Masson pine extracts are 5-10 fold less expensive than maritime pine bark extracts, making Masson pine more attractive as an economic adulterant (Yannick Piriou [DRT (les Dérivés Résiniques et Terpéniques)] email to Maria J. Monagas, May 3, 2018). Galloylated PACs have been reported from maritime pine and Masson pine, but don't seem to be abundant.[22] The monomer units consist mainly of catechin and epicatechin, although small amounts of epigallocatechin and gallocatechin have been reported as well. [47,48] Typically, pine bark extracts contain only B-type PACs. The average DP of a hot water extract of *P. pinaster* is between 6 and 7. [47,49] Similar results were reported for Scots pine (*P. sylvestris*) by Bianchi et al.[50] The PAC fraction of a hot water extract consisted of exclusively B-type procyanidins with average DP of 6.7. A comparison of HPLC-UV fingerprints between grape seed and Masson pine extract did not show a substantial difference, except that the Masson pine extract had a larger concentration of more highly polymerized PACs and exhibited the peak of an A-type dimer.[7]

Prunus dulcis: The composition of almond skin extract, a byproduct of the almond industry, has been described in a patent by Bartolome et al.[51] Almond skin extract contains PACs with afzelechin, catechin, gallocatechin, epiafzelechin, epicatechin, and epigallocatechin as monomers. Besides catechin and epicatechin, the extract contains mainly procyanidins B1, B2, B3, B5, B7 and C1, and unidentified A-type dimers and trimers, although MALDI-TOF data showed presence of molecules with DPs of up to 10. A-type dimers and trimers made up ca. 19-22% of the total amount of PAC dimers and trimers in almond skin extract. Almond skin extract also contains propelargonidin and prodelphinidin dimers and trimers, albeit at low concentrations (less than 5% of the total amount of monomers, dimers, and trimers). The main flavonoids in the skin have been reported as kaempferol-3-*O*-rutinside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, and naringenin-7-*O*-glucoside.[52,53] The occurrence of prodelphinidins and propelargonidins, and the absence of galloylated PACs may provide a means to distinguish almond skin extracts from GSEs.

Sorghum bicolor: A large number of phenolic compounds have been isolated from *S. bicolor*: These include hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, anthocyanins, flavan-3-ols, and flavan-4-ols.[54-56] The composition of phenolic compounds in sorghum is strongly dependent on the genotype. Ferulic and *p*-coumaric acids are the most abundant phenolic acids, and are mostly bound to cell walls. High amounts of bound protocatechuic acid were reported from white and red sorghum. The flavonoids apigenin and luteolin are predominantly found in tan-pigmented genotypes. Other flavonoids from sorghum include eriodictyol, eriodictyol 5-*O*-glucoside, taxifolin, taxifolin 7-*O*-glucoside,

and kaempferol 3-*O*-rutinoside-7-*O*-glucuronide. Sorghum anthocyanins are unusual by the fact that they do not contain a hydroxyl group in position 3. The most common anthocyanins are apigeninidin and luteolinidin. Sorghums with a black pericarp (bran) have higher amounts of anthocyanins compared to those with red and brown pericarp. The occurrence of flavan-4-ols seems to be restricted to red bran sorghum.[54,55]

Condensed tannins are found only in sorghum genotypes II and III, which have dominant B1 and B2 genes. Sorghum PACs are mainly of the B-type with epicatechin as extension unit and catechin as terminal unit.[55,57] However, Krueger et al. reported the presence of some variations in the PAC composition from Ruby Red sorghum (a type III genotype), e.g., the inclusion of gallocatechin/epigallocatechin units, PACs containing proluteolinidin and proapigenidin monomers, and the occurrence of eriodictyol, or eriodictyol-5-*O*-glucoside as terminal units.[58] White sorghum genotypes contain the monomers catechin and epicatechin, but no polymers.[55,59]

8.2 Laboratory methods

Note: Unless otherwise noted, all methods summarized below are based only on the analysis of the powdered GSE and its adulterants. It should also be emphasized that there is no substitute for a strong working knowledge of PAC chemistry and their methods of analysis when venturing into authentication work.

8.2.1 Chemical and botanical reference materials

With any analytical method, it is important to have reliable standards and or reference materials. This requirement is especially compounded when conducting investigations into adulteration. The question for the analyst may be what potential adulterants to test for. The answers may be logically deduced by asking what classes of compounds and their botanical sources could be used to replace or substitute authentic GSE. Adulterants containing gallic acid, and/or PACs would all be logical substitutes with increasing compositional similarities to GSE. With respect to botanical sources, waste streams from agricultural and food industries would be the most economically attractive. In any scenario, the researcher should consider other qualitative features of a given chromatogram noting and spectroscopically characterizing peaks that appear inconsistent with an authentic GSE reference material, or use the entirety of the chromatographic or spectroscopic/spectrometric fingerprint to assess the authenticity of the material. In addition to an authentic GSE, the analyst should compare the results with those from extracts from rational/potential adulterating materials. A reference grape seed extract can be obtained from the United States Pharmacopeia (USP), while authentic grape seeds are available from Alkemist Labs and ChromaDex. A grape seed/skin mixture is offered by the American Herbal Pharmacopoeia (AHP). However, some of the adulterating materials may not be easily available as botanical reference materials to quality control personnel.

Table 2: Proanthocyanidin characteristics of low-cost materials containing condensed tannins

Ingredient	Monomer(s)	Galloylation	PAC-type	Average degree of polymerization[a]
Grape seed	Catechin, epicatechin	Yes	B-type	2-12[b23,24,60]
Almond skin	Afzelechin, catechin, epiafzelechin, epicatechin, epigallocatechin, gallocatechin	No	A-type, B-type	8-9[61]
Apple peel	Catechin, epicatechin	No	B-type	3-10[40,43,44,62]
Green tea leaf	catechin, epicatechin, epiafzelechin, epigallocatechin, gallocatechin	Yes	B-type	1-1.1[63]
Maritime pine bark	Catechin, epicatechin, epigallocatechin, gallocatechin	Yes	B-type	3-7[49]
Masson pine bark	Catechin, epicatechin, epigallocatechin, gallocatechin	Yes	A-type, B-type	no data
Peanut skin	Catechin, epicatechin	No	A-type, B-type	1-9[64]
Sorghum seed	Catechin, epicatechin	No	B-type	8[61] Red: 10-20[59] White: 5-12[59]

[a]Measured by thiolysis

[b]The number depends on the processing method. For grape seed, average degrees of polymerization between 1-37 have been reported on isolated fractions.[65-67]

An important factor is the use of chemical reference standards for the chromatographic assays. Afzelechin, catechin, epiafzelechin, epicatechin, epigallocatechin, gallocatechin, and some PAC standards (e.g., procyanidins A1, A2, B1, B2, and C1) can be sourced commercially, but many of the grape seed PAC trimers and more highly polymerized molecules are not available from leading manufacturers of chemical reference standards.

8.2.2 Colorimetric assays

The information discussed below is provided as a background for subsequent discussions on more specific approaches to assess adulteration of GSEs. Colorimetric methods for PACs include the Bate Smith reaction, Folin-Ciocalteu method, butanol/hydrochloric acid (HCl) assay, vanillin assay and 4-(dimethylamino)cinnamaldehyde (DMAC) assay. Thorough descriptions with applications, strengths, and limitations of the various colorimetric methods are available.[61,68] A summary on colorimetric methods to measure color and anthocyanins in fruits has been published by Wrolstad.[69] This review lists the strengths and limitations of the various methods.

Comments: Colorimetric assays typically are simple and affordable to run. These assays utilize reagents that react with phenolics to form colored products that are readily quantifiable by absorption measurements. These methods are of great utility for screening of plant materials for phenolics and to measure gross phenolic content.

However, colorimetric assays are non-specific in that they measure total phenolic content (i.e., phenolic acids, flavonoids, PACs, etc.) but fail to distinguish among molecules that may be characteristic of a particular compound class. Since phenolic compounds are found throughout the plant kingdom, these assays provide no information to determine the botanical source or assess adulteration. Regardless, these assays are commonly employed to study GSEs, and often represent the only chemical assay on a certificate of analysis for ingredients that are rich in PACs. With respect to adulteration with peanut skin extracts, colorimetric assays have had limited use.[7,70] Based on the generic nature of the approach, the utility of colorimetry to detect the presence of adulterants in GSE is not supported. It should be noted that colorimetry in conjunction with protein precipitation steps can allow for the determination of total phenolic content and those phenolic compounds that interact with proteins. As such, a purported GSE that does not show an interaction with protein may be considered a low molecular fraction thereof or represent non-authentic material.

The analysis of total anthocyanins using the colorimetric method by Niketic-Aleksic showed that concentrations above 0.1% (w/w) in GSE powder can be detected.[71] Therefore the simple *Total Anthocyanin* colorimetric method can easily be used as a screening tool for the presence of anthocyanins prior to HPLC analysis. Residues from grape skin may make their way into GSEs, but unintentional inclusion of grape skin materials is expected to be at low amounts and therefore lead to anthocyanin concentrations below the 0.1% mentioned above. Presence of anthocyanins in GSE above 0.1% is an indication of adulteration.

8.2.3 TLC/HPTLC

Methods described in the following literature were covered in this review: Lea and Arnold,[72] Villani et al.,[7] and Sudberg et al.[70]

***Comments*:** One of the first thin layer chromatographic (TLC) separations of procyanidins according to their DP was carried out by Lea and Arnold in 1978 using apple ciders.[72] Villani et al.[7] developed a new TLC method in 2015 for a quick and inexpensive qualitative test to detect presence of peanut skin and pine bark extracts in GSEs. A mixture of acetone-acetic acid-toluene was used to develop the samples on a silica TLC plate. A-type PAC dimers were used as markers for peanut skin. This approach can be used to easily determine the presence of A-type PAC dimers in GSEs. However, the authors noted that given similar chemistries, the method does not allow for pine bark and GSE differentiation.

High-performance thin-layer chromatography (HPTLC) was successfully used to detect adulteration with peanut skin extract in commercial GSEs by Sudberg et al.[70] Catechin, authentic grape seed, and peanut skin extracts were used as reference materials. Separations were effected over silica gel

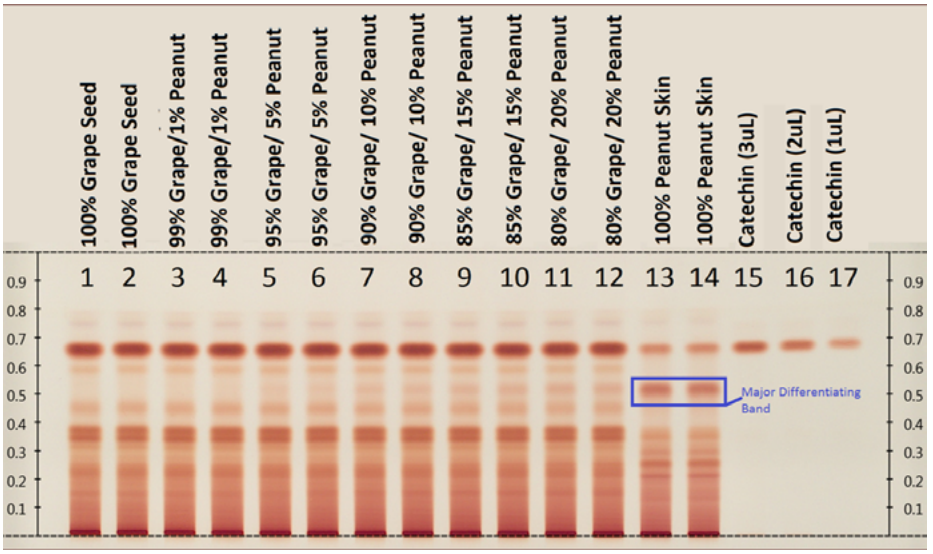


Figure 2: HPTLC analysis of authentic GSE (lanes 1-2), mixtures of GSE and peanut skin extract (lanes 3-12), authentic peanut skin extract (lanes 13-14), and the standard compound catechin (lanes 15-17). Concentrations for extracts were ca. 100 mg/mL, and 0.5 mg/mL for catechin.
Image courtesy of Alkemists Labs; Costa Mesa, CA.

plates using a toluene: acetone: formic acid mobile phase. Bands were observed under visible light post treatment with Fast Blue Salt B derivatizing reagent (Figure 2). The validated HPTLC approach described is a viable diagnostic tool for readily assessing the presence of PACs that typify peanut skin extracts. Limits of visual detection of presence of peanut skin detection were approximated at 5% (w/w).

8.2.4 HPLC

Numerous high-performance liquid chromatographic (HPLC) methods utilizing a range of stationary phases (e.g., C18, silica, diol, cyano, amide, various polymeric phases, etc.) and detectors (e.g., ultraviolet [UV], photodiode array [PDA], fluorescence [FLD], mass spectrometric [MS], coulometric, etc.) were utilized for the analyses of PACs and anthocyanins from various botanical sources. The sample preparation for both PACs and anthocyanins is largely matrix dependent.

Regarding PACs, some materials may require a defatting step to remove lipids and waxes prior to extraction. Extraction solvents can range from mixtures of aqueous alcohol or acetone or acidified versions thereof. Many commercial botanical extracts typically require only dissolution in an HPLC-compatible solvent and filtration prior to injection. Solid phase extraction may be required to remove interfering or other extraneous materials. There are essentially two approaches to analysis; destructive and non-destructive. The former approach can involve acid catalyzed cleavage of a sample in the presence of nucleophiles (e.g., benzyl mercaptan, phloroglucinol, etc.). The HPLC analysis of reaction products provides qualitative information with respect to subunit structure (e.g., flavan-3-ol substitution pattern) and therefore allows the user to determine the presence of atypical PACs (e.g., propelargonidins) in a suspect GSE. Thiolysis is one widely used approach that is undertaken in the characterization of PACs,[73] and to calculate the average DP. Non-destructive methods analyze the GSEs without prior chemical reaction. The disadvantage of non-destructive HPLC methods is the often poor resolution of PAC molecules with four or more units, in particular in methods using reverse phase chromatography, giving yield to broad humps of unseparated PAC-polymers that are useless for species distinction. The use of polyvinylpolypyrrolidone to bind polyphenols, or filters with a molecular weight of 3000 or 5000 Da to remove the larger PACs prior to analysis may improve the chromatograms to some extent,[28,74] but these sample preparation steps carry the risk of eliminating potentially characteristic molecules as well. Both approaches rely on analytical standards or reference materials for qualitative and quantitative HPLC analyses. The combination of both approaches is complementary in evaluating GSEs for adulterants.

A crucial part of an HPLC identity test involves comparative HPLC of intact suspect and authentic samples (see 8.2.1 and Figure 3). As stated above, the analyst may use an array of separation modes. One of the earliest examples of HPLC use is the normal phase separation of grape seed procyanidins by DP using a gradient of acidified methanol in dichloromethane.[75] Later, Waterhouse et al.[76] applied the same conditions using a cacao extract as a reference material to approximate the DP in GSEs.

To obtain separation of PAC molecules according to size, gel permeation chromatography (GPC) may also be employed. However, this approach tends to suffer from poor resolution and a need for derivatization. Elimination of derivatization and minor improvements in resolution were achieved by Kennedy and Taylor whereby fractionated GSEs were separated over two porous polystyrene–divinylbenzene columns connected in series.[77]

With the goal of eliminating the use of methylene chloride and minimizing florescence quenching as was the case with Rigaud's silica method,[75] a hydrophilic interaction chromatographic (HILIC) method was developed by Kelm

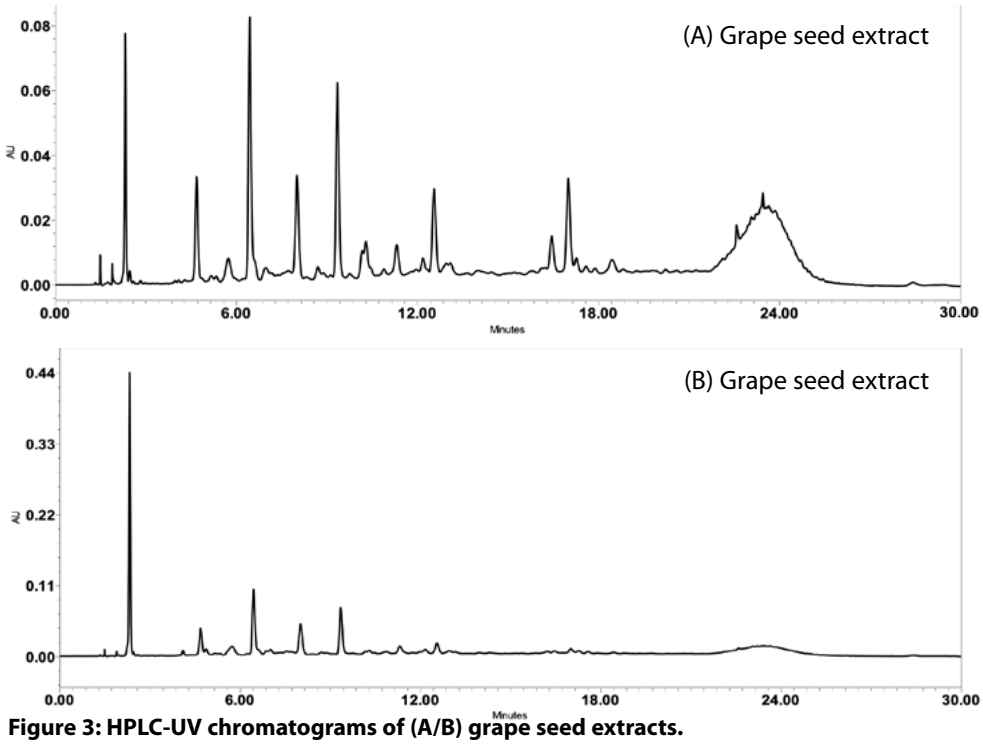


Figure 3: HPLC-UV chromatograms of (A/B) grape seed extracts.

HPLC-UV conditions: Column: Agilent Zorbax SB-C18, 4.6 x 150 mm; 3.5 µm; Solvent: (A) Water (0.3% phosphoric acid) and (B) acetonitrile, gradient 10 – 20% (B) in 18.9 min, 20 – 60% (B) in 8.4 min, 60 – 10% (B) in 0.4 min. Conditioning: 8 min. Flow rate: 1.0 mL/min. Detection: UV at 278 nm.

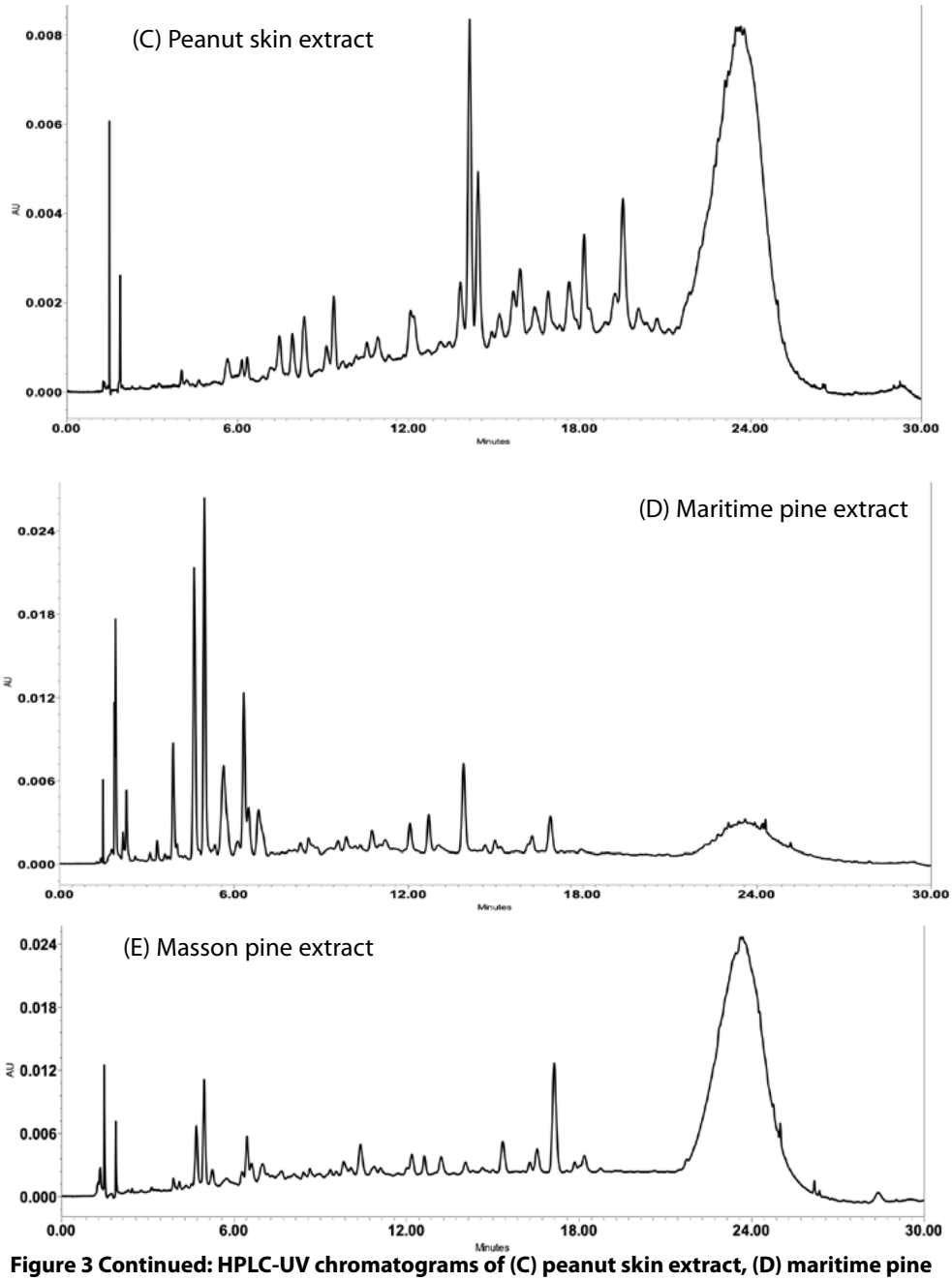


Figure 3 Continued: HPLC-UV chromatograms of (C) peanut skin extract, (D) maritime pine bark, (E) Masson pine bark.

HPLC-UV conditions: Column: Agilent Zorbax SB-C18, 4.6 x 150 mm; 3.5 µm; Solvent: (A) Water (0.3% phosphoric acid) and (B) acetonitrile, gradient 10 – 20% (B) in 18.9 min, 20 – 60% (B) in 8.4 min, 60 – 10% (B) in 0.4 min. Conditioning: 8 min. Flow rate: 1.0 mL/min. Detection: UV at 278 nm.

et al.[78] to separate cacao procyanidins based on DP. Separation was achieved by an acidified gradient of methanol into acetonitrile over a diol stationary phase. Normal phase and HILIC separations formed the basis of methods aimed at MS characterization of GSEs described below.

8.2.5 HPLC-Vis

The methods described by Wang et al.,[79] and Oh et al.[80] were evaluated and used in this review.

Comments: The publications describe the separation and identification of anthocyanins using HPLC-Vis using a photo diode array detector and HPLC-MS. Anthocyanins are generally detected at wavelengths between 520-546 nm.[81] These pigments are not found naturally in grape seeds, but trace amounts may be transferred on the grape seed from the process of crushing and separation from red grape skins (see section 8.2.2). The brownish colored GSE would be visually reddish purple in color if large amounts of anthocyanins were transferred into the GSE extract.

8.2.6 HPLC-UV/FLD

Methods described in the following literature were evaluated in this review: Kelm et al.,[78] Nakamura et al.,[25] and Robbins et al.[82]

Comments: While the addition of a FLD reportedly leads to a better signal-to-noise ratio for catechin monomers, dimers, and trimers compared to HPLC-UV,[25] data on the ability of HPLC-FLD to distinguish grape seed extracts from its adulterants are lacking.

8.2.7 HPLC-MS, UHPLC-MS, HPLC-UV/MS, UHPLC-UV/MS, and HPLC-MS/MS

Methods described in the following literature were discussed in this review: Appeldoorn et al.,[20] Gu et al.,[73] Kelm et al.,[83] Kuhnert et al.,[84] Li et al.,[85] Ma et al.,[86] Sica et al.,[28] Villani et al.,[7] and Zhang et al.[87]

***Comments*:** The combination of normal or hydrophilic interaction (HILIC) phase separations by DP coupled with MS offers a powerful qualitative tool to characterize and assess the distribution of monomers, oligomeric sets and polymers. Ambiguity of closely-eluting trimers and their galloylated forms are largely negated when one can differ-

entiate between masses. These and other separation modes (i.e., amide-C18) can also be used to elucidate the presence of adulterants.

Kelm et al. extended their earlier work to the separation of grape seed tannins over a diol stationary phase: ESI-MS analysis confirmed the presence of procyanidin dimers through octamers with 0 to 3 units of galloylation.[83] Kuhnert et al. essentially adapted this approach for separation and characterization of dimers through pentamers. Characterization and semi-quantification was achieved by aid of a tetramer standard.[84] However, no attempt was made to evaluate these conditions for the ability to detect adulteration.

Appeldoorn utilized normal and reverse phase HPLC-MS to characterize various A- and B-type PACs derived from grape seed and peanut skins. Since the conditions were optimized for isolation of PACs, it is not clear how well these conditions are suited for authentication purposes.[20]

Villani et al., used high-performance liquid chromatography with ultraviolet and mass spectrometric detection (HPLC-UV/MS) to obtain a chemical fingerprint of grape seed, peanut skin, and pine extracts.[7] Due to the similarity of GSE and pine (*P. massoniana*) bark extract, distinction between the two materials is difficult, in particular in case of grape seed and pine bark extract mixtures. Peanut skin is readily distinguished from GSE using HPLC-UV/MS.

Diol HPLC and HPLC-MS/MS analyses were used by Kelm et al. to characterize 20 authentic and commercially obtained bulk materials labeled as GSEs.[6] Atypical peaks observed in HPLC profiles were further evaluated by HPLC-MS/MS, which lead to the detection of both A-type procyanidins and B-type propelargonidins in one and four samples, respectively. Therefore, this approach can be used to detect adulteration with extracts containing A-type procyanidins and/or propelargonidins.

Li et al., (2002) utilized HPLC-atmospheric-pressure chemical ionization (APCI)-MS to characterize gallic acid, procyanidin B2 and B4, (+)-catechin, (-)-epicatechin, and (+)-epicatechin gallate in grape seed extract.[85] Despite the lack of any structural information of DP>2, the presence of procyanidins B2 and B4 dimers could serve as ancillary information in the authentication of GSE.

In 2018, Ma et al., identified epicatechin vanillate in grape seed and red wine at ppb levels using HPLC-HRMS/MS.[86] Given the peculiarity of this compound, it may serve as a unique marker compound for grape seed extracts. However, a more thorough survey of authentic grape seed extracts would need to be undertaken in order to affirm its universal presence in grape seeds, and to evaluate the usefulness of the method in GSE authentication.

Zhang et al.[87] developed a 65 min. long ultra high-performance liquid chromatography combined with a high resolution mass spectrometric detection (UHPLC-HRMS) method using a computer algorithm to extract, identify and quantify catechin monomers and oligomers up to octamers. The approach allowed distinguishing grape seed PACs from those of apple, chocolate, and other fruit samples. Based on the available data, HPLC-MS methods can distinguish among grape seed extracts and its adulterants. Ideally, these methods are combined with a robust chemometric analysis. More samples need to be included to account for the variability in extracts due to natural variations and differences in the manufacturing processes.

The paper by Sica et al. describes a rather comprehensive characterization of GSE using ultrahigh-performance liquid chromatography–ultraviolet–charged aerosol detector–high resolution mass spectrometry (UHPLC-UV-CAD-HRMS). A rough separation of the GSE constituents was achieved on a C18 column in 75 min. Using the CAD chromatogram, GSE was easily distinguished from peanut skin and maritime pine by comparison of the chromatographic fingerprint. The 39 major peaks of the chromatogram were partially or completely assigned to 83 different compounds, including a broad hump comprising the PACs with a DP of 6 or higher that was considered as one single compound. Structural assignment was based on comparing high-resolution MS data to those of known compounds or literature values, and by using MS fragmentation patterns of known compounds to assign molecules to a specific class of compounds. The authors noted an important difference between untreated and enzymatically-treated GSEs, but were able to distinguish both types of GSEs from the potential adulterants.

Comments: The HPLC-MS methods evaluated were mainly developed to identify the various PACs in GSE and other materials. As such, it is not clear how well these methods would work as routine quality control assays to authenticate GSE, and to detect potential adulterants. The methods published by Kelm et al.,[6] Zhang et al.,[87] Sica et al.,[28] and Villani et al.[7] indicate that comparison of the GSE chromatographic fingerprint with those of adulterating materials will allow detection with peanut skin (and other ingredients having A-type PACs) and propelargonidin-containing materials. The use of HILIC chromatography to separate PACs is promising, but more data are needed to evaluate its ability to confirm the authenticity of GSE.

8.2.8 MALDI-TOF

Methods described in the following literature were evaluated in this review: Ricci et al.,[88] Weber et al.,[22] Hümmer and Schreier,[89] and Yang and Chien.[90]

Comments: Matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry is commonly used in research settings to determine the general structural features (e.g., type(s) of monomer [although stereochemical assignments cannot be made using MALDI-TOF], A-type or B-type PAC, number of monomer units, and extent of galloylation) and distribution of PACs of various DPs within materials containing condensed tannins. The sample preparation is quick and easy, and consists mainly of dissolving the extract in methanol or an acetone-water mixture and then diluting it 1:1 in the matrix system (most often a methanolic solution of 2,5-dihydrobenzoic acid). Both, linear and reflectron modes can be used; a comparison between the two suggests that linear mode provides better data on PACs with a high DP.[22] MALDI-TOF has shown to distinguish among GSE, oak (*Quercus robur*, Fagaceae) bark, and green tea extract,[88] as well as between GSE and pine bark extract.[22] The main disadvantage is the cost of the instrument ($180,000 – 350,000) and

a lack of MALDI-TOF capacity in many contract labs.

8.2.9 NMR

The following method was evaluated in this review: Anstasiadi et al.[91]

^{1}H and/or ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy may be used for comparison of suspect samples against authentic samples. Obvious discrepancies inconsistent with proanthocyanidin molecules such as missing carbonyl carbon peaks or methine protons at the 2 and

Table 3. Comparison among the different approaches to authenticate grape seed extract

Method	Applicable to	Pro	Contra
Macroscopic	- Unprocessed plant parts	- Quick - Inexpensive	- No automation/statistics - Outcome relies on analyst's expertise
Microscopic	- Unprocessed plant parts - Powdered seeds	- Quick - Inexpensive	- Automation/statistics only with high-end microscopes - Outcome relies on analyst's expertise - Challenge to distinguish closely related species
Genetic	- Unprocessed plant parts - Powdered seeds	- Able to distinguish closely related species - Newer approaches can detect fragmented DNA in processed materials - Adulterant may be readily identified	- Labor-intensive sample preparation and analysis - Expensive equipment - Not applicable to highly processed powdered extracts - Detection limits in powders need to be evaluated
HPTLC	- Extracts	- Quick - Affordable equipment -Adulteration as low as 5% peanut skin detected - No statistics required	- No statistics - Detection of adulteration below 5% is challenging - Need for reference standard compounds
HPLC-UV-Vis/FLD	- Extracts	- Standard equipment in many laboratories - Ideal for compounds with strong chromophore (e.g., phenolic acids) - Adulteration with peanut skin and sources of propelargonidins can be detected - Anthocyanins	- Expensive equipment - Mostly quantitative (less specific than HPLC-UV/MS) - Unable to distinguish overlapping peaks - Detection of adulteration often challenging - Need for reference standard compounds
HPLC-UV/MS	- Extracts	- Qualitative and quantitative - High sensitivity	- Expensive equipment - Detection of adulteration often challenging
HPLC-MS/MS	- Extracts	- Qualitative and quantitative - High sensitivity and specificity	- Expensive equipment - Detection of adulteration often challenging
MALDI-TOF	- Extracts	- Provides structural information for PACs - Can detect relatively high molecular weight PACs - Adulteration can be detected using PAC fingerprint	- Expensive equipment

3 carbons on the flavanol C-ring would suggest admixture or substitution with extraneous, non PAC-containing materials. When coupled to pattern recognition software, NMR is a powerful and rugged tool for the characterization and authentication of botanical extracts. With regards to grape procyanidins, NMR has been used to effectively fingerprint and differentiate via principle component analysis, wines by variety, vintage and region.[91] Accordingly, it would stand to reason that a combination of chemometrics and NMR spectroscopy could be used to detect presence of adulterants in a GSE. However, the ability of NMR to discriminate between GSE and PAC-containing materials still needs to be demonstrated using a validated method.

9. Conclusions

Authentication of GSE can be challenging. Authentication or detection of adulteration in extracts may require more than one method. Adulteration with PACs represents a level of sophistication discernable only by chromatographic approaches hyphenated with a variety of detection method (UV-Vis, fluorescence detector [FLD], mass spectrometry [MS], tandem mass spectrometry [MS/MS], etc.), or by stand-alone spectroscopic/spectrometric methods such as nuclear magnetic resonance (NMR) or matrix-assisted laser desorption/ionization time-of-flight [MALDI-TOF] MS. However, adulteration with B type procyanidin-rich materials presents a greater challenge not readily resolved by chromatographic or spectroscopic/spectrometric methods. The best results are obtained when chemical fingerprints, e.g., those obtained by HPLC-UV, HPLC-MS, or MALDI-TOF, are compared against authentic GSE, as well as the potential adulterating materials, and analyzed using state-of-the-art statistical software.

10. References

1. Feringa HHH, Laskey DA, Dickson JE, Coleman CI. The effect of grape seed extract on cardiovascular risk markers: A meta-analysis of randomized controlled trials. *J Am Diet Assoc.* 2011;111(8):1173-1181.
2. Ma Z, Zhang H. Phytochemical constituents, health benefits, and industrial applications of grape seeds: A mini-review. *Antioxidants.* 2017;6(3):71.
3. McGuffin M, Kartesz JT, Leung AY, Tucker AO. *Herbs of Commerce.* 2nd ed. Silver Springs, MD: American Herbal Products Association; 2000.
4. Tropicos.org. Missouri Botanical Garden. https://www.tropicos.org/Name/34000217. Accessed January 17, 2019.
5. The Plant List (2013). Version 1.1. http://www.theplantlist.org/tpl1.1/record/kew-2457006. Accessed January 17, 2019.
6. Kelm MA, Kupina S, Shrikhande A. Grape seed extract authentication. 250th American Chemical Society National Meeting & Exposition; 2015; Boston, MA.
7. Villani TS, Reichert W, Ferruzzi MG, Pasinetti GM, Simon JE, Wu Q. Chemical investigation of commercial grape seed derived products to assess quality and detect adulteration. *Food Chem.* 2015;170:271-280.
8. Reed KA. *Identification of phenolic compounds from peanut skin using HPLC-MSn.* Blacksburg, VA, Virginia Polytechnic Institute and State University; 2009.
9. Cadot Y, Miñana-Castelló MT, Chevalier M. Anatomical, histological, and histochemical changes in grape seeds from *Vitis vinifera* L. cv Cabernet franc during fruit development. *J Agric Food Chem.* 2006;54(24):9206-9215.
10. Nowshehri JA, Bhat ZA, Shah MY. Pharmacognostic standardisation and phytochemical evaluation on the seeds of two *Vitis vinefera* L. varieties grown in Kashmir valley, India. *Pharmacogn J.* 2016;8(5):465-470.
11. *Vitis vinifera* (seed). American Herbal Products Association; 2014. http://www.botanicalauthentication.org/index.php. Accessed January 17, 2019.
12. Wang X, Liu Y, Wang L, Han J, Chen S. A nucleotide signature for the identification of *Angelicae sinensis* radix (danggui) and its products. *Sci Rep.* 2016;6:34940.
13. Little DP. Authentication of *Ginkgo biloba* herbal dietary supplements using DNA barcoding. *Genome.* 2014;57(9):513-516.
14. Ivanova NV, Kuzmina ML, Braukmann TWA, Borisenko AV, Zakharov EV. Authentication of herbal supplements using next-generation sequencing. *PLoS ONE.* 2016;11(5):e0156426. .
15. Lu Z, Rubinsky M, Babajanian S, Zhang Y, Chang P, Swanson G. Visualization of DNA in highly processed botanical materials. *Food Chem.* 2018;245:1042-1051.
16. Reynaud DT, Mishler BD, Neal-Kababick J, Brown PN. The capabilities and limitations of DNA barcoding of botanical dietary supplements [white paper]. 2015; https://gallery.mailchimp.com/2d47ec72fa1542de734a46f71/files/Reynaud_DNA_Barcoding_White_Paper.pdf. Accessed June 28, 2018.
17. Parveen I, Gafner S, Techen N, Murch SJ, Khan IA. DNA Barcoding for the identification of botanicals in herbal medicine and dietary supplements: strengths and limitations. *Planta Med.* 2016;82(14):1225-1235.
18. Monagas M, Quintanilla-López JE, Gómez-Cordovés C, Bartolomé B, Lebrón-Aguilar R. MALDI-TOF MS analysis of plant proanthocyanidins. *J Pharm Biomed Anal.* 2010;51(2):358-372.
19. Hellström JK, Törrönen AR, Mattila PH. Proanthocyanidins in common food products of plant origin. *J Agric Food Chem.* 2009;57(17):7899-7906.
20. Appeldoorn MM, Sanders M, Vincken J-P, et al. Efficient isolation of major procyanidin A-type dimers from peanut skins and B-type dimers from grape seeds. *Food Chem.* 2009;117(4):713-720.
21. Weseler AR, Bast A. Masquelier's grape seed extract: from basic flavonoid research to a well-characterized food supplement with health benefits. *Nutr J* 2017;16(1):5.
22. Weber HA, Hodges AE, Guthrie JR, et al. Comparison of proanthocyanidins in commercial antioxidants: grape seed and pine bark extracts. *J Agric Food Chem.* 2007;55(1):148-156.
23. La VD, Bergeron C, Gafner S, Grenier D. Grape seed extract suppresses lipopolysaccharide- induced matrix metalloproteinase (MMP) secretion by macrophages and inhibits human MMP-1 and -9 activities. *J Periodontol.* 2009;80(11):1875-1882.
24. Monagas M, Hernández-Ledesma B, Garrido I, J Martín-Alvarez P, Gómez-Cordovés C, Bartolomé B. Quality assessment of commercial dietary antioxidant products from *Vitis vinifera* L. grape seeds. *Nutr Cancer.* 2005;53(2):244-254.
25. Nakamura Y, Tsuji S, Tonogai Y. Analysis of proanthocyanidins in grape seed extracts, health foods and grape seed oils. *J Health Sci.* 2003;49:45-54.
26. Chamorro S, Viveros A, Alvarez I, Vega E, Brenes A. Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment. *Food Chem.* 2012;133(2):308-314.
27. Ras RT, Zock PI, Zebregs YEMP, Johnston NR, Webb DJ, Draijer R. Effect of polyphenol-rich grape seed extract on ambulatory blood pressure in subjects with pre- and stage I hypertension. *Br J Nutr.* 2013;110:2234-2241.
28. Sica VP, Mahony C, Baker TR. Multi-detector characterization of grape seed extract to enable in silico safety assessment. *Front Chem.* 2018;6(334).
29. O'Keefe SF, Wang H. Effects of peanut skin extract on quality and storage stability of beef products. *Meat Sci.* 2006;73(2):278-286.
30. Constanza KE, White BL, Davis JP, Sanders TH, Dean LL. Value-added processing of peanut skins: antioxidant capacity, total phenolics, and procyanidin content of spray-dried extracts. *J Agric Food Chem.* 2012;60(43):10776-10783.
31. Dudek MK, Gliński VB, Davey MH, Sliva D, Kaźmierski S, Gliński JA. Trimeric and tetrameric A-type procyanidins from peanut skins. *J Nat Prod.* 2017;80(2):415-426.
32. Bansode RR, Randolph P, Ahmedna M, et al. Bioavailability of polyphenols from peanut skin extract associated with plasma lipid lowering function. *Food Chem.* 2014;148:24-29.
33. Lee L-S, Kim S-H, Kim Y-B, Kim Y-C. Quantitative analysis of major constituents in green tea with different plucking periods and

their antioxidant activity. *Molecules.* 2014;19(7):9173.
34. Friedman M, Levin CE, Choi SH, Kozukue E, Kozukue N. HPLC analysis of catechins, theaflavins, and alkaloids in commercial teas and green tea dietary supplements: comparison of water and 80% ethanol/water extracts. *J Food Sci.* 2006;71(6):C328-C337.
35. Zhao Y, Chen P, Lin L, Harnly JM, Yu L, Li Z. Tentative identification, quantitation, and principal component analysis of green pu-erh, green, and white teas using UPLC/DAD/MS. *Food Chem.* 2011;126(3):1269-1277.
36. Roman MC, Hildreth J, Bannister S. Determination of catechins and caffeine in *Camillia sinensis* raw materials, extracts, and dietary supplements by HPLC-UV: Single-laboratory validation. *J AOAC Int.* 2013;96(5):933-941.
37. Blaschek W, Frohne D, Loew D. *Theae viridis folium*. In: Blaschek W, ed. *Wichtl – Teedrogen und Phytopharmaka*. Stuttgart, Germany: Wissenschaftliche Verlagsgesellschaft mbH; 2016:644-646.
38. Lin L-Z, Chen P, Harnly JM. New phenolic components and chromatographic profiles of green and fermented teas. *J Agric Food Chem.* 2008;56(17):8130-8140.
39. Del Rio D, Stewart AJ, Mullen W, et al. HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea. *J Agric Food Chem.* 2004;52(10):2807-2815.
40. Pastene E, Troncoso M, Figueroa G, Alarcón J, Speisky H. Association between polymerization degree of apple peel polyphenols and inhibition of *Helicobacter pylori* urease. *J Agric Food Chem.* 2009;57(2):416-424.
41. Kschonsek J, Wolfram T, Stöckl A, Böhm V. Polyphenolic compounds analysis of old and new apple cultivars and contribution of polyphenolic profile to the in vitro antioxidant capacity. *Antioxidants.* 2018;7(1):20.
42. Vrhovsek U, Rigo A, Tonon D, Mattivi F. Quantitation of polyphenols in different apple varieties. *J Agric Food Chem.* 2004;52(21):6532-6538.
43. Jakobek L, García-Villalba R, Tomás-Barberán FA. Polyphenolic characterisation of old local apple varieties from Southeastern European region. *J Food Comp Anal.* 2013;31(2):199-211.
44. Guyot S, Marnet N, Laraba D, Sanoner P, Drilleau J-F. Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a French cider apple variety (*Malus domestica* var. Kermerrien). *J Agric Food Chem.* 1998;46(5):1698-1705.
45. Shoji T, Mutsuga M, Nakamura T, Kanda T, Akiyama H, Goda Y. Isolation and structural elucidation of some procyanidins from apple by low-temperature nuclear magnetic resonance. *J Agric Food Chem.* 2003;51(13):3806-3813.
46. Feliciano RP, Meudt JJ, Shanmuganayagam D, Krueger CG, Reed JD. Ratio of "A-type" to "B-type" proanthocyanidin interflavan bonds affects extra-intestinal pathogenic *Escherichia coli* invasion of gut epithelial cells. *J Agric Food Chem.* 2014;62(18):3919-3925.
47. Kim SM, Kang S-W, Jeon J-S, Um B-H. A comparison of Pycnogenol® and bark extracts from *Pinus thunbergii* and *Pinus densiflora*: extractability, antioxidant activity and proanthocyanidin composition. *J Med Plants Res.* 2012;6(14):2839-2849.
48. Navarrete P, Pizzi A, Pasch H, Rode K, Delmotte L. MALDI-TOF and 13C NMR characterization of maritime pine industrial tannin extract. *Ind Crops Prod.* 2010;32(2):105-110.
49. Jerez M, Pinelo M, Sineiro J, Núñez MJ. Influence of extraction conditions on phenolic yields from pine bark: assessment of procyanidins polymerization degree by thiolysis. *Food Chem.* 2006;94(3):406-414.
50. Bianchi S, Kroslakova I, Janzon R, Mayer I, Saake B, Pichelin F. Characterization of condensed tannins and carbohydrates in hot water bark extracts of European softwood species. *Phytochemistry.* 2015;120:53-61.
51. Bartolome SB, Monagas JM, Lafuente IG, et al., Inventors; Consejo Superior De Investigaciones Cientificas, Madrid (Spain); Exxentia Grupo Fitoterapeutico S.A., Madrid (Spain) assignee. Phenolic extracts of almond peel containing procyanidins, propelargonidins, and prodelphinidins, and method for preparation thereof. 2010.
52. Monagas M, Garrido I, Lebrón-Aguilar R, Bartolome B, Gómez-Cordovés C. Almond (*Prunus dulcis* (Mill.) D.A. Webb) skins as a potential source of bioactive polyphenols. *J Agric Food Chem.* 2007;55(21):8498-8507.
53. Bolling BW. Almond polyphenols: methods of analysis, contribution to food quality, and health promotion. *Compr Rev Food Sci Food Saf.* 2017;16(3):346-368.
54. Dykes L, Rooney LW. Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World.* 2007;52(3):105-111.
55. Dykes L, Rooney LW. Sorghum and millet phenols and antioxidants. *J Cereal Sci.* 2006;44(3):236-251.
56. Yang L, Allred CD, Awika JM. Emerging evidence on the role of estrogenic sorghum flavonoids in colon cancer prevention. *Cereal Foods World.* 2014;59(5):244-251.
57. Gu L, Kelm MA, Hammerstone JF, et al. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J Nutr.* 2004;134(3):613-617.
58. Krueger CG, Vestling MM, Reed JD. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of heteropolyflavan-3-ols and glucosylated heteropolyflavans in sorghum [*Sorghum bicolor* (L.) Moench]. *J Agric Food Chem.* 2003;51(3):538-543.
59. Bröhan M, Jerkovic V, Wilmotte R, Collin S. Catechins and derived procyanidins in red and white sorghum: their contributions to antioxidant activity. *J Inst Brew.* 2011;117(4):600-607.
60. Labarbe B, Cheynier V, Brossaud F, Souquet J-M, Moutounet M. Quantitative fractionation of grape proanthocyanidins according to their degree of polymerization. *J Agric Food Chem.* 1999;47(7):2719-2723.
61. Prior RL, Gu L. Occurrence and biological significance of proanthocyanidins in the American diet. *Phytochemistry.* 2005;66(18):2264-2280.
62. Oszmiański J, Wolniak M, Wojdyło A, Wawer I. Influence of apple

pureé preparation and storage on polyphenol contents and antioxidant activity. *Food Chem.* 2008;107(4):1473-1484.
63. Jiang X, Liu Y, Wu Y, et al. Analysis of accumulation patterns and preliminary study on the condensation mechanism of proanthocyanidins in the tea plant [*Camellia sinensis*]. *Sci Rep.* 2015;5:8742.
64. Sarnoski PJ, Johnson JV, Reed KA, Tanko JM, O'Keefe SF. Separation and characterisation of proanthocyanidins in Virginia type peanut skins by LC–MSn. *Food Chem.* 2012;131(3):927-939.
65. Prieur C, Rigaud J, Cheynier V, Moutounet M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry.* 1994;36(3):781-784.
66. Sun B, Leandro C, Ricardo da Silva JM, Spranger I. Separation of grape and wine proanthocyanidins according to their degree of polymerization. *J Agric Food Chem.* 1998;46(4):1390-1396.
67. Spranger I, Sun B, Mateus AM, de Freitas V, Ricardo da Silva JM. Chemical characterization and antioxidant activities of oligomeric and polymeric procyanidin fractions from grape seeds. *Food Chem.* 2008;108(2):519-532.
68. Hagerman AE. *The Tannin Handbook*. 2011; http://www.users.miamioh.edu/hagermae/. Accessed June 28, 2018.
69. Wrolstad RE. Color and pigment analyses in fruit products. Vol Station Bulletin 624. Corvallis, OR: Agricultural Experiment Station, Oregon State University; 1993:1-17.
70. Sudberg E, Sudberg S, Nguyen J. Validation of a high performance thin-layer chromatographic fingerprint method for the simultaneous identification of grape seed and peanut skin and the adulteration of commercial grape seed extract with peanut skin. AHPA (American Herbal Products Association) Botanical Congress; 2014; Las Vegas, NV.
71. Niketic-Aleksic G, Hrazdina G. Quantitative analysis of the anthocyanin content in grape juices and wines. *Lebensm Wiss Technol.* 1972;5:163-165.
72. Lea AGH, Arnold GM. The phenolics of ciders: Bitterness and astringency. *J Sci Food Agric.* 1978;29(5):478-483.
73. Gu L, Kelm MA, Hammerstone JF, et al. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J Agric Food Chem.* 2003;51(25):7513-7521.
74. Peng Z, Hayasaka Y, Iland PG, Sefton M, Høj P, Waters EJ. Quantitative analysis of polymeric procyanidins (tannins) from grape (*Vitis vinifera*) seeds by reverse phase high-performance liquid chromatography. *J Agric Food Chem.* 2001;49(1):26-31.
75. Rigaud J, Escribano-Bailon MT, Prieur C, Souquet JM, Cheynier V. Normal-phase high-performance liquid chromatographic separation of procyanidins from cacao beans and grape seeds. *J Chromatogr A.* 1993;654(2):255-260.
76. Waterhouse AL, Ignelzi S, Shirley JR. A comparison of methods for quantifying oligomeric proanthocyanidins from grape seed extracts. *Am J Enol Vitic.* 2000;51(4):383-389.
77. Kennedy JA, Taylor AW. Analysis of proanthocyanidins by high-performance gel permeation chromatography. *J Chromatogr A.* 2003;995(1):99-107.
78. Kelm MA, Johnson JC, Robbins RJ, Hammerstone JF, Schmitz HH. High-performance liquid chromatography separation and purification of cacao (*Theobroma cacao* L.) procyanidins according to degree of polymerization using a diol stationary phase. *J Agric Food Chem.* 2006;54(5):1571-1576.
79. Wang H, Race EJ, Shrikhande AJ. Characterization of anthocyanins in grape juices by ion trap liquid chromatography-mass spectrometry. *J Agric Food Chem.* 2003;51(7):1839-1844.
80. Oh YS, Lee JH, Yoon SH, et al. Characterization and quantification of anthocyanins in grape juices obtained from the grapes cultivated in Korea by HPLC/DAD, HPLC/MS, and HPLC/MS/MS. *J Food Sci.* 2008;73(5):C378-C389.
81. Lorrain B, Ky I, Pechamat L, Teissedre P-L. Evolution of analysis of polyhenols from grapes, wines, and extracts. *Molecules.* 2013;18(1):1076.
82. Robbins RJ, Leonczak J, Li J, et al. Determination of flavanol and procyanidin (by degree of polymerization 1-10) content of chocolate, cocoa liquors, powder(s), and cocoa flavanol extracts by normal phase high-performance liquid chromatography: collaborative study. *J AOAC Int.* 2012;95(4):1153-1160.
83. Kelm MA, Versari A, Parpinello GP, Thorngate JH. Mass spectral characterization of *Uva longanesi* seed and skin extracts. *Am J Enol Vitic.* 2012;63(3):402-406.
84. Kuhnert S, Lehmann L, Winterhalter P. Rapid characterisation of grape seed extracts by a novel HPLC method on a diol stationary phase. *J Funct Foods.* 2015;15:225-232.
85. Li W, Fong HHS, Singletary KW, Fitzloff JF. Determination of catechins in commercial grape seed extract. *J Liq Chromatogr Relat Technol.* 2002;25(3):397-407.
86. Ma W, Waffo-Téguo P, Jourdes M, Li H, Teissedre P-L. First evidence of epicatechin vanillate in grape seed and red wine. *Food Chem.* 2018;259:304-310.
87. Zhang M, Sun J, Chen P. A computational tool for accelerated analysis of oligomeric proanthocyanidins in plants. *J Food Comp Anal.* 2017;56:124-133.
88. Ricci A, Parpinello GP, Palma AS, et al. Analytical profiling of food-grade extracts from grape (*Vitis vinifera* sp.) seeds and skins, green tea (*Camellia sinensis*) leaves and Limousin oak (*Quercus robur*) heartwood using MALDI-TOF-MS, ICP-MS and spectrophotometric methods. *J Food Comp Anal.* 2017;59:95-104.
89. Hümmer W, Schreier P. Analysis of proanthocyanidins. *Mol Nutr Food Res.* 2008;52(12):1381-1398.
90. Yang Y, Chien M. Characterization of grape procyanidins using high-performance liquid chromatography/mass spectrometry and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J Agric Food Chem.* 2000;48(9):3990-3996.
91. Anastasiadi M, Zira A, Magiatis P, Haroutounian SA, Skaltsounis AL, Mikros E. 1H NMR-based metabonomics for the classification of Greek wines according to variety, region, and vintage. Comparison with HPLC data. *J Agric Food Chem.* 2009;57(23):11067-11074.