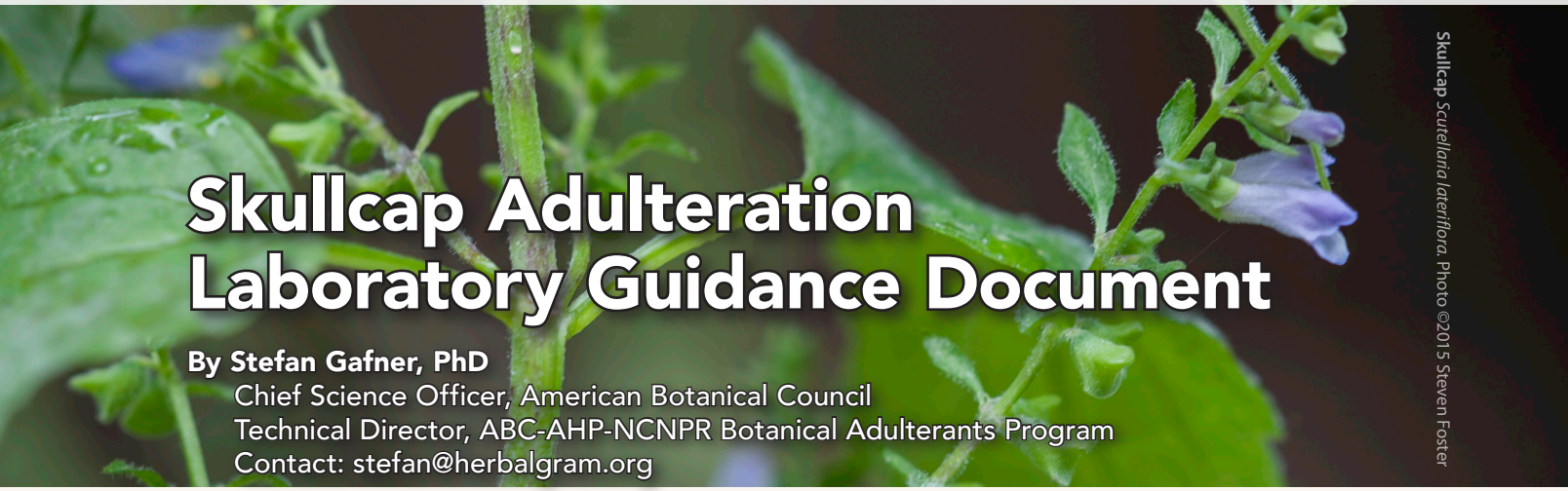
ABC AHP NCNPR
Botanical Adulterants Program
American Botanical Council • the American Herbal Pharmacopoeia • the University of Mississippi's National Center for Natural Products Research



Skullcap *Scutellaria lateriflora*. Photo ©2015 Steven Foster

Skullcap Adulteration Laboratory Guidance Document

By Stefan Gafner, PhD
Chief Science Officer, American Botanical Council
Technical Director, ABC-AHP-NCNPR Botanical Adulterants Program
Contact: stefan@herbalgram.org

Keywords: Adulterant, adulteration, skullcap adulteration, germander, *Scutellaria lateriflora*, *Scutellaria* spp., skullcap, scullcap, *Teucrium canadense*, *Teucrium chamaedrys*

1. Purpose

Skullcap (*Scutellaria lateriflora*, family Lamiaceae) herb has a long history of adulteration, evidenced in comments from over 100 years ago by Felter and Lloyd that "*Scutellaria versicolor* Nuttall and *Scutellaria canescens* Nuttall are the species generally collected by herbalists and substituted for *Scutellaria lateriflora*."[1] Besides the substitutions with other species from the genus *Scutellaria*, adulteration with germander (*Teucrium*) species containing hepatotoxic furano neo-clerodane diterpenes has been reported in the early 1990s and seems to persist in the herb trade in North America and possibly elsewhere.[2] This Laboratory Guidance Document presents a review of the various publicly-available analytical technologies and methods used to differentiate between authentic *S. lateriflora* and its potentially adulterating species, listed in Table 1.

2. Scope

The various analytical methods described below were reviewed with the specific purpose of identifying strengths and limitations of existing methods for differentiating *S. lateriflora* from its potentially adulterating species. Analysts can use this review to help guide the appropriate choice of techniques for their specific skullcap products for qualitative purposes. The positive evaluation of a specific method for testing *S. lateriflora* material in the products' particular matrix in this Laboratory Guidance Document does not reduce or remove the responsibility of laboratory personnel to demonstrate adequate method performance in their own laboratory using accepted protocols outlined in the US Food and Drug Administration's Final Rule for Current Good Manufacturing Practices for Dietary Supplements (as published in 21 CFR Part 111) and by AOAC (Association of Official Analytical Chemists) International, International Organization for Standardization (ISO), World Health Organization (WHO), and International Conference on Harmonisation (ICH).

3. Common and scientific names

3.1 Common Name: skullcap[3]

3.2 Other Common Names

English: blue skullcap, helmet flower, hoodwort, European or greater skullcap, Quaker bonnet, mad-dog skullcap, mad weed, scullcap, Virginia skullcap
French: scutellaire, scutellaire latériflore, scutellaire de Virginie, toque, toque bleue, toque casquée, toque des marais
German: Helmkraut, Fieberkraut, Fleckenkraut, Blaues Gnadenkraut, Kappenhelmkraut, Gemeines Schildkraut
Italian: scutellaria
Spanish: escutelaria, escutelaria de Virginia

3.3 Latin Binomial: *Scutellaria lateriflora* L.[4,5]

3.4 Synonyms: *Cassida lateriflora* (L.) Moench; *Scutellaria polybotrya* Bernh.[4,5]

3.5 Botanical Family: Lamiaceae

4. Botanical Description

Botanical descriptions for *Scutellaria* and *Teucrium* species are provided in local, national, and international floras, including *Flora of North America*, *Flora Europaea*, and *Flora of China*. Additionally, the genus *Scutellaria* (including tables distinguishing them from *Teucrium* species), is described in the skullcap monograph of the American Herbal Pharmacopoeia (AHP), accompanied by illustrations and images.[6] Morphological identification to the species level requires personnel trained in botany as well as authenticated materials with intact and characteristic botanical features.

Sections 5-8 of the present document discuss macroscopic, microscopic, genetic, and chemical authentication methods for *S. lateriflora*. A comparison among the various approaches is presented in Table 3 at the end of section 8.

5. Identification and Distinction using Macroanatomical Characteristics

Macroscopic identification criteria for *S. lateriflora* have been published in the AHP monograph by Upton et al.,[6] in the Ph.D. thesis by Brock,[8] and in Applequist.[9] Descriptions in the AHP are more detailed and include the potential adulterants *T. chamaedrys*, *T. canadense*, *S. galericulata*, and *S. incana* in a table format. High-quality drawings illustrate the text in the table and make it more easily understandable. The text by Brock contains details on *S. lateriflora*, *T. chamaedrys*, *T. canadense*, *S. galericulata*, and *S. ovata*, and many of her comments are based on the 2009 AHP monograph. In addition to a table listing the main features of each plant, Brock also provides helpful guidance outlining the main distinctive features between the different *Scutellaria* species and *Teucrium*.

6. Identification and Distinction using Microanatomical Characteristics

Detailed microscopic descriptions of *S. lateriflora*, and the germander species *T. canadense* and *T. chamaedrys*, are found in a number of references.[6,10,11] The textbook by Upton et al.[10] also contains a section on the roots of *S. baicalensis*. However, there are no microscopic descriptions in the recent literature for other *Scutellaria* species listed in Table 1.

Comments: While outside the scope of this document, botanical microscopy is one of the easiest ways to detect adulteration with inert materials and undisclosed fillers (e.g., cellulose, starch, sand). However, it is unclear if a microscopic distinction of powdered aerial material of *S. lateriflora* and closely related *Scutellaria* species can be

Table 1. Scientific names, family, and common names of known* skullcap (*Scutellaria lateriflora*) adulterants

Species[a]	Synonyms[b]	Family	Common name[c]	Other common names[d]
Scutellaria alpina L.	*Cassida alpina* (L.) Moench	Lamiaceae		Alpine skullcap
Scutellaria baicalensis Georg	*S. lanceolaria* Miq.; *S. macrantha* Fisch.	Lamiaceae	Chinese skullcap	Baikal skullcap, scute
Scutellaria galericulata L.	*Cassida galericulata* (L.) Scop.	Lamiaceae		Marsh skullcap, marsh skullwort
Scutellaria incana Biehler	*S. canescens* Nutt.	Lamiaceae		Hoary skullcap, downy skullcap
Scutellaria ovata Hill	*S. versicolor* Nutt.	Lamiaceae		Heartleaf skullcap
Teucrium[e] *canadense* L.	*T. boreale* E.P. Bicknell; *T. bracteosum* Raf.; *T. menthifolium* E.P. Bicknell; *T. mexicanum* Sessé & Moc.; *T. nashii* Kearney; *T. occidentale* A. Gray; *T. roseum* E.P. Bicknell; *T. virginicum* L.	Lamiaceae		Canada germander, American germander, wood sage
Teucrium[e] *chamaedrys* L.	*T. stevenianum* Klokov	Lamiaceae	Germander	Wall germander[c]

[a]According to The Plant List and the Tropicos database. [4,5]
[b]According to The Plant List and the Tropicos database. [4,5] A comprehensive list of synonyms can be accessed through The Plant List website. [4]
[c]According to the American Herbal Products Association's *Herbs of Commerce*, 2nd ed. (2000). [3]
[d]According to The Plant List, the Tropicos database, *Herbs of Commerce*, 2nd ed., and the USDA PLANTS Database. [3,4,5,7]
[e]*Teucrium* species have also been referred to as "pink skullcap" which contributes to the nomenclatural confusion.

*Note: The list of known adulterants is based on published data, e.g., references 1, 2, and 6. Some of the listed species may represent incidences of historical significance but occurrence may be rare or absent in the current marketplace.

achieved. The use of microscopy for the authentication of *S. lateriflora*, in addition to a macroscopic assessment, may be adequate for companies that grow their own plant material, or when whole plant material is purchased. In all other cases, a microscopic examination should be combined with other appropriate methods (genetic or chemical) for authentication or detection of adulteration. The identification of *S. lateriflora* extracts cannot be achieved using microanatomical characteristics.

7. Genetic Identification and Distinction

Methods described in the following literature were evaluated in this review: Hosokawa et al.[12] and Hosokawa et al.[13]

Comments: The approach using direct sequencing[13] has been tested successfully with a number of closely related skullcap species and is considered the more reliable of the two genetic methods to authenticate *S. lateriflora*. However, genetic assessment will not identify the plant part, which is a legal requirement of dietary supplement ingredient identification. Thus, genetic testing must typically be coupled with another appropriate test for distinguishing the plant parts. As DNA methods are usually inappropriate and unreliable for identity testing of extracts or certain other processed material (see Table 3), the authentication and detection of adulteration has to be done by chemical means in these cases.

8. Chemical Identification and Distinction

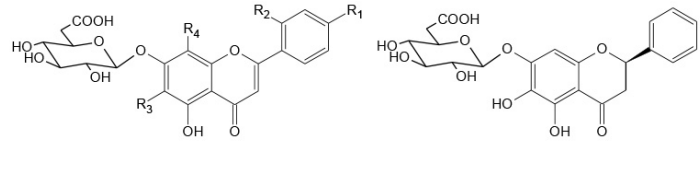
There are numerous analytical methods available for authenticating *S. lateriflora* and differentiating it from other *Scutellaria* species as well as potential adulterants, such as *Teucrium* species. These methods are cited in the Laboratory Methods section below. Distinction based on the phytochemical profile requires a detailed knowledge of the constituents of *S. lateriflora* and its adulterants. Below is a summary of the phytochemical composition of skullcap and its known adulterants, including chemical structures of the principal flavonoids occurring in *S. lateriflora* (Figure 1) and phenylpropanoid glycosides in *Teucrium* species (Figure 2).

8.1 Chemistry of *Scutellaria lateriflora* and Potential Adulterants

Scutellaria lateriflora: According to a review of the analytical literature, the main flavonoid in dried *S. lateriflora* aerial parts is baicalein-7-O-glucuronide (syn: baicalin, **1**). Other important flavonoids are dihydrobaicalin (**2**), lateriflorein-7-O-glucuronide (syn: lateriflorin, **3**), wogonoside (**4**), ikonnikoside I (**5**), and oroxylin A-7-*O*-glucuronide (**6**).[11,14-18] Larger amounts of the aglycone, baicalein, point to a cleavage of the glucuronic acid moiety in **1** and are often indicative of stability issues. Since most of the flavone-glucuronides (**1**, **4**, **6**, and scutellarein-7-O-glucuronide [syn: scutellarin, **7**]) are found in many species of the genus *Scutellaria*, methods for authentication of *S. lateriflora* relying on its major constituents must be based on the totality of compounds present (phytochemical fingerprint) in regard to both the composition and the relative amounts.

Other compounds reported from *S. lateriflora* aerial parts include waxes, essential oil, neo-clerodane diterpenes, amino acids, coumarins, and stilbenes.[15,16,19-22] According to several authors who used authenticated samples in their analysis, neither verbascoside (**8**) nor teucrioside (**9**) (Figure 2; see also *Teucrium* species below) occur in *S. lateriflora*;[11,17,23,24] therefore, the phenylpropanoid glycosides were proposed as markers to detect adulteration of skullcap with germander. The findings of two studies[18,25] that reported **8** from skullcap materials remain controversial, since the analysis was performed on unauthenticated commercial products[18] or showed a very untypical chemical composition.[25]

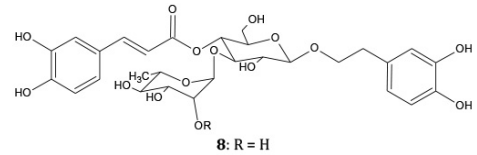
Scutellaria alpina: Seven flavonoids are known from *S. alpina* leaves:[26] chrysin, 2'-methoxychrysin, apigenin, scutellarein, **7**, chrysin-7-*O*-glucuronide (**10**), and apigenin-7-*O*-glucuronide (**11**). While the lack of **1**, **2**, **4**, and **6** is easily detected in a substitution, a mixture of *S. alpina* with *S. lateriflora* may be difficult to detect based on flavonoids only. The presence of the scutalpins, neo-clerodane diter-



1: $R_1 = R_2 = R_4 = H, R_3 = OH$
3: $R_1 = R_4 = H, R_2 = OCH_3, R_3 = OH$
4: $R_1 = R_2 = R_3 = H, R_4 = OCH_3$
5: $R_1 = R_4 = H, R_2 = R_3 = OH$
6: $R_1 = R_2 = R_4 = H, R_3 = OCH_3$
7: $R_1 = R_3 = OH, R_2 = R_4 = H$

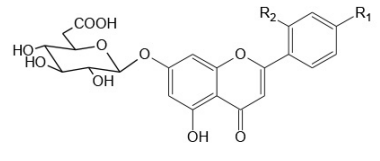
2

Figure 1: Chemical structures of principal flavonoids found in aerial parts of *S. lateriflora*



8: R = H
9: R = lyxose

Figure 2: Chemical structures of phenylpropanoid glycosides from *Teucrium* species



10: $R_1 = R_2 = H$
11: $R_1 = H, R_2 = OCH_3$
12: $R_1 = OH, R_2 = H$

Figure 3: Chemical structures of important flavone-glucuronides found in *Scutellaria* species

penes characteristic to *S. alpina*, can be used to unequivocally identify this species.[27,28]

Scutellaria galericulata: The flavonoid compositions of aerial parts of *S. galericulata* and *S. lateriflora* grown in North America are very similar, the only difference being the presence of **10** and the absence of **6** in *S. galericulata*.[29] Phenylpropanoid glycosides [2-(4-hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside, calceolarioside B, osmanthuside E, and martynoside] have been reported from the aerial parts of *S. galericulata* material from Turkey.[30] These four phenylpropanoid glycosides represent appropriate marker compounds to distinguish *S. galericulata* from *S. lateriflora*. Additionally, the scutegalins, neo-clerodane diterpenes characteristic of *S. galericulata*, can be used for authentication.[31,32]

Scutellaria incana: The only quantitative phytochemical characterization of aerial parts of *S. incana* available is summarized in Table 2, indicating large amounts of **4** and **7**, but no **1**-**3**, **5**, or **6**.[29] Qualitatively, a total of 40 flavonoids have been reported from *S. incana*, including a number of flavone C-glycosyl compounds, **1**, **4**, and **6**, **7**, and **10**.[33] In addition, the authors detected **8** in the plant material. The identification was mainly based on HPLC-MS, a sensitive technique that may have allowed detecting very low amounts of **1** and **6**. Structure assignments were tentative in some cases, but the presence of flavone C-glycosides and **8** should allow a distinction between *S. incana* and other species.

Scutellaria ovata: The major flavone-glucuronide from the aerial parts of *S. ovata* is **6**, with smaller amounts of **1** and **10**.[29] In addition, oroxylin A-7-*O*-glucoside and ovatin (5,6-dimethoxyflavone-7-*O*-glucoside) were also reported from the species.[34] Presence of oroxylin A-7-*O*-glucoside and absence of **2**, **3**, and **5** differentiates *S. ovata* and *S. lateriflora*.

Scutellaria baicalensis: The composition of Baikal skullcap has been extensively studied, and a large number of chemical structures have been reported from this plant.[35] The main flavonoids in the roots have been identified as **1**, **4**, **6**, baicalein, and wogonin,[36-38] and the contents of **1** have to be no less than 10% in the crude root drug according to the *Japanese Pharmacopoeia*.[39] The aerial parts contain mainly **1**, **4**, and **7**.[25] It is distinct from *S. lateriflora* by the absence of **3** and **5** in aerial parts and roots, and by the presence of large amounts of **4** in root material.

Teucrium canadense: The aerial parts are characterized by the presence of **8** as the major component, with smaller amounts of flavone-glycosides (e.g., **11**), but not **1**-**3**, **5**, or **7**.[11,17] The lipophilic fraction is dominated by the neo-clerodane diterpenes teuflin and teucvidin.[17,40]

Teucrium chamaedrys: The major compound in *T. chamaedrys* aerial parts is **9**, with other phenylpropanoid glycosides (**8**, teucrioside 3''''-O-methyl ether, and teucrioside-3'''', 4''''-O-dimethyl ether)[41] as minor compounds. Also present are flavonoids, mainly glycosides of apigenin, diosmetin, and luteolin,[17] and the reportedly characteristic hypolaetin- and isoscutellarein-7-*O*-(6'''-*O*-acetyl) allosyl-(1→2)-*O*-glucosides.[42] In the lipophilic fraction, the predominant neo-clerodane diterpene is teucrin A.[17,40]

A complicating factor with regard to establishing a phytochemical profile is the known instability of some of the major components in *S. lateriflora*. This is of particular concern if fresh material is used for extraction, or if the material is improperly dried, since the high amount of water will expose the flavonoids to oxidative degradation. Degradation has also been observed in tinctures with low amounts of ethanol, leading to a complete absence of known skullcap metabolites in certain products.[43]

8.2 Laboratory Methods

Note: Unless otherwise noted, all methods summarized below use only aerial parts of *Scutellaria* spp. and/or *Teucrium* spp.

8.2.1 HPTLC

Methods from the following sources were evaluated in

Table 2. Comparison of contents (in % [w/w]) of flavone- and flavanone-glucuronides in dried hydroethanolic (70% ethanol) extracts of *S. lateriflora*, *S. alpina*, *S. galericulata*, *S. incana*, and *S. ovata* [29]

Species[a]	1	2	3	4	5	6	7	10	12
S. lateriflora	7.0-17.2	3.2-14.6	0.8-2.3	0.6-0.9	0.3-1.1	0.7-0.9	0.5-0.7	0	0-0.3
S. alpina[b]	*	0	0	0.5	0	0	6.0	0.9	*
S. galericulata	1.6-16.7	9.2-24.2	0.2-0.6	0-0.1	0-0.8	0	0.8-3.4	0.5-8.9	0-9.1
S. incana	0	0	0	1.4-3.9	0	0	6.4-8.7	0.1-0.3	0
S. ovata	1.1	0	0	0	0	9.9	0.1	0.9	0

1: Baicalein-7-*O*-glucuronide; **2**: Dihydrobaicalin; **3**: Lateriflorein-7-*O*-glucuronide; **4**: Wogonoside; **5**: Ikonnikoside I; **6**: Oroxylin A-7-*O*-glucuronide; **7**: Scutellarein-7-*O*-glucuronide; **10**: Chrysin-7-*O*-glucuronide; **12**: 2'-Methoxychrysin-7-*O*-glucuronide

[a]There are no comparative results for *S. baicalensis* available.
[b]**1** and **12** were present at low levels in one of two voucher samples of *S. alpina*. The material with **1** and **12** was not quantitatively analyzed. [Gafner, unpublished]

this review: Upton et al.,[6] Gafner et al.,[11] and Hong et al.[44]

Comments: The conditions described in references 11 and 44 are the same with the exception of the extraction process. Using methanol[44] as the extraction solvent will shorten the application time (methanol dries easier than a 70% aqueous ethanol solution) and may lead to more uniform bands.

Hong et al. have been able to detect as little as 0.5% *T. chamaedrys* in *S. baicalensis* using the HPTLC conditions initially published by Gafner et al.[11] Upton et al.[6] have modified the solvent system to include suitable conditions for the less polar components, in particular **1**. The modification comes at the expense of a lower resolution between the abundant flavone-glucuronides (e.g., **1**, **7**) in *S. baicalensis* and the phenylpropanoid glycosides (**8**, **9**) in *Teucrium*.

Both systems are suitable for authentication of *S. lateriflora*. For dried skullcap raw material where the flavone-glucuronides are predominant, the mobile phase developed by Gafner et al.[11] and Hong et al.[44] may be preferred. System suitability parameters have not been published for any of the methods and will need to be included in the validation process.

8.2.2 HPLC and UHPLC

Methods described in the following literature were evaluated in this review: Upton et al.,[6] Bergeron et al.,[15] Li et al.,[16] Lin et al.,[17] Zhang et al.,[18] Sun and Chen,[24] Islam et al.,[25] Gao et al.,[43] Makino et al.,[45] Parajuli et al.,[46] Tascan et al.,[47] Cole et al.,[48] and Brock et al.[49] A comparison among the various HPLC and UHPLC methods are given in Appendix 1, Table 4. Specific comments on strengths and weaknesses of each of the methods are listed in Appendix 1, Table 5.

***Comments*:** In most cases, sample preparation is the most time-consuming part of an analysis. For routine quality control, a quick and easy method is helpful, e.g., the sample preparation outlined by Lin et al.[17] The solvent of choice in most cases is a mixture of MeOH-water (between 6:4 and 8:2, v/v) or EtOH-water (6:4 or 7:3, v/v), which will give adequate extraction of the flavone-glucuronides. All published methods will be able to detect adulteration with *Teucrium* species, if the adulterant is present in a sufficient quantity. The methods which have been the most thoroughly validated[6,15] should be preferred. If the run time is of essence, the conditions developed by Sun and Chen[24] are the best option. System suitability parameters (e.g., tailing factor, resolution) have not been published for any of the methods and will need to be included in the validation process.

8.2.3 MS-Fingerprinting

Chen et al.[23]:

Comments: A statistics-based authentication method is state of the art for analytical technologies. The analysis is very short and environmentally friendly due to low solvent use. The method will provide a "yes" or "no" answer without relying on the interpretation of an expert after constructing an appropriate library of authenticated materials; however, an expert analyst is required to setup the parameters for the instrument and the statistical evaluation, and to run the instrument. Initial costs for the instrumentation are high. A sonication time of 1 hour and small sample volume may lead to a high temperature extraction and increase the risk of degradation. Very small sample amounts are used for extraction. The method has not been validated and no system suitability parameters were described.

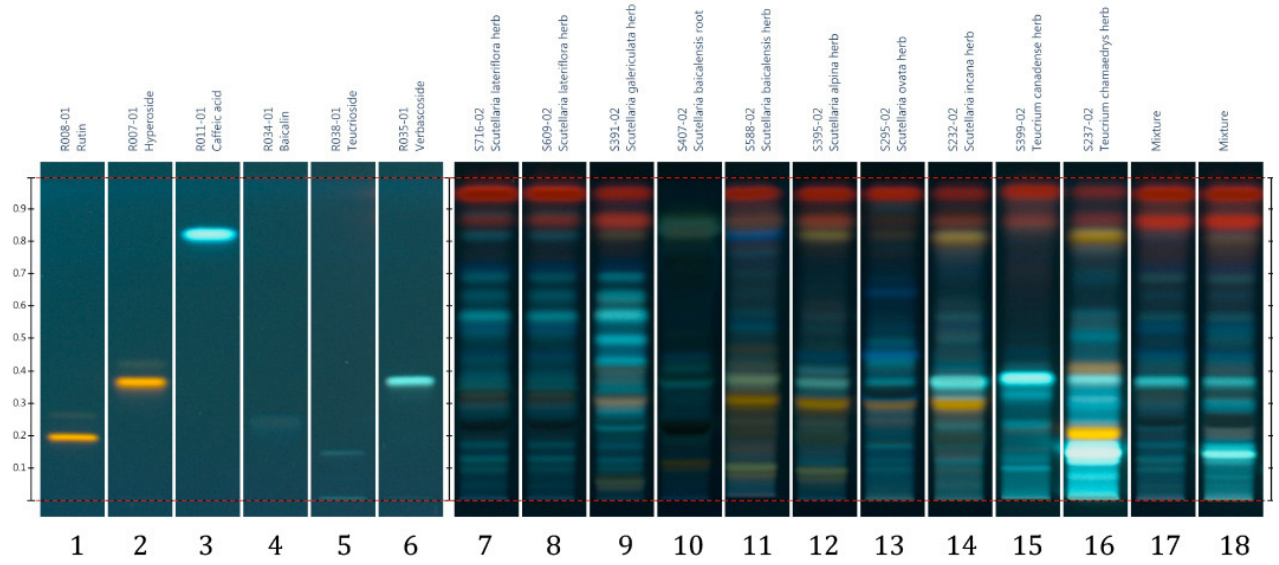


Figure 4: HPTLC analysis of *S. lateriflora* and its adulterants according to [6]. For lanes 1-16, the identities of the materials are indicated above the lane. Lane 17: mixture of *Scutellaria lateriflora*: *Teucrium canadense* (80:20); Lane 18: mixture of *Scutellaria lateriflora*: *Teucrium chamaedrys* (80:20). Detection: Natural products/polyethylene glycol (NP/PEG) reagent, UV at 366 nm. Image provided by Camag AG; Switzerland.

8.2.4 NMR

Colson et al.[50]:

Comments: A statistics-based authentication method is state of the art for analytical technologies. The results show that this approach is able to clearly distinguish *S. lateriflora* from *Teucrium* samples and the instrument will provide a "yes" or "no" answer without relying on expert interpretation. As with other statistics-based evaluations, added materials (e.g., carriers, processing aids) will modify the outcome of the PCA and thus may cluster the material outside the acceptable range. Therefore, the construction of a library containing authenticated materials of the same composition as the analyte is necessary. Expert analysts are required to setup the appropriate parameters and run the instrument. The analysis time is short and ecologically responsible due to the low amount of solvent used. As a result of the reproducibility using NMR, new samples can be directly compared to samples run earlier without having to rerun the whole series. System suitability for any botanical analysis is the same: the ^{1}H line shape and the ^{1}H sensitivity have to comply with the probe specifications. In addition, the temperature must be stable to 0.1°C. However, the sample preparation is very time-consuming due to the need to freeze-dry the extract before analysis in order to avoid a large signal from residual water. Data on method validation are not available.

8.2.5 NIR Hyperspectral Imaging

Sandasi et al.[51]:

***Comments*:** Near-infrared hyperspectral imaging (NIR-HSI) is a fast, non-destructive, environmentally friendly, and affordable method. Samples of raw material, extracts, or even ingredients in heterogeneous matrices can be analyzed with little or no pre-processing. Sandasi et al. subjected the hyperspectral images to multivariate statistical analysis in order to differentiate the three species analyzed (*S. lateriflora*, *T. canadense*, and *T. chamaedrys*). Using the statistical approach, a "yes" or "no" answer can be obtained without the need to rely on the interpretation of an expert. HSI also enables large quantities of material to be analyzed thus avoiding sampling problems. The technique has good selectivity; nevertheless, the sensitivity is lower compared to conventional chromatographic techniques. In the case of skullcap, the method could detect only admixtures with *Teucrium* spp. at levels ≥ 40%, as the error of detection (deviation between the exact and predicted values) becomes larger with decrease in percentage adulteration. Sandasi et al. have used only one authenticated sample of each species to create their library, so it needs to be seen how differences due to agricultural or processing variations impact the results, and how well the method can distinguish the various known adulterants from within the *Scutellaria* genus.[51]

9. Conclusion

There are numerous techniques and analytical methods that readily allow for the differentiation of *Scutellaria lateriflora* from the potentially toxic adulterant *Teucrium* species even when admixtures occur at small concentrations. Differentiating among closely related species of *Scutellaria* is more challenging. Authentication of *Scutellaria* species solely based on the presence of marker compounds (e.g., baicalein-7-O-glucuronide, scutellarein-7-O-glucuronide, wogonoside, or baicalein) is insufficient unless a thorough statistical evaluation (e.g., with HPLC, direct MS,[23] or NMR[50]) is performed.

For species authentication of commercially available whole plant material, confirmation of species identity and purity may be achieved by organoleptic methods, if conducted by qualified personnel (e.g., a botanist). For cut or powdered raw material, a combination of a physical assessment test (e.g., macroscopic or microscopic) and/or a genetic approach (e.g., Hosokawa et al., 2005)[13] combined with chemical identification methods is recommended. HPTLC and HPLC methods can be used for chemical characterization of raw material and extracts. Suggested HPTLC methods include the methods described in references 6, 11, and 44. For laboratories with an HPLC, the suggested methods are detailed in 6 and 15; for laboratories with UHPLC equipment, the method of choice is presented in 24. It should be noted that with all the available methods, data on method validation is limited, and system suitability data are lacking.

Note: A number of identity tests for skullcap materials are offered by third-party analytical laboratories.

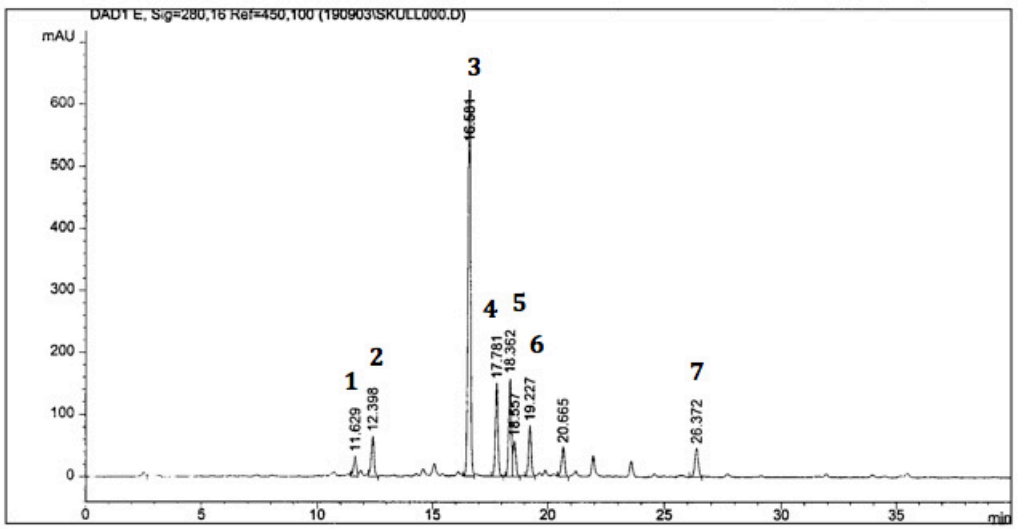


Figure 5: Typical HPLC-UV trace for *S. lateriflora*. Conditions as outlined in [15]. 1: Scutellarin; 2: Ikonnikoside I; 3: Baicalin; 4: Lateriflorin; 5: Dihydrobaicalin; 6: Oroxylin A-7-*O*-glucuronide; 7: Baicalein

Table 3. Comparison among the different techniques to authenticate *S. lateriflora*

Method	Applicable to	Pro	Con
Macroscopic	Raw material[a]	Quick Inexpensive No solvents required	No automation/statistics Outcome relies on analyst's expertise Difficult or impossible for c/s material
Microscopic	Raw material	Quick Inexpensive Can readily detect adulterating *Teucrium* species Few solvents required	No automation/statistics Outcome relies on analyst's expertise Challenge to distinguish closely related *Scutellaria* species
Genetic	Raw material	Able to distinguish closely-related species Reliable Able to detect small amounts of adulterants	Labor-intensive sample preparation and analysis Expensive equipment Cannot distinguish among plant parts
HPTLC	Raw material, extracts	Quick Basic systems affordable for smaller labs Able to detect small amounts of adulterants	No statistics High-end equipment expensive Need for standard compounds[b]
HPLC-UV	Raw material, extracts	Standard equipment in many laboratories Able to detect small amounts of adulterants Mostly quantitative (less specific than HPLC-UV/MS)	Equipment expensive Often no statistics applied (although software is available) Need for standard compounds[b]
HPLC-UV/MS	Raw material, extracts	Standard equipment in many laboratories Able to detect small amounts of adulterants Qualitative and quantitative	Equipment very expensive Often no statistics applied (although software is available) Quality of data depends on ability to ionize analyte Need for standard compounds[b]
Standalone MS (flow-injection MS)	Raw material, extracts	Short analysis time Reliable State-of-the-art statistical evaluation Independent of analyst's expertise after method is set up Qualitative and quantitative	Equipment very expensive[c] Initial setup of parameters complex Quality of data depends on ability to ionize analyte
NMR	Raw material, extracts	Short analysis time Reliable and highly reproducible State-of-the-art statistical evaluation Independent of analyst's expertise after method is set up Qualitative and quantitative	Equipment and maintenance very expensive[c] Initial setup of parameters complex Labor- and time-intensive sample preparation Needs at least 4' x 7' floor space
NIR-HSI	Raw material, extracts	No sample preparation needed Short analysis time State-of-the-art statistical evaluation Independent of analyst's expertise after method is set up	Ability to detect adulterants in mixtures is lower than with other methods No quantitative analysis

[a]Only whole and cut and sifted (c/s)

[b]Some useful standard compounds (dihydrobaicalin, ikonnikoside I, 2'methoxychrysin-7-*O*-glucuronide, teucrioside, teuflin) are not commercially available.

[c]Costs for high-resolution mass spectrometers and NMR instruments are generally above $250,000. A low-cost NMR for natural products analysis can be obtained for ca. $150,000.

According to input from five contract laboratories, the testing methods include microscopy, HPTLC, and HPLC-UV. Additional testing methods (HPLC-MS or near-infrared [NIR] methods) can be developed upon request.

10. References

1. Felter HW, Lloyd JU. *King's American Dispensatory*. 19th ed. Cincinnati, OH: The Ohio Valley Company; 1905:1739-1741.
2. Foster S. Adulteration of skullcap with American germander. *HerbalGram*. 2012:(93);34-41.
3. McGuffin M, Kartesz JT, Leung AY, Tucker AO. *Herbs of Commerce*. 2nd ed. Silver Spring, MD: American Herbal Products Association; 2000.
4. The Plant List. Version 1.1 (September 2013). http://www.theplantlist.org. Accessed November 6, 2014.
5. Tropicos.org. Missouri Botanical Garden. http://www.tropicos.org. Accessed November 6, 2014.
6. Upton R, Graff A, Soria T, Swisher D. *American Herbal Pharmacopoeia and Therapeutic Compendium: Skullcap Aerial Parts:* Scutellaria lateriflora *L.: Standards of Analysis, Quality Control and Therapeutics*. Scotts Valley, CA: American Herbal Pharmacopoeia; 2009.
7. USDA PLANTS Database. http://plants.usda.gov. Accessed November 6, 2014.
8. Brock CA. American skullcap (*Scutellaria lateriflora* L): a study on its effects on mood in healthy volunteers. PhD thesis. London, United Kingdom: University of Westminster; 2012. Available at http://westminsterresearch.wmin.ac.uk/12194/1/Christine_BROCK.pdf. Accessed November 6, 2014.
9. Applequist W. *The Identification of Medicinal Plants: A Handbook of the Morphology of Botanicals in Commerce*. Austin, TX: American Botanical Council; Saint Louis, MO: Missouri Botanical Garden; 2006.
10. Upton R, Graff A, Jolliffe G , Länger R, Williamson E, eds. *American Herbal Pharmacopoeia: Botanical Pharmacognosy—Microscopic Characterization of Botanical Medicines.* Boca Raton, FL: CRC Press; 2011:599-603.
11. Gafner S, Bergeron C, Batcha LL, et al. Analysis of *Scutellaria lateriflora* and its adulterants *Teucrium canadense* and *Teucrium chamaedrys* by LC-UV/MS, TLC, and digital photomicroscopy. *J AOAC Int*. 2003;86(3):453-460. Abstract available at http://www.ingentaconnect.com/content/aoac/jaoac/2003/00000086/00000003/art00003.
12. Hosokawa K, Minami M, Kawahara K, Nakamura I, Shibata T. Discrimination among three species of medicinal *Scutellaria* plants using RAPD markers. *Planta Med*. 2000;66(3):270-272. Abstract available at https://www.thieme-connect.com/ejournals/abstract/10.1055/s-2000-8557.
13. Hosokawa K, Minami M, Nakamura I, Hishida A, Shibata T. The sequences of the plastid gene *rpl16* and the *rpl16-rpl14* spacer region allow discrimination among six species of *Scutellaria. J Ethnopharmacol.* 2005;*99(1):105-108. Abstract available at* http://www.sciencedirect.com/science/article/pii/S0378874105001194.
14. Gafner S, Bergeron C, Batcha LL, et al. Inhibition of [3H]-LSD binding to 5-HT_7 receptors by flavonoids from *Scutellaria lateriflora*. *J Nat Prod*. 2003;66(4):535-537. Abstract available at http://pubs.acs.org/doi/abs/10.1021/np0205102.
15. Bergeron C, Gafner S, Clausen E, Carrier DJ. Comparison of the chemical composition of extracts from *Scutellaria lateriflora* using accelerated solvent extraction and supercritical fluid extraction versus standard hot water or 70% ethanol extraction. *J Agric Food Chem*. 2005;53(8):3076-3080. Abstract available at http://pubs.acs.org/doi/abs/10.1021/jf048408t.
16. Li J, Wang YH, Smillie TJ, Khan IA. Identification of phenolic compounds from *Scutellaria lateriflora* by liquid chromatography with ultraviolet photodiode array and electrospray ionization tandem mass spectrometry. *J Pharm Biomed Anal*. 2012;63:120-127. Abstract available at http://www.sciencedirect.com/science/article/pii/S0731708512000477.
17. Lin LZ, Harnly JM, Upton R. Comparison of the phenolic component profiles of skullcap (*Scutellaria lateriflora*) and germander (*Teucrium canadense* and *T. chamaedrys*), a potentially hepatotoxic adulterant. *Phytochem Anal*. 2009;20(4):298-306. Abstract available at http://onlinelibrary.wiley.com/doi/10.1002/pca.1127/abstract.
18. Zhang Z, Lian XY, Li S, Stringer JL. Characterization of chemical ingredients and anticonvulsant activity of American skullcap (*Scutellaria lateriflora*). *Phytomedicine*. 2009;16(5):485-493. Abstract available at http://www.sciencedirect.com/science/article/pii/S0944711308001499.
19. Yaghmai MS, Benson GG. The wax hydrocarbons of *Scutellaria lateriflora* L. *J Nat Prod*. 1979;42(2):228-230. Abstract available at http://pubs.acs.org/doi/abs/10.1021/np50002a014?journalCode=jnprdf.
20. Yaghmai MS. Volatile constituents of *Scutellaria lateriflora*. *Flavour Fragr J*. 1988;3(1):27-31. Abstract available at http://onlinelibrary.wiley.com/doi/10.1002/ffj.2730030106/abstract.
21. Bruno M, Cruciata M, Bondi ML, et al. Neo-clerodane diterpenoids from *Scutellaria lateriflora*. *Phytochemistry*. 1998;48(4):687-691. Abstract available at http://www.sciencedirect.com/science/article/pii/S0031942297010327.
22. Li J, Ding Y, Li XC, et al. Scuteflorins A and B, dihydropyranocoumarins from *Scutellaria lateriflora*. *J Nat Prod*. 2009;72(6):983-987. Abstract available at http://pubs.acs.org/doi/abs/10.1021/np900068t.
23. Chen P, Lin LZ, Harnly JM. Mass spectroscopic fingerprinting method for differentiation between *Scutellaria lateriflora* and the germander (*Teucrium canadense* and *T. chamaedrys*) species. *J AOAC Int*. 2010;93(4):1148-1154. Abstract available at http://www.ingentaconnect.com/content/aoac/jaoac/2010/00000093/00000004/art00013.
24. Sun J, Chen P. A flow-injection mass spectrometry fingerprinting method for authentication and quality assessment of *Scutellaria lateriflora*-based dietary supplements. *Anal Bioanal Chem*. 2011;401(5):1577-1584. Abstract available at http://link.springer.com/article/10.1007%2Fs00216-011-5246-2.
25. Islam MN, Downey F, Ng CKY. Comparative analysis of bioactive phytochemicals from *Scutellaria baicalensis, Scutellaria lateriflora*, *Scutellaria racemosa*, *Scutellaria tomentosa* and *Scutellaria wrightii* by LC-DAD-MS. *Metabolomics*. 2011;7(3):446-453. Abstract available at http://link.springer.com/article/10.1007%2Fs11306-010-0269-9.
26. Kikuchi Y, Miyaichi Y, Yamaguchi Y, Kizu H, Tomimori T, Vetschera K. Studies on the constituents of *Scutellaria* species. XIV. On the constituents of the roots and the leaves of *Scutellaria alpina* L. *Chem Pharm Bull (Tokyo)*. 1991;39(1):199-201. Available at https://www.jstage.jst.go.jp/article/cpb1958/39/1/39_1_199/_pdf.
27. de la Torre MC, Rodríguez B, Bruno M, et al. *Neo*-clerodane diterpenoids from *Scutellaria alpina. Phytochemistry. 1995;38(1):181-187. Abstract available at* http://www.sciencedirect.com/science/article/pii/0031942294006173.
28. Muñoz DM, de la Torre MC, Rodríguez B, Simmonds MSJ, Blaney WM. Neo-clerodane insect antifeedants from *Scutellaria alpina* subsp. *javalambrensis* [*sic*]. *Phytochemistry*. 1997;44(4):593-597. Abstract available at http://www.sciencedirect.com/science/article/pii/S0031942296006085.

29. Gafner S, Bergeron C, Sudberg S, et al. Adulteration of skullcap (*Scutellaria lateriflora* L.): from digital photo-microscopy to HPLC-NMR. Presented at: First Natural Health Product Conference; February 2004; Montreal, QC, Canada.
30. Ersöz T, Taşdemir D, Çaliş İ, Ireland CM. Phenylethanoid glycosides from *Scutellaria galericulata*. *Turk J Chem*. 2002;26:465-471. Available at http://pdf.easechem.com/pdf/20/kim-26-4-2-0108-2.pdf.
31. Rodríguez B, de la Torre MC, Rodríguez B, et al. Neoclerodane insect antifeedants from *Scutellaria galericulata*. *Phytochemistry*. 1993;33(2):309-315. Abstract available at http://www.sciencedirect.com/science/article/pii/003194229385509P.
32. Rodríguez B, de la Torre MC, Rodríguez B, Gómez-Serranillos P. Neo-clerodane diterpenoids from *Scutellaria galericulata*. *Phytochemistry*. 1996:41(1):247-253. Abstract available at http://www.sciencedirect.com/science/article/pii/0031942295005218.
33. Islam MN, Downey F, Ng CKY. Comprehensive profiling of flavonoids in *Scutellaria incana* L. using LC-Q-TOF-MS. *Acta Chromatographica*. 2013;25(3):555-569. Abstract available at http://www.akademiai.com/content/667663217411h723/.
34. Nicollier GF, Thompson AC, Salin ML. Flavones of *Scutellaria ovata*. *J Agric Food Chem*. 1981;29(6):1179-1181. Abstract available at http://pubs.acs.org/doi/abs/10.1021/jf00108a021?journalCode=jafcau.
35. Shang X, He X, He X, et al. The genus *Scutellaria* an ethnopharmacological and phytochemical review. *J Ethnopharmacol*. 2010;128(2):279-313. Abstract available at http://www.sciencedirect.com/science/article/pii/S037887411000022X.
36. De Smet PAGM. *Scutellaria* species. In: De Smet PAGM, Keller K, Hänsel R, Chandler RF, eds. *Adverse Effects of Herbal Drugs*. Volume 2. Berlin-Heidelberg, Germany: Springer-Verlag; 1993:289-296.
37. Tang W, Eisenbrand G. *Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine*. Berlin-Heidelberg, Germany: Springer-Verlag; 1992:919-929.
38. World Health Organization Regional Office for the Western Pacific. *Medicinal Plants in Mongolia*. Geneva, Switzerland: WHO Press; 2013:191-193.
39. Japanese Ministry of Health, Labour and Welfare. *Japanese Pharmacopoeia*. 16th ed. Tokyo, Japan: Japanese Ministry of Health, Labour and Welfare; 2011:1747. Available at http://jpdb.nihs.go.jp/jp16e. Accessed November 6, 2014.
40. Sundaresan PR, Slavoff SA, Grundel E, et al. Isolation and characterisation of selected germander diterpenoids from authenticated *Teucrium chamaedrys* and *T. canadense* by HPLC, HPLC-MS and NMR. *Phytochem Anal*. 2006;17(4):243-250. Abstract available at http://onlinelibrary.wiley.com/doi/10.1002/pca.912/abstract.
41. Avula B, Manyam RB, Bedir E, Khan IA. Rapid separation and determination of four phenylpropanoid glycosides from *T. chamaedrys* by capillary electrophoresis method. *Chromatographia*. 2003;58(11-12):751-755. Abstract available at http://link.springer.com/article/10.1365/s10337-003-0110-y.
42. Harborne JB, Tomás-Barberán FA, Williams CA, Gil MI. A chemotaxonomic study of flavonoids from European *Teucrium* species. *Phytochemistry*. 1986;25(12):2811-2816. Abstract available at http://www.sciencedirect.com/science/article/pii/S0031942200837474.
43. Gao J, Sanchez-Medina A, Pendry BA, Hughes MJ, Webb GP, Corcoran O. Validation of a HPLC method for flavonoid biomarkers in skullcap (*Scutellaria*) and its use to illustrate wide variability in the quality of commercial tinctures. *J Pharm Pharm Sci*. 2008;11(1):77-87. Available at http://ejournals.library.ualberta.ca/index.php/JPPS/article/viewFile/1122/1150.
44. Hong T, Jeong ML, Zahn M, et al. Detection of the potential adulterant *Teucrium chamaedrys* in *Scutellaria baicalensis* raw material and extract by high-performance thin-layer chromatography. *J AOAC Int*. 2009;92(3):785-788. Abstract available at http://www.ingentaconnect.com/content/aoac/jaoac/2009/00000092/00000003/art00015.
45. Makino T, Hishida A, Goda Y, Mizukami H. Comparison of the major flavonoid content of *S. baicalensis*, *S. lateriflora*, and their commercial products. *J Nat Med*. 2008;62(3):294-299. Abstract available at http://link.springer.com/article/10.1007%2Fs11418-008-0230-7.
46. Parajuli P, Joshee N, Rimando AM, Mittal S, Yadav AK. In vitro antitumor mechanisms of various *Scutellaria* extracts and constituent flavonoids. *Planta Med*. 2009;75(1):41-48. Abstract available at https://www.thieme-connect.com/ejournals/abstract/10.1055/s-0028-1088364.
47. Tascan A, Adelberg J, Tascan M, Rimando A, Joshee N, Yadav AK. Hyperhydricity and flavonoid content of *Scutellaria* species in vitro on polyester-supported liquid culture systems. *HortScience*. 2010;45(11):1723-1728. Available at http://hortsci.ashspublications.org/content/45/11/1723.full.
48. Cole IB, Cao J, Alan AR, Saxena PK, Murch SJ. Comparisons of *Scutellaria baicalensis*, *Scutellaria lateriflora* and *Scutellaria racemosa*: genome size, antioxidant potential and phytochemistry. *Planta Med*. 2008;74(4):474-481. Abstract available at https://www.thieme-connect.com/ejournals/abstract/10.1055/s-2008-1034358.
49. Brock C, Whitehouse J, Tewfik I, Towell T. Identity issues surrounding American skullcap (*Scutellaria lateriflora*) and an optimised high performance liquid chromatography method to authenticate commercially available products. *J Herb Med*. 2013;3(2):57-64. Abstract available at http://www.sciencedirect.com/science/article/pii/S2210803313000043.
50. Colson KL, Fischer C, Gafner S, Hicks J, Luchsinger S. Quality control analysis of skullcap using ^{1}H NMR. Presented at: 52nd Annual Meeting of the American Society of Pharmacognosy; 2011; San Diego, CA.
51. Sandasi M, Vermaak I, Chen W, Viljoen AM. Skullcap and germander: preventing potential toxicity through the application of hyperspectral imaging and multivariate image analysis as a novel quality control method. *Planta Med*. 2014;80(15):1329-1339. Abstract available at https://www.thieme-connect.com/DOI/DOI?10.1055/s-0034-1383037.
52. LaBudde RA, Harnly JM. Probability of identification: a statistical model for the validation of qualitative botanical identification methods. *J AOAC Int*. 2012;95(1):273-285. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3620024/.

Appendix 1

Table 4. Comparison among different published HPLC methods for *S. lateriflora*

Reference	Number of samples[a]	Origin of samples (aerial parts when not specified)	Sample preparation: handling[b] / duration [min][c]	Column type	Run time [min][d]	Detection wavelength (UV) or ion mode (MS)
[6]	1[e]	AHP	6 / 1460	C18	46	UV: 280
[15]	1[e]	Commercial raw material	9[f] / 2460[f]	C18	46	UV: 280
[16]	1	Commercial raw material	15 / 200	C18	75	MS (negative)
[17]	9	AHP	5 / 90	C18	75	UV: 280, 310, 330, 350 MS (pos/neg)
[18]	10	Commercial products off shelf	ASE[g]	C18	85	UV: 278
[24]	17	AHP & Internet	7 / 95	C18	18	MS (negative)
[25]	1	Grown from seeds	8 / 150	C18	36	UV: 280
[43]	7	Commercial products off shelf	3[g] / 15[h]	C18	32	UV: 270
[45]	8	Research Center for Medicinal Plant Resources, Tsukuba & commercial sources	15 / 180	C18	24	UV: 277
[46,47]	1	Grown from seeds	ASE[g]	C18	33	UV: 270
[48]	1	Tissue culture from seeds	10 / 185	C18	60	MS (positive)
[49]	2	AHP & commercial raw material	7 / 170	C18	30	UV: 280

[a]Number of *S. lateriflora* samples analyzed
[b]Number of sample preparation steps involved (see Table 6 in Appendix 1)
[c]Estimated based on description provided in the reference (see Table 6 in Appendix 1)
[d]Not including the time used to return to initial conditions and equilibrate
[e]Method has been used with over 20 authenticated reference samples and commercial products as part of QC
[f]Extraction with ethanol-water (7:3, v/v)
[g]ASE = accelerated solvent extraction
[h]Indications refer to tinctures only

Table 5. Comments on the published HPLC methods for *S. lateriflora*

Reference	Comments
Upton, [6] Bergeron [15]	The method was validated by 2 independent laboratories, and has good separation and peak shapes. The sample extraction was optimized for flavone-glucuronides (unpublished data) but is quite lengthy and therefore addition of citric/ascorbic acid is needed to prevent degradation. The Zorbax column is superior to the Phenomenex column, but some peaks (e.g., **6**) are not resolved. There is no indication about detection levels of *Teucrium* species. System suitability parameters have not been published.
Li [16]	This is a reliable fingerprinting method, and the MS fractionation gives excellent data on the peak identity for phenolics. There are no data on *Teucrium* species detection. The sample preparation time of over 2 hours with a number of handling steps is not ideal for a routine lab method. The run time is lengthy without noticeable improvement in peak separation over other methods. The method calls for the injection of a fairly concentrated solution, so samples high in **1** could lead to column overload. There are no data on system suitability parameters and the method has not been validated.
Lin [17]	The chromatogram includes both flavonoids and diterpenes. The method provides good separation but the high injection volume is likely the reason for the tailing observed with **1**. The sample extraction is easy and quick, optimized for flavone-glucuronides. Some diterpenes may not be very soluble in 60% aqueous methanol. *Teucrium* species at levels as low as 1% in *S. lateriflora* can be detected. The run time is lengthy. The sample size is small (100 mg) and sonication (1 hr) longer than in other methods. There are no data on system suitability parameters and the method has not been validated.
Zhang [18]	This is a validated method and the conditions give good peak shapes. The sample preparation is specific to research project, but is not applicable in routine QC. There are no data on *Teucrium* species detection, but **8** can be quantified down to 0.5 mg/g (500 ppm) of plant material. The method is lengthy (no peaks of interest are eluting in the first 30 min) and the separation between chrysin and oroxylin A is insufficient. There are no data on system suitability parameters.
Sun [24]	This method has a short run time due to use of UPLC-MS. The sample preparation method is easy and quick, although the sampling size (10 mg) is low for a quantitative method and therefore some of the precision may be lost. There is no indication on detection levels of *Teucrium* species. The chromatogram contains some unresolved peaks. There are no data on system suitability parameters and the method has not been validated.
Islam [25]	The HPLC-UV method is reasonably short (the HPLC-MS method is very short) and shows a good separation. Some broadening of later eluting peaks (e.g., chrysin) is observed. The internal standard (digoxin) for HPLC-MS is chemically very different from the target analytes. MS parameters are not fully detailed and there is no indication about detection levels of *Teucrium* species; however, **8** can be quantified down to 0.51 mg/g (510 ppm, UV/vis) and 0.38 mg/g (380 ppm, MS) dry plant material. There are no data on system suitability parameters and the method has not been validated.
Gao [43]	The method is validated, reasonably short, and has good peak shapes. It has been tested only on tinctures. Only very small volumes are used for sample preparation (as low as 50 µL), possibly leading to lower precision. The internal standard (IS) helps to correct for imprecisions in the injection system, but the additional dilution due to the IS addition using small volumes might actually introduce a larger error than any contribution from injection imprecisions. There are no data on *Teucrium* species detection. Not all peaks are well separated. Data on system suitability parameters are lacking.
Makino [45]	This is an official method (JP XV for *S. baicalensis*). It is short and gives good peak shapes. The extraction procedure using MeCN-phosphoric acid is labor intensive. There are no data on *Teucrium* species detection. Not all peaks are well separated. Data on system suitability parameters are lacking.
Parajuli, [46] Tascan [47]	The method is reasonably short and has good peak shapes. The sample preparation is specific to the research project, and not applicable in routine QC. Addition of HCl during the sample preparation may hamper stability of phytochemicals; the HCl concentration is unclear. There are no data on *Teucrium* species detection. The method works only for medium-to-low polar compounds. Flavone-glucuronides are not sufficiently well separated. There is no information on column temperature. Data on system suitability parameters are lacking and the method has not been validated.
Cole [48]	The high injection volume (50 µL) into the 100% aqueous initial mobile phase may lead to precipitation of compounds on column. Materials analyzed were from tissue cultures, which will differ from wild-crafted or cultivated plant material. The HPLC parameters are unclear: the text describes a 45 min run time but states that the elution of peaks was monitored up to 60 min, which would mean eluting for 30 min with 100% MeCN. The HPLC-MS trace also shows a run time of 60 min, with no peak of interest eluting after 25 min. MS detection parameters and validation data for skullcap flavonoids are not available. No *Teucrium* samples were analyzed. There are no data on system suitability parameters.
Brock [49]	The chromatography is reasonably short. The sample preparation time is short; and the sonication should be 30 min rather than twice for 15 min (personal communication to S. Gafner, September 19, 2013). There are no data on the method's ability to detect *Teucrium* species. The separation is insufficient and a reprint of the chromatogram shows prominent peak tailing (indicative of contamination, bad column, or some precipitation of flavonoids in the mobile phase during the injection process). There are no data on system suitability parameters and the method has not been validated.

Note: The use of the term "validated" indicates a method has been validated for quantitative analysis, not for qualitative identification according to LaBudde and Harnly. [52]

Table 6. Various sample process steps and time requirements. *If no specific duration is given* in a paper, the time is estimated according to the table (e.g., if the authors sonicate a sample for 15 min, then 15 min is used for calculation rather than 30 min as indicated in the table). Plant collection, drying, and grinding are not included in order to better compare the actual processing time among the various methods.

Processing step	Time [min]
Cooling down	30
Combining fractions	5
Decanting and centrifugation	10
Dissolving	5
Diluting	5
Evaporation (organic solvents only)	60
Evaporation (solvent mixtures containing water)	120
Filling to volume	0
Filtration (paper, organic solvents only)	30
Filtration (paper, solvent mixtures containing water)	60
Filtration (0.2 or 0.45 µm HPLC filters)	5
Initial addition of solvent	5
Lyophilization (including time to freeze the sample)	720
Mixing (by inversion)	0
Partitioning	60
Sonication	30
Sonication (including solvent addition, e.g., in repeated extractions)	30
Washing (flasks, beakers)	5
Weighing	5

Skullcap *Scutellaria lateriflora*
Photo ©2015 Steven Foster