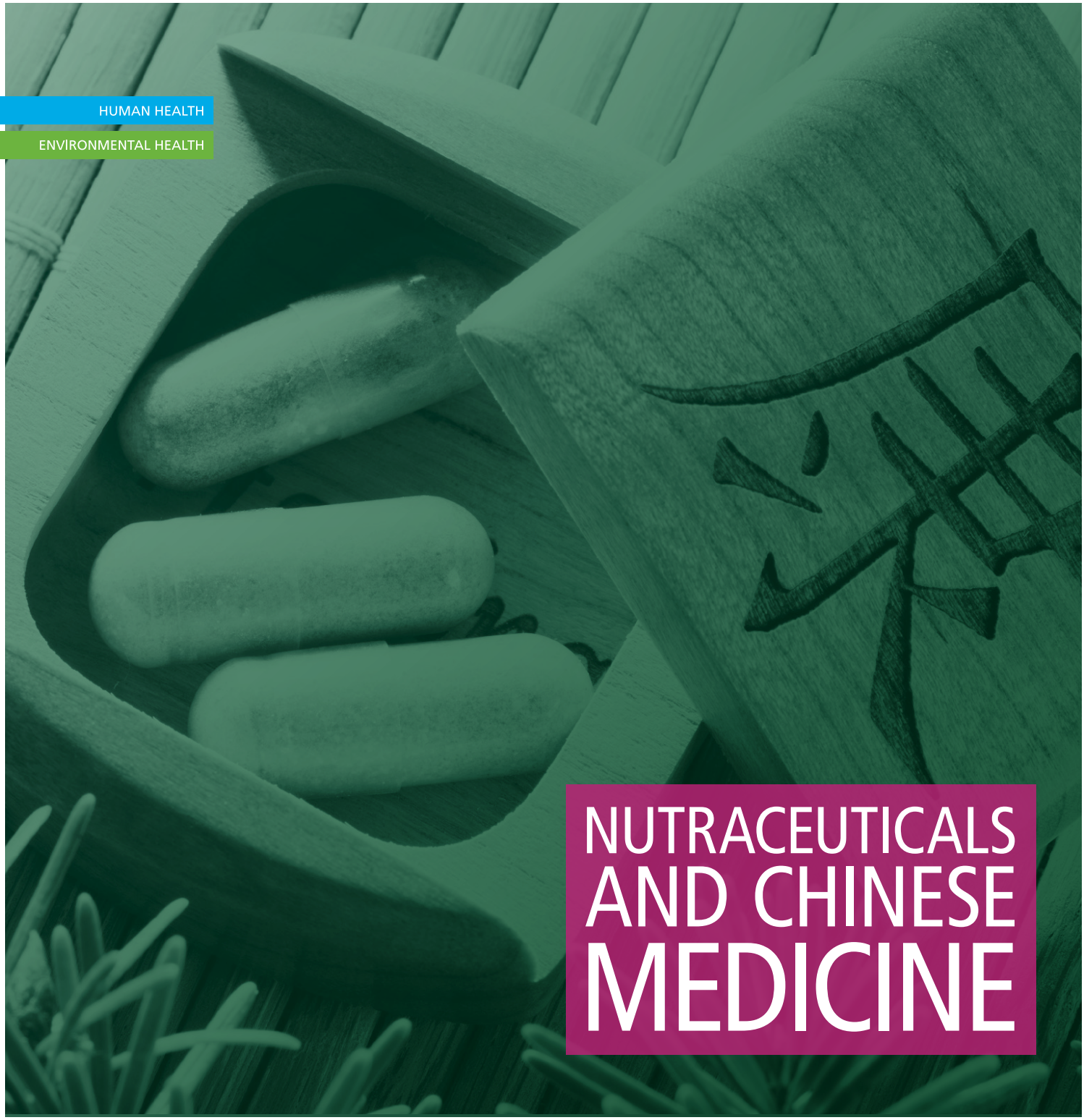
HUMAN HEALTH

ENVIRONMENTAL HEALTH



NUTRACEUTICALS AND CHINESE MEDICINE

Nutraceuticals and
Chinese Medicine Compendium

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APPLICATION NOTE

FT-IR Spectroscopy

Authors

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Rapid Authentication of Larch Fiber Dietary Supplement Ingredient by FT-IR Using Diamond Single Bounce uATR Sampling Device

Introduction

Larch fiber is a complex botanical material rich in arabinogalactin containing polymers that has shown promise as an immune-stimulating and pre-biotic dietary supplement. However, due to the highly complex nature of the material, it does not lend itself to easy identification using microscopy or thin layer chromatography; two common methods used to authenticate dietary supplements. In order to comply with the new Dietary Supplement current Good Manufacturing Practices (cGMP's), all dietary ingredients must be identified using a valid scientific method. A PerkinElmer MIR/NIR spectrometer equipped with a single-bounce diamond uATR sampling accessory allows for rapid identification of larch fibers of various grades. Even though there is some variance among samples, the fingerprint region has a consistent profile that is unique and easily recognized. Differentiation between authentic larch fiber and common potential economic adulterants (cellulose and carageenan) is achieved using this method. FT-IR is a very well established methodology for identification of chemical compounds and can be utilized to comply with the 21 CFR Part 111 cGMP's for Dietary Supplements. Using the Enhanced Security software meets the requirements of 21 CFR Part 11 which is also called for in the Dietary Supplement cGMP's.

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Authentication of Dietary Supplement Ingredients

An ideal identity method would be a) fast, b) simple, c) able to differentiate larch fiber from other commonly utilized ingredients and d) robust enough to tolerate slight changes in the finished product as a result of manufacturing differences. As will be shown here, the use of FT-IR (MIR) coupled with the uATR sampling accessory meets these conditions.

Larch fiber polymers can range from 10,000-120,000 Daltons. The typical ratio of galactose to arabinose units is 6:1. An analysis of the sugar ratios can help confirm identity but this is both a time and resource intensive process. Yariv reagents can also be utilized in the analysis of larch fiber arabinogalactins but these are proprietary and costly reagents and, like sugar profiling, requires a high level of technical skill.

The color of larch fiber is white to off-white and it has a powder consistency which can create challenges for visual identification. Various processing methods create variations in the appearance. Further, it can easily be substituted with other products commonly used in manufacturing supplements making economic or unintentional adulteration a concern.

Experimental

Materials used in this study are the following:

1. Larch Arabinogalactin – (Fluka® brand) Sigma-Aldrich®, St. Louis, MO
2. Gum Arabic (from acacia) – Sigma-Aldrich®, St. Louis, MO
3. Cellulose Powder & Maltodextrin – gifts of Dr. Andrew Halpner
4. Carageenan – Research Organics®, Cleveland, OH
5. Larch Fibers (Lonza & Spectrum) – gifts of Dr. Anthony Smith

The PerkinElmer MIR/NIR spectrometer is capable for both near infrared (NIR) and mid infrared (MIR) operation, used with a single bounce diamond uATR sampling accessory fitted with conical pressure plate and adaptor for 0.5 mm shoe for 1 or 3 bounce plates (Part No. L1202049).

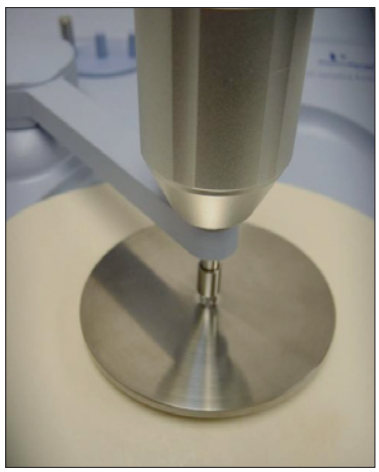

Figure 1. Close-up view of pressure arm and uATR crystal with conical shoe and adaptor.

Data acquisition was carried out using the following procedure:

- Place a small amount of sample on the uATR accessory to cover the crystal
- Compress with pressure arm until ~70% transmission is achieved
- Collect spectra from 4000 – 650 cm^{-1}

Sample analysis involved post acquisition use of automated data processing using "Data Tune Up" to correct for the distortion caused by uATR. Data were compared on-screen and interpreted by visual inspection. Maltodextrin and larch arabinogalactin were further analyzed using the data comparison software to demonstrate automated matching.

Results and Discussion

A comparison of the spectra obtained from the three larch materials used in this study show close agreement in the fingerprint region (Figures 2 and 3). Slight differences from water bands are due to differing levels of moisture.

Conclusion

While some materials may require library matching or the formation of derivatives, the unique profile of larch fiber readily allows for authentication by FT-IR based on visual inspection. A "Compare" analysis of larch fiber and maltodextrin showed a match quality of 0.56 (2000 cm^{-1} to 650 cm^{-1}) whereas arabinogalactin larch standard match quality was >0.80 demonstrating that comparison against a reference set using automated software analysis is also possible. The mid-IR data differs from near-IR in that a visual inspection of the transmission spectra is often adequate to confirm identity. This is especially true when working with pure chemical compounds. Finally, the ability to compress samples with real-time instrument read back of force gauge pressure helps assure consistent spectra and good reproducibility.

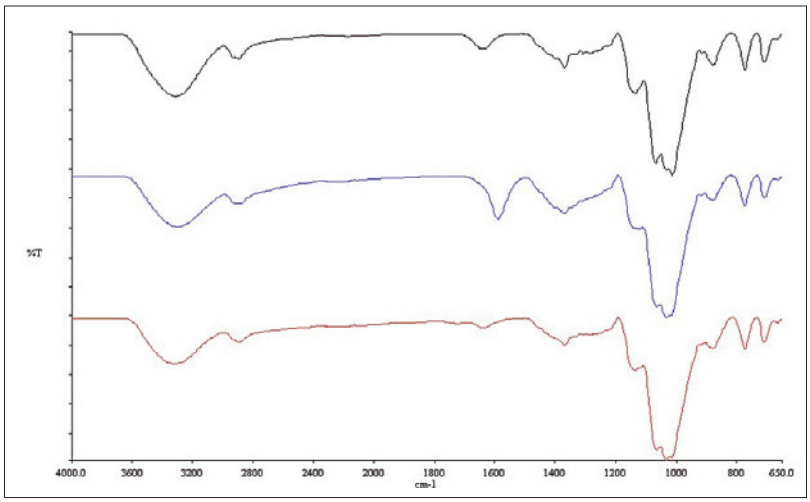

Figure 2. Larch fiber samples stacked full scan for three genuine samples.

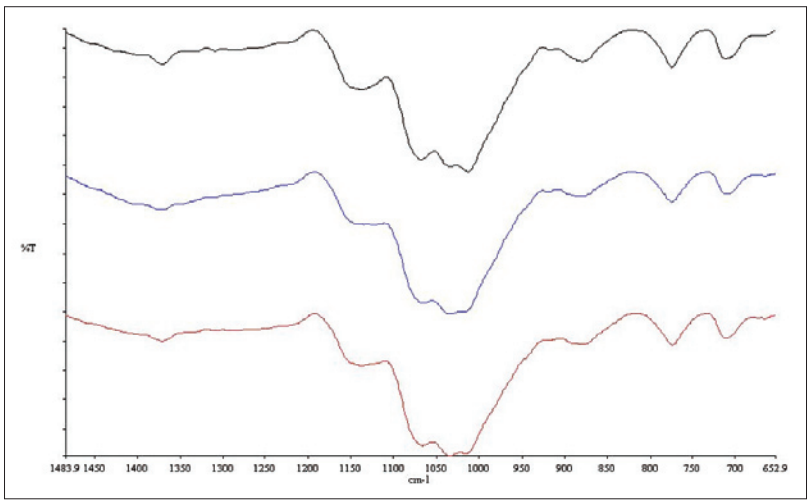

Figure 3. Fingerprint region (1450 – 650 cm^{-1}) of larch samples showing consistent profile between three genuine samples of larch fiber.

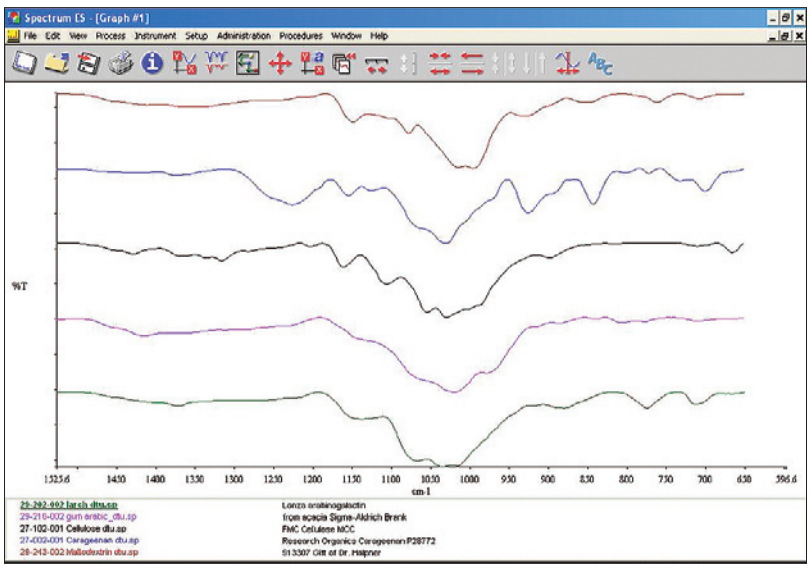

Figure 4. Screen shot of fingerprint region of larch fiber and various potential adulterants (top-red Maltodextrin, middle-upper-blue Carageenan, middle-black Cellulose, middle-lower-pink Acacia Gum Arabic, bottom-green Arabinogalactin).

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2. Alternative Medicine Review Vol 5 #5 2000 pg 463-466

3. 2009-2010 USP Dietary Supplements Compendium.

4. Federal Register – 21 CFR Part 111 (Code of Federal Regulations).

5. Infrared Spectral Interpretation – A Systematic Approach, Brian Smith CRC Publishers 1999.

Acknowledgement

A special thanks to Dr. Andrew Halpner and Dr. Anthony Smith for their generous gifts of authenticated commercial dietary ingredients.

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009489_01

APPLICATION NOTE

Atomic Absorption

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The Determination of Minerals and Metals in Multi-Mineral/Multi-Vitamin Tablets by Flame Atomic Absorption Spectroscopy

Introduction

There are many mineral dietary supplements available in today's marketplace to ensure that mineral deficiencies do not occur in one's diet. The mineral content of these products must be verified for quality control (QC) purposes. Furthermore, the Nutritional Labeling and Education Act of 1990 mandates accurate labeling of all food supplements sold in the U.S. which means accurate testing of the products is mandatory. In many labs, this task is accomplished by the technique of flame atomic absorption spectroscopy (FAAS). FAAS has the advantages of lower initial cost, low cost per analysis, and requires less operator training than many other trace elemental techniques. The objective of this work is to demonstrate the applicability of FAAS using the PerkinElmer® PinAAcle™ 900T to accomplish this task. Seven elements are determined in two commercially available multi-mineral tablets, a NIST® Standard Reference Material, and a commercial reference material which simulates a mixed food diet.

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Experimental

Instrumentation

The PinAAcle 900T flame and longitudinal Zeeman furnace atomic absorption spectrometer controlled by WinLab32™ for AA software, running under Microsoft® Windows® 7, was used for all analyses (PerkinElmer, Inc., Shelton, CT). A high-sensitivity nebulizer (Part No. N3160112) with a spacer was employed. Single-element Lumina™ hollow cathode lamps (HCLs) were used and the instrumental operating conditions for each element in this application are shown in Table 1. A four-second integration time and three replicates were used for all elements.

Figure 1. PinAAcle 900T atomic absorption spectrometer.

Sample and Standard Preparation

One NIST® SRM 3280 Multi-vitamin/Multi-mineral Tablet and two over the counter multi-vitamin/multi-mineral tablets were coarsely ground. Nominally, 0.75 g were weighed out and rinsed into a 1000 mL volumetric flask containing about 20 mL of ASTM® Type I deionized water (>16MΩ • cm). Four mL of HCl (trace metal grade), two mL of HNO_3 (trace metal grade), and a magnetic stirring bar were then added to the flask. This sample digestion solution was stirred for approximately one hour and warmed, not to boiling, on a hot plate. Deionized water was then added to the volumetric mark after removal of the magnetic stir bar. The sample digestion solution was then filtered through Whatman® 46 paper (passes < 8 micron) to remove waxy undissolved material, presumably from the tablet's coating. The filtrate was then analyzed, after appropriate dilution, against single-element aqueous standards (PerkinElmer Pure) for elemental quantitation. A Certified Reference Material Mixed Food Diet (CRM-MFD) (High Purity Standards, Charleston, SC) was prepared by dissolution, following the manufacturer's instructions, to an appropriate concentration for individual elements.

All tablets also contained about 2 mg/tablet of silicon, much of which may have remained undissolved. For an accurate measurement of the silicon, HF (trace metal grade) and plastic volumetric flasks should be employed for the dissolution.

For calcium and magnesium determinations, $La(NO_3)_2$ (reagent grade) was added to all standards, blank and samples to a concentration level of 0.2% to remove chemical inferences from other matrix elements. For potassium determinations, CsCl (reagent grade) was added to all test solutions to a concentration level of 0.2% as an ionization buffer.

Results

All elements showed acceptable calibration criteria. The results obtained for the FAAS analyses of the multi-vitamin/multi-mineral tablets are shown in Table 2. The values are corrected for laboratory sample dissolution steps and dilutions. The commercial tablets were calculated and reported in mg/tablet as this was the unit shown and guaranteed on each label. All elements were found to be within the certified range for the NIST® 3280 tablet. Both commercial tablets showed good correlation to labeled quantity guaranteed by the manufacturer.

Table 1. **PinAAcle 900T Instrumental Parameters.**

	Element						
Parameter	**Ca**	**Mg**	**K**	**Fe**	**Zn**	**Cu**	**Mn**
Wavelength (nm)	422.7	285.2	766.5	248.3	213.9	324.8	279.5
Slit Width (nm)	0.7	0.7	0.7	0.2	0.7	0.7	0.2
Oxidant Flow (L/min)	10	10	10	10	10	8.7	10
C_2H_2 Flow (L/min)	2.5	2.5	2.5	2.2	2.5	2.0	2.5
Working Range (mg/L)	5	1	4	4	1	4	2
HCL Part Nos.	N3050114	N3050144	N3050139	N3050126	N3050191	N3050121	N3050145
Standard Part Nos.	N9303763	N9300179	N9303779	N9303771	N9300178	N9300183	N9303783

Table 2. **Corrected FAAS results for one certified reference material and two commercial multi-vitamin/multi-mineral samples.**

	NIST® 3280 (mg/g)			Commercial Tablet 1 (mg/Tab)			Commercial Tablet 2 (mg/Tab)		
Element	**Certified**	**Found**	**SD**	**Label**	**Found**	**SD**	**Label**	**Found**	**SD**
Ca	110.7	107	0.8	162	163	0.6	200	211	0.6
Mg	67.8	69.1	0.2	100	107	0.4	100	109	0.4
K	53.1	53.8	0.3	80	83.8	0.4	80	90.7	0.4
Fe	12.35	12.9	0.05	18	19.1	0.08	n/a	0.23	0.006
Zn	10.15	10.1	0.02	15	15.5	0.04	15	15.2	0.03
Cu	1.4	1.42	0.01	2	2.19	0.01	2	2.04	0.01
Mn	1.44	1.48	0.01	2	1.91	0.01	2	2.04	0.01

The results for the FAAS analysis of the Certified Reference Material – Mixed Food Diet from High Purity Standards – are given in Table 3. All elements were found to be within 5% of the certified value, showing excellent agreement with the reference material.

Table 3. **Mixed Food Diet CRM results using aqueous standards and FAAS on a PinAAcle 900T.**

	CRM-MFD (mg/L)		
Element	**Certified**	**Found**	**SD**
Ca	40	39.8	0.2
Mg	12	11.8	0.03
K	160	159	0.005
Fe	0.8	0.802	0.005
Zn	0.3	0.290	0.001
Cu	0.06	0.061	0.001
Mn	0.2	0.191	0.003

Conclusions

The mineral content of multi-vitamin/multi-mineral tablets must be determined in order to ensure the quality of commercial dietary supplements. This method demonstrates the ability of the PinAAcle 900T flame atomic absorption system to accurately measure minerals in both commercial tablets and food simulation diets. It is an efficacious method that allows for cost effectiveness and ease of use, with less operator training than other analytical methods. The PinAAcle 900H (Flame and Deuterium Furnace) and PinAAcle 900F (Flame only) spectrometers can also be used for this application.

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009636A_01

APPLICATION NOTE

FT-IR Spectroscopy

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Classification of Samples of the Traditional Remedy "Chinese Goldthread" by FT-IR Spectrometry and AssureID software

Introduction

Coptidis Rhizoma (the root of the Coptis chinensis plant), which is also known as goldthread, is one of the most commonly used traditional Chinese medicines (TCM) in China. It can clear heat and reduce fire and is usually used for diarrhoea, vomiting, jaundice, fever, toothache, conjunctivitis and some other conditions.[1]

In a separate note,[2] we have shown that FT-IR spectrometry can be used to identify the presence of the marker compound (berberine) in Coptidis Rhizoma samples, and importantly, to distinguish it from other berberine-containing materials (Phellodendri Chinensis Cortex and Phellodendri Amurensis Cortex). Second-derivative processing and peak location methods were used for the identification.

TCM materials are normally processed before being used clinically, and different processing methods lead to different medicinal effects. The Chinese Pharmacopoeia[1] lists three processing methods for Coptidis Rhizoma. Coptidis Rhizoma processed by rice wine is used for toothache and conjunctivitis, while that processed by ginger juice or the extract of Corni Fructus is used as an anti-emetic and to comfort the stomach. These different processing methods do not bring about an obvious difference in the appearance of the herb, so it is important to develop an analytical method that is capable of distinguishing these materials. In terms of chemical composition, the differences between the raw and the processed herbs may be not as significant as those between different kinds of herbs, so we can expect to require a more careful analysis of the spectra using chemometric tools.

In this note, we show that FT-IR spectrometry combined with AssureID™ software and the COMPARE™ and SIMCA algorithms can be used to distinguish the raw and processed Coptidis Rhizoma samples.

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Experimental

Samples of raw Coptidis Rhizoma were obtained from the Institute of Chinese Materia Medica of the China Academy of Chinese Medical Sciences. Some raw Coptidis Rhizoma samples were processed according to the standard procedures of the Chinese Pharmacopoeia[1] to obtain three types of processed Coptidis Rhizoma. Coptidis Rhizoma processed by rice wine was obtained by mixing 1 kg of raw Coptidis Rhizoma with 125 g of rice wine and stir-baking to dryness. Coptidis Rhizoma processed by ginger juice was obtained by mixing the juice from 125 g of raw ginger with 1 kg of raw Coptidis Rhizoma and stir-baking to dryness. Coptidis Rhizoma processed by Corni Fructus was obtained by mixing 1 kg of raw Coptidis Rhizoma with the liquid part from decocting 100 g of Corni Fructus with water and then stir-baking to dryness.

Spectra of the raw and the processed Coptidis Rhizoma samples were measured on a PerkinElmer® Spectrum™ 100 FT-IR spectrometer equipped with a single-bounce diamond attenuated total reflectance (ATR) sampling accessory (Figure 1). Spectra were acquired at 4 cm^{-1} resolution and for an accumulation time of 60 seconds. The COMPARE and SIMCA analyses were carried out using AssureID software.

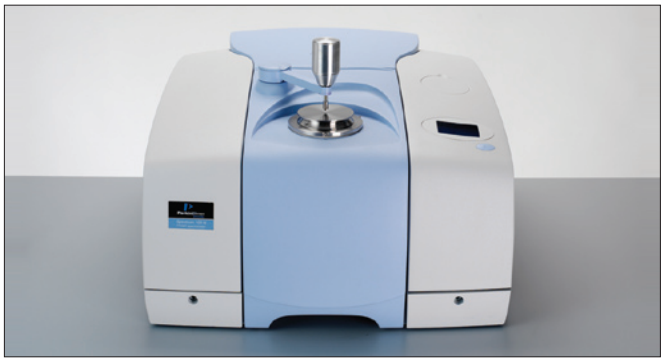

Figure 1. Spectrum 100 FT-IR with single-bounce diamond ATR accessory.

Results and Discussion

As-measured and processed FT-IR spectra

Typical spectra of the raw and processed samples are shown in Figure 2. There are no obvious differences between the spectra of single samples of the raw and the processed Coptidis Rhizoma.

As the differences between materials become smaller, it becomes increasingly important to develop and validate classification models with large sets of data. If a small dataset is relied on too heavily, a classification may be developed based on differences that are due to chance, and not reproducible.

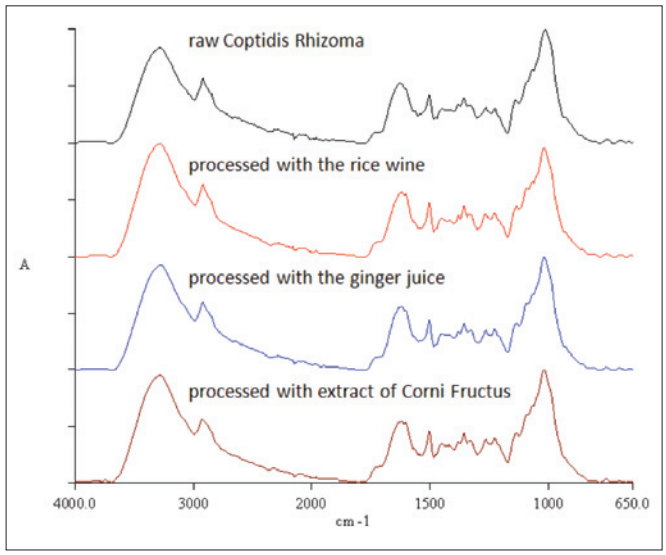

Figure 2. FT-IR spectra of the raw Coptidis Rhizoma and processed by different methods.

Spectra of 7 raw samples and 7 samples processed by each of rice wine, ginger juice and extract of Corni Fructus were used to develop the classification models. Separate sets of 3 samples of each material were measured in the same way and used to validate the performance of the classification models.

The spectra were pre-processed by AssureID software using second derivative and standard normal variate (SNV) transformations. SNV normalizes spectra to a common intensity, removing variations due to variable contact between the sample and the ATR crystal. The processed spectra are shown in Figure 3.

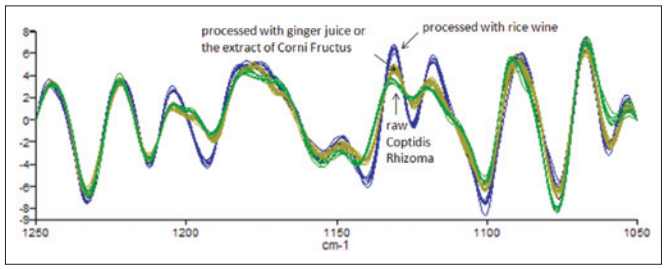

Figure 3. Normalized second-derivative FT-IR spectra of the raw Coptidis Rhizoma (green) and processed by different methods (rice wine, blue; ginger juice or extract of Corni Fructus, yellow).

These processing steps greatly emphasize the differences between the sample spectra, and the presence of several samples for each material allows the comparison of within-group and between-group variations. The samples treated with ginger juice or extract of Corni Fructus cannot be distinguished from each other, but are clearly distinct from the raw and rice-wine-processed samples.

These spectra, using the wavenumber range 1250-1050 cm^{-1}, were used to develop classification models using the COMPARE and SIMCA algorithms in the AssureID software. Three materials were configured: one for raw Coptidis Rhizoma, one the rice wine treatment, and one for ginger juice or Corni Fructus (combined).

Discrimination using the COMPARE algorithm

COMPARE functions by measuring the correlation between each sample spectrum and the mean sample spectrum for each material. A threshold correlation coefficient is established for each material that allows the classification of future samples.

Figure 4 shows the correlation coefficients of all samples with the average spectrum for each group. If the correlation within each group is significantly higher than the correlations between the groups, a reliable classification model can be built – indicated by the dashed correlation thresholds. In Figure 4a, for example, we can see that the "raw" spectra all have a correlation of >0.99 with the mean raw spectrum, while the "rice wine" and "ginger juice/Corni Fructus" spectra have correlations with the mean "raw" spectrum of around 0.97 or lower. Similar observations can be made about the processed materials from Figures 4b and 4c. From all of this, and assuming that the samples are representative, we can conclude that the three materials can be distinguished.

This conclusion was supported by the correct classification of all of the independent validation samples.

Discrimination using the SIMCA algorithm

While the COMPARE algorithm appears sufficient here, it cannot cope as well when there is variability within each class. For example, spectra of samples of the raw root taken from plants grown in different areas, or dried in different ways may show a greater degree of variation. In this case, a single mean spectrum is no longer representative of the set of spectra.

The SIMCA algorithm builds a principal components model of the spectra within each class, so that future spectra exhibiting variation from the mean that is consistent with the spectra used to calibrate the model can be correctly recognized as coming from the same material. With this approach, even materials exhibiting a large degree of within-class variability can be successfully distinguished, provided that the differences between classes are not the same as the differences within each class.

The classification model is developed by building individual principal components models for each class, and establishing thresholds on the distance from the center of each model. These calculations are performed automatically, and the Troubleshooting tool warns the user of any potential problems (such as materials that are poorly separated and cannot be distinguished).

Figure 5 shows a scatter plot of all the samples in the space defined by the first three principal components of the complete data set. Samples of each group are gathered in a region enveloped by an ellipsoid, and a good degree of separation is evident.

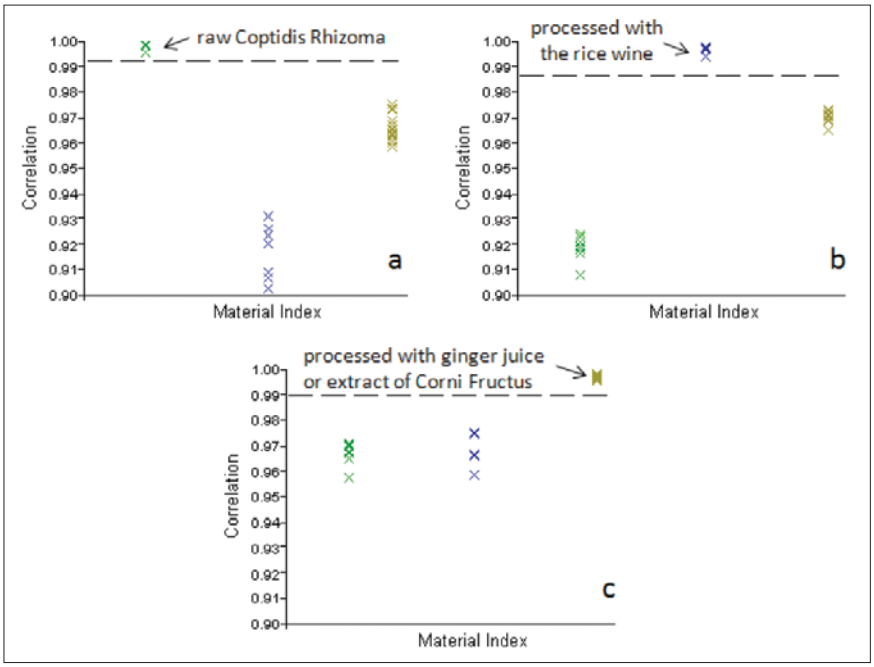

Figure 4. Correlation coeffiients of all samples with the average spectrum for each group. (a) Raw Rhizoma Coptidis (b) Rhizoma Coptidis processed by rice wine (c) Rhizoma Coptidis processed by either ginger juice or extract of Corni Fructus. The dashed line indicates the classification threshold.

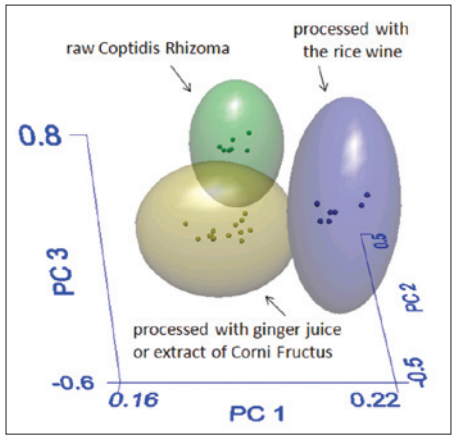

Figure 5. Principal components scatter plot of all the samples.

As with the COMPARE model, 100% successful classification of both the calibration and validation samples was achieved.

AssureID in routine use

After a classification model is built and validated, it can be incorporated into a workflow that can be run using the dedicated Analyzer software module. The workflow automatically guides the analyst through the necessary sample preparation steps, configures the instrument, carries out the calculations and reports the result as a simple pass/fail for the selected material. All of the results are stored in a database and full traceability is maintained for compliance with electronic data-management regulations like 21 CFR Part 11.

Summary

In this note we have shown that the FT-IR spectrometry combined with AssureID software is a simple and powerful tool for quality control of TCMs. Samples of raw Coptidis Rhizoma and that processed by various means can readily be distinguished. It proved impossible to distinguish root processed by ginger juice from that processed by extract of Corni Fructus, but this is a less important distinction, as both are used to treat the same ailments.

The Spectrum 100 FT-IR equipped with a diamond ATR accessory and AssureID Analyzer software provides a very quick and simple way for analysts to identify samples.

References

1. Chinese Pharmacopoeia Commission, Chinese Pharmacopoeia (vol. 1), Beijing: China Medical Science and Technology Press, 2010, p 285-286.

2. Chen Jian-Bo, Zhou Qun, Sun Su-Qin, Ben Perston and Patrick Courtney, Rapid Quality Control of the Traditional Remedy "Chinese Goldthread" by FT-IR Spectrometry PerkinElmer Application Note 009318_01.

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APPLICATION NOTE

FT-IR Spectroscopy

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Rapid Quality Control of the Traditional Remedy "Chinese Goldthread" by FT-IR Spectrometry

Introduction

Coptidis Rhizoma (the root of the *Coptis chinensis* plant), which is also known as goldthread, is one of the most commonly used traditional Chinese medicines (TCM). It can clear heat and reduce fire and is usually used for diarrhoea, vomiting, jaundice, fever, toothache, conjunctivitis, and some other conditions.[1]

In the Chinese Pharmacopoeia, the isoquinoline alkaloid berberine is considered as the marker component of Coptidis Rhizoma and is used for the identification of this herb.[1] However, berberine is also present in some other herbs, such as Phellodendri Chinensis Cortex and Phellodendri Amurensis Cortex, which are used to treat different conditions.[2,3]

In general, the strategy of relying on a single marker compound to identify a herb is clearly not reliable, as there may be other herbs containing the same compound. Robust identification requires consideration of the entire chemical composition of the sample. Among analytical methods that can provide this information, infrared spectroscopy offers a unique combination of specificity, sensitivity, speed, and convenience of sampling. In recent years, numerous academic papers have been published describing the use of infrared spectroscopy for characterisation of herbal medicines.[4-6] Practical guidelines for successful implementation of the method have recently been published.[7]

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In this note, we show that Fourier Transform Infrared Spectroscopy (FT-IR) can be used to identify the existence of berberine in Coptidis Rhizoma samples and to distinguish Coptidis Rhizoma from Phellodendri Chinensis Cortex and Phellodendri Amurensis Cortex.

Experimental

Berberine hydrochloride was purchased from Sigma-Aldrich®. Samples of raw Coptidis Rhizoma, Phellodendri Chinensis Cortex, and Phellodendri Amurensis Cortex were obtained from the Institute of Chinese Materia Medica of the China Academy of Chinese Medical Sciences.

Spectra of berberine hydrochloride, Coptidis Rhizoma, and the Phellodendri Chinensis Cortex and Phellodendri Amurensis Cortex samples were measured on a PerkinElmer® Spectrum™ 100 FT-IR spectrometer equipped with a single-bounce diamond attenuated total reflectance (ATR) sampling accessory (Figure 1). Spectra were acquired at 4 cm^{-1} resolution and for an accumulation time of 60 seconds. The spectra were analyzed in Spectrum software (version 10).

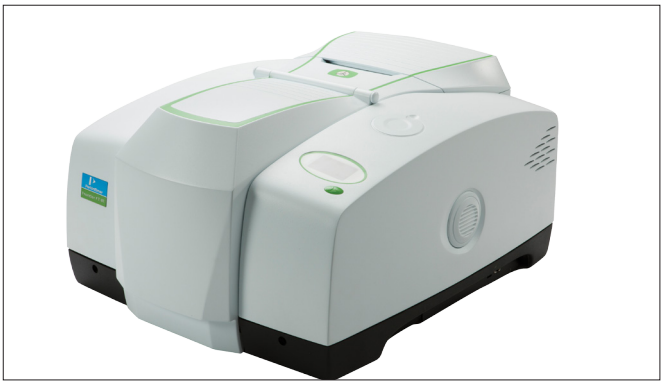

Figure 1. The Frontier FT-IR.*

Results and Discussion

Confirmation of the presence of the marker compound

Comparing the FT-IR spectra of berberine hydrochloride and the raw Coptidis Rhizoma (Figure 2), we can see that all of the major absorption peaks of berberine, including those at 1507, 1385, 1362, 1340, 1271, and 1232 cm^{-1}, are also present in the spectrum of Coptidis Rhizoma. This gives us confidence that the marker compound is indeed present in the sample.

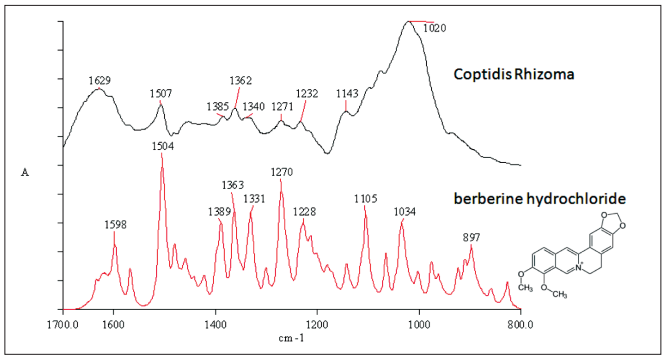

Figure 2. FT-IR spectra of berberine hydrochloride and the raw Coptidis Rhizoma.

Berberine is just one component of the herb and so its peaks are overlapped with peaks from other compounds. Taking the second derivative of the IR spectra can help to identify peak locations in the presence of overlapping peaks. Using this approach (as shown in Figure 3) gives a clearer match between the locations of the berberine peaks and those of the Coptidis Rhizoma sample.

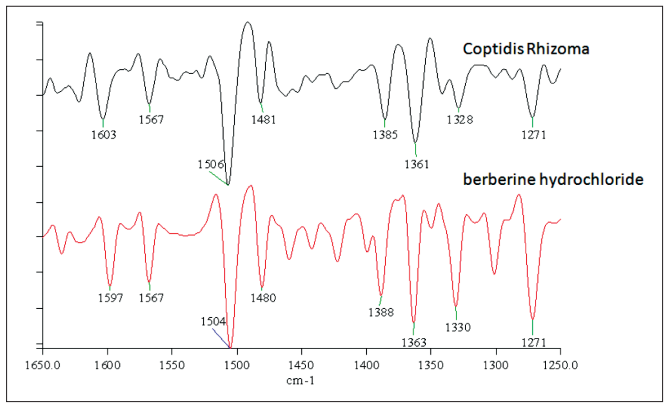

Figure 3. Second derivative IR spectra of berberine hydrochloride and the raw Coptidis Rhizoma.

Discrimination of Coptidis Rhizoma from some other similar herbs

Figure 4 shows the FT-IR spectra of Coptidis Rhizoma and the bark of two other berberine-containing herbs, Phellodendri Chinensis Cortex and Phellodendri Amurensis Cortex. These are quite similar materials, and peaks due to berberine and other compounds commonly found in plants are visible in all three spectra. However, there are some clear differences among the spectra. All three materials have a peak at around 1730 cm^{-1}, but this peak is very weak in the Coptidis Rhizoma spectrum and markedly stronger in the cortex Phellodendri spectra. The broad band at around 1600 cm^{-1} peaks at 1629 cm^{-1} for Coptidis Rhizoma, but at about 1604 cm^{-1} for the other two herbs. Finally, Coptidis Rhizoma lacks the peak at about 1420 cm^{-1} that is present for the cortex Phellodendri samples. It is easy to discriminate Coptidis Rhizoma from Phellodendri Chinensis Cortex and Phellodendri Amurensis Cortex even though they all contain berberine. Note, however, that a distinction between the two Phellodendri species would not be possible by this simple inspection approach.

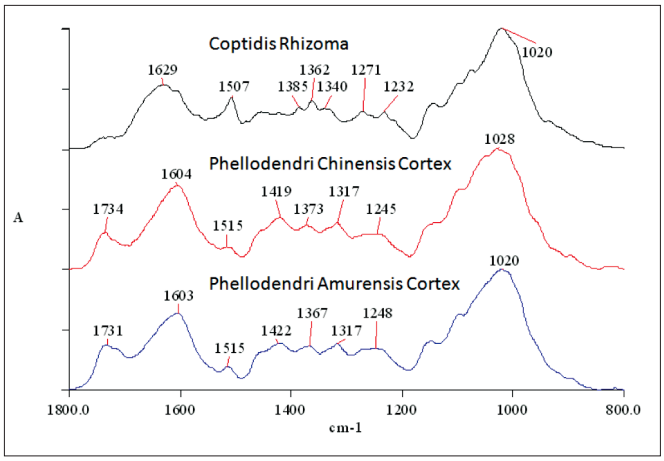

Figure 4. FT-IR spectra of the raw Coptidis Rhizoma, Phellodendri Chinensis Cortex, and Phellodendri Amurensis Cortex.

Conclusions

FT-IR can be a simple and powerful method for quality control of TCM. In contrast to methods relying on a single marker compound, FT-IR allows discrimination among similar herbs – such as the three berberine-containing herbs considered here. ATR accessories (particularly diamond ATR as used here) provide a very quick and simple way to measure the spectra of sufficiently homogeneous TCM samples.

*The Frontier FT-IR supersedes the Spectrum 100.

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APPLICATION NOTE

FT-IR Spectroscopy

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Rapid Quality Control for the Traditional Chinese Remedy "Red Flower Oil" by FT-IR Spectroscopy

Introduction

Red flower oil is a popular remedy in China and Southeast Asia. It is used for the treatment of muscle and joint pain, sprains, and bruising. It is prepared by blending several natural essential oils: principally wintergreen, clove, cinnamon, and turpentine, plus other oils or herbal extracts in smaller quantities.

The most important chemical components in red flower oil are methyl salicylate (from wintergreen oil), eugenol (from clove or cinnamon), and α-pinene (from turpentine). This is recognized in the publication WS3-B-2699-97[1] from the Ministry of Health of the People's Republic of China, which states that these three compounds must be quantified by a gas chromatography (GC) method before the product is approved for sale to the public. According to this document, the minimum contents of methyl salicylate and eugenol are 33.5% v/v and 38% v/v, respectively. Poor quality red flower oil typically has less eugenol, as clove and cinnamon essential oils are more expensive than wintergreen, and natural oil of wintergreen is more easily substituted by industrial methyl salicylate. Another possible source of poor quality is adulteration of the product by addition of excess amounts of inert vegetable or paraffin oil.

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In this note, we show that visual inspection of the FT-IR spectrum can yield important information about the quality of red flower oil with a total analysis time of a few minutes or less. In another note,[2] we describe work showing that an accurate and precise quantitative calibration for the three major components is possible.

Experimental

Methyl salicylate, eugenol, and α-pinene were obtained from SigmaAldrich®. Twenty samples of commercial red flower oil were obtained from nine manufacturers.

Spectra were measured on a PerkinElmer® Spectrum™ 100 FT-IR spectrometer equipped with a 9-bounce diamond/ZnSe attenuated total reflectance (ATR) sampling accessory. Spectra were acquired at 4 cm^{-1} resolution and for an accumulation time of 60 seconds.

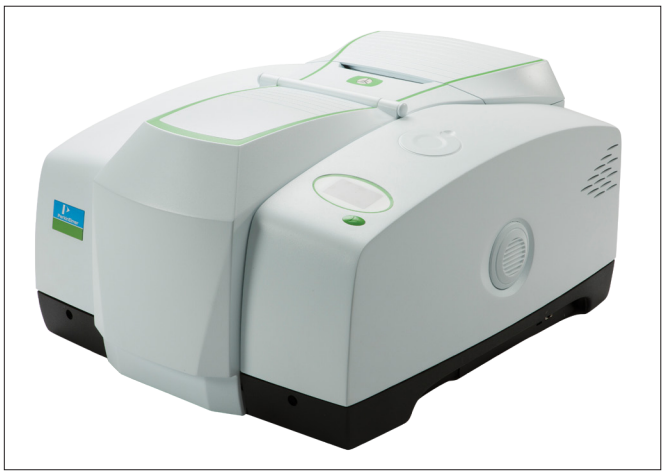

Figure 1. The Frontier FT-IR*.

Results and discussion

Spectra of the pure compounds and some commercial samples

Molecular structures and spectra of methyl salicylate, eugenol, and α-pinene are shown in Figure 2, along with spectra from two commercial red flower oil samples, Sample A and Sample B. The O–H and C–H stretch regions are especially informative. While methyl salicylate and eugenol both have phenolic OH groups, the stretch frequency for methyl salicylate is approximately 300 cm^{-1} lower. This phenomenon is due to intramolecular hydrogen bonding with the adjacent carbonyl group, and allows the two materials to be readily distinguished by visual inspection of the spectrum. α-Pinene can be recognized by the stronger bands in the C–H stretch region, particularly at 2916 cm^{-1}, although this does overlap with bands due to eugenol and does not distinguish it from other hydrocarbon compounds.

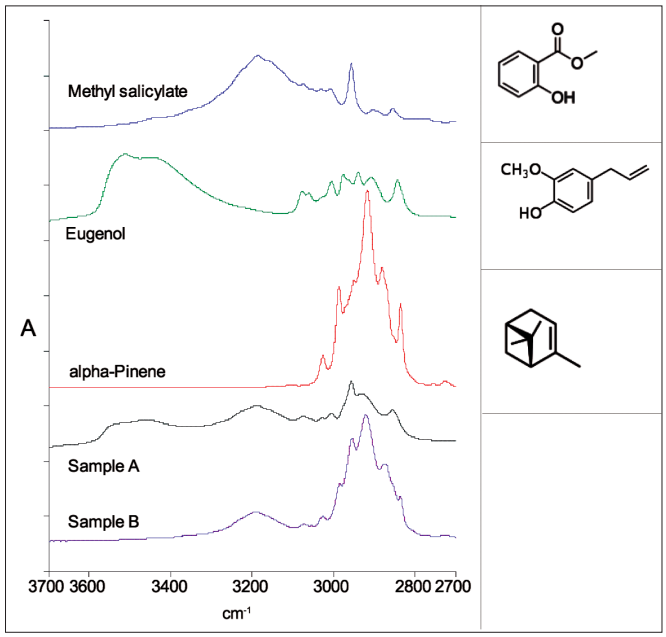

Figure 2. Molecular structures and IR spectra (O–H and C–H stretching region) of the main components in red flower oil, and of two commercial samples from different suppliers.

Comparing the pure-compound spectra with the two commercial samples, we can immediately conclude that Sample A contains significant amounts of methyl salicylate and eugenol, while Sample B appears to consist of methyl salicylate diluted in some hydrocarbon oil, without any eugenol. Clearly, Sample B is of an inferior red flower oil product.

Spectra of commercial red flower oil samples

Spectra were measured for 20 commercial samples obtained from nine manufacturers. Representative spectra from each manufacturer are shown in Figure 3 below. A wide variation in quality was found, with many of the samples having low or even non-detectable amounts of eugenol. Some of these samples appear to make up the difference with more methyl salicylate, but several samples have low levels of both eugenol and methyl salicylate, perhaps indicating dilution with some other oil.

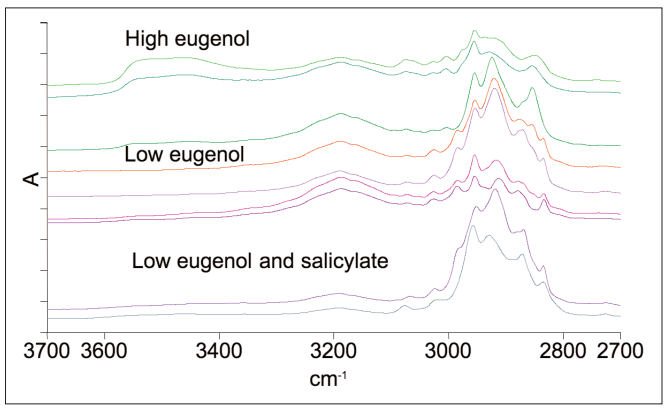

Figure 3. Representative spectra of the commercial red flower oil samples (one from each manufacturer).

Closer inspection of the spectra (Figure 4) revealed that several of the samples had an absorption band at 1745 cm^{-1}, characteristic of ester groups and suggesting that these samples contained vegetable oil as a diluent (although further investigation with other techniques would be required to prove this).

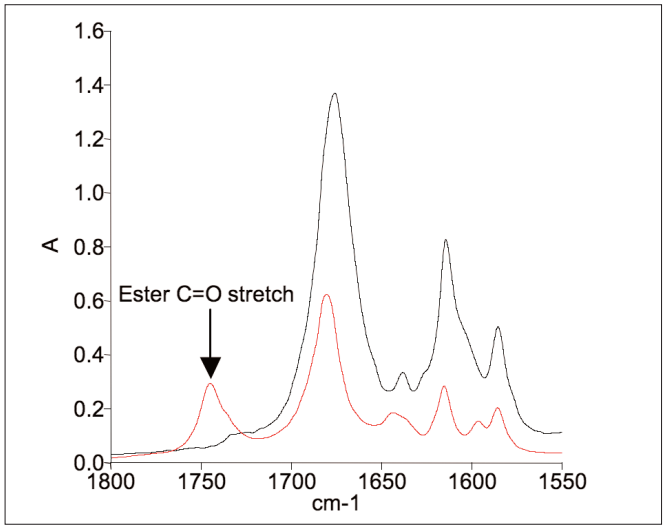

Figure 4. Spectra of two red flower oil samples, one showing a prominent band at 1745 cm^{-1}, consistent with the C=O stretch mode in triglycerides and indicating possible use of vegetable oil in the product.

While much variation was seen between products from different manufacturers, samples of different batches from the same manufacturer had nearly identical spectra, indicating that – at least for these manufacturers – the variation is due to differences in formulation rather than poor process control.

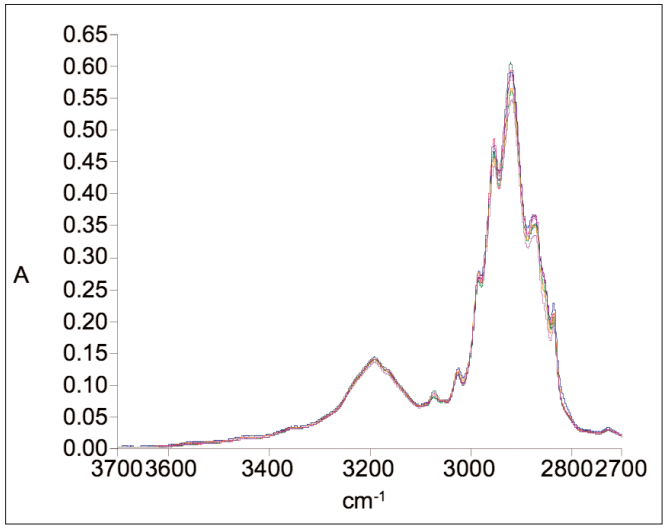

Figure 5. Spectra of seven red flower oil samples from one manufacturer.

Conclusions

Infrared spectroscopy with ATR sampling can be used to obtain an indicative determination of the quality of red flower oil in less than a minute. Qualitative inspection of the spectra allows the easy identification of the key components, methyl salicylate and eugenol.

*The Frontier FT-IR supersedes the Spectrum 100.

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APPLICATION NOTE

UHPLC

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Analysis of Ginsenosides in Ginseng Root with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

The root of the panax genus plant (also called Ginseng) has been used as an herbal medicine in Asia for over two thousand years for its purported various health benefits, including (but not limited to), antioxidant, anticarcinogenic, anti-inflammatory, antihypertensive and anti-diabetic. The pharmacologically active compounds behind the claims of ginseng's efficacy are ginsenosides; their underlying mechanism of action although not entirely elucidated appears to be similar to that of steroid hormones. There are a number of ginseng species, and each has its own set of ginsenosides. In fact, more than forty different ginsenosides have been identified. Ginsenosides are a diverse group of steroidal saponins with a four ring-like steroid structure with sugar moieties (Figure 1); they are found exclusively in ginseng plants and are in higher concentration in their roots. There are two main groups of ginsenosides: the panaxadiol group or Rb1 group that includes Rb1, Rb2, Rc, Rd, Rg3, Rh2, and Rh3; and the panaxatriol group or Rg1 group that includes Rg1, Re, Rf, Rg2 and Rh1.

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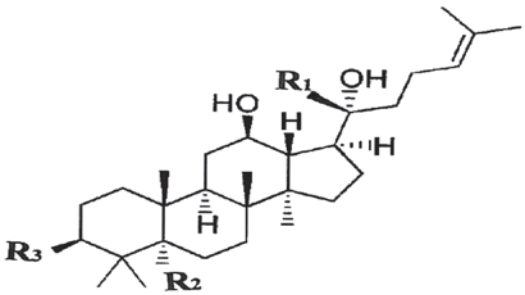
Qualitative and quantitative analytical techniques for the analysis of ginsenosides are in demand to ensure quality control in ginseng root processing, as well as for the study of their metabolism and bioavailability. This application note presents a robust liquid chromatography method to simultaneously test seven ginsenosides. Method conditions and performance data including precision, accuracy and linearity are presented. The method is applied to a panax ginseng (Korean Ginseng) root capsules and the types of ginsenosides are confirmed.



Ginsenoside	R3	R2	R1
Rb1 group	-O-Glc$_{(2-1)}$Glc	-H	-O-Glc$_{(6-1)}$Glc
Rg1 group	-OH	-O-Glc	-O-Glc

Glc Glucopyranoside (β-D-Glucose).
Subscripts indicate the carbon in the glucose rings linking the two carbohydrates.

Figure 1. Molecular structure of ginsenosides.

Experimental

Seven stock standard solutions of each ginsenoside at 1 mg/mL concentration were prepared by dilution with 70:30 methanol/water (diluent), followed by one minute vortex. A working standard of 0.14 mg/mL was prepared by mixing together 0.5 mL of each of the stock solution.

Precision was evaluated with eight injections of the working standard. Linearity was determined across a range of 7 µg/mL to 140 µg/mL. To assess the accuracy of the method, purified water was spiked with the working standard to obtain a solution with 7 µg/mL ginsenosides. About 3 g of a panax ginseng powder from a popular brand capsules was transferred into 50 mL volumetric flask, 30 mL of diluent was added followed by about a minute vortexing and 30 min. sonication. The solution was then centrifuged at 5000 RPM for 10 min. and the supernatant was collected and set aside. 15 mL of diluent was added in the remaining precipitate followed by vortexing, sonication and centrifugation similar to that described above. This latter supernatant was collected and added to the first collected supernatant in a 50 mL volumetric flask; the solution was brought volume with diluent, mixed well and filtered with a 0.2 µm nylon membrane prior to testing.

A PerkinElmer® Flexar™ FX-15 UHPLC system fitted with a Flexar FX PDA photodiode array detector served as a platform for this experiment. The separation was achieved using a PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm, 2.7 µm (superficially porous particles) column.

Table 1. **Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar FX UHPLC
Setting:	50 µL loop and 15 µL needle volume, partial loop mode
	350 µL mixer volume; injector wash and carrier: water
Injection:	2 µL
PDA Detector:	Scanned from 190-400 nm, recording setting 203 nm
UHPLC Column:	PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm, 2.7 µm (superficially porous particles) at 45 °C, Part No. N9308402
Mobile Phase:	A: water
	B: acetonitrile

Time (min)	Flow rate (mL/min)	B %	Curve
2.5	0.4	30-35	1
3.5	0.4	35-50	1

	3 minutes equilibration after each run
	(HPLC grade solvent and ACS grade reagent)
Sampling Rate:	5 pt/s
Software:	Chromera® Version 3.0

Results and Discussion

The optimal flow rate of this method was determined to be 0.4 mL/min. at 45 °C, the pressure stabilized around 5150 PSI (355 bar) and all the peaks eluted within six minutes. A representative chromatogram of the standards solution and the Korean ginseng tested are in Figure 2 and 3. Excellent method performance was achieved: the linearity of the analysis had a R-squared of not less than 0.997 for each ginsenoside and a precision (relative standard deviation %RSD) with values ranging from 0.6% to1.2%. The spiked purified water tested had an average recovery of 99.9% with values ranging from 91.2% to108.0% (Figure 4). Details of the method performance and results of the panax ginseng and spiked sample tested are presented in Table 2.

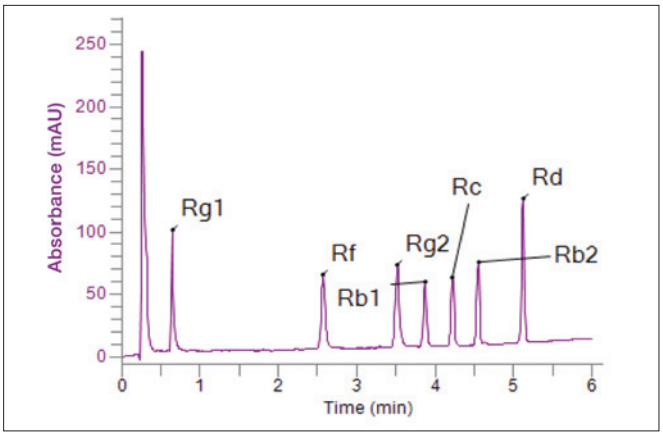


Figure 2. Chromatogram from the analysis of a standard.

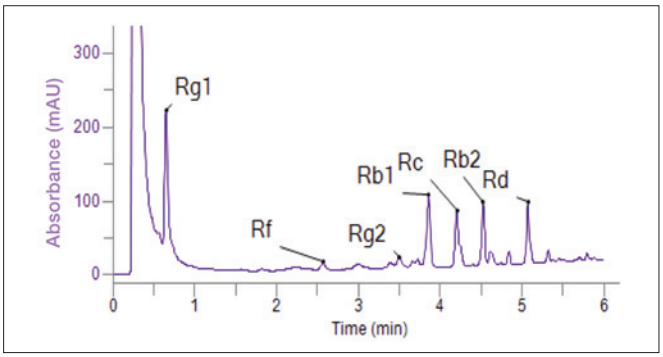


Figure 3. Chromatogram from the analyses of panax ginseng.

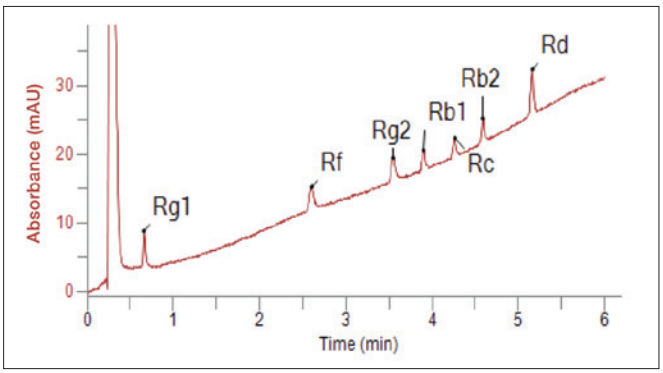


Figure 4. Chromatogram from the analyses of 7 ppm ginsenosides in water.

Table 2. **Precision, linearity, accuracy and samples.**

Compound	%RSD n = 8	r^2	Range (µg/mL)	Korean Ginseng (mg/g)	7 ppm Spiked Water
Rg1	0.9	0.9997	7 - 140	13	97.5
Rf	0.6	0.9971	7 - 140	1	91.2
Rg2	1.2	0.9983	7 - 140	1	98.7
Rb1	1.1	1	7 - 140	10	102.1
Rc	1.2	0.9994	7 - 140	10	100.3
Rb2	1.0	0.9996	7 - 140	7	101.4
Rd	1.2	0.9997	7 - 140	4	108.0
Avg/Tot.	**1.0/NA**	**0.9988/NA**	**NA**	**NA/46**	**99.9/NA**

NA = Not Applicable

Conclusion

The seven ginsenosides were well resolved within six minutes. The method was shown to be linear with R-squared ≥ 0.997, precise with %RSD ≤1.2 and accurate with a recovery averaging 99.9%. The Korean ginseng capsule tested has 46 mg/g of ginsenosides. PerkinElmer's Flexar FX PDA detector provides rugged and accurate detection over a range of 190 nm to 700 nm, encompassing UV and visible wavelengths. PerkinElmer's Chromera software offers many data acquisition and processing features: spectral library creation, and peak purity, spectra 3-D and contour maps, which are powerful tools that give insight to the information content of a 3-D photodiode array chromatogram. The spectra library search function allowed the storage of standard peaks spectra that could later be used for peak identification confirmation in the sample.

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Note: This application note is subject to change without prior notice.

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