Application Note: 51819

Nutraceutical Ingredient Identification by FT-NIR

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Key Words

- Antaris
- cGMP
- Dietary Supplements
- FT-NIR
- Near-infrared
- Nutraceuticals

Introduction

In recent years, the United States FDA has brought companies that manufacture, package, or label dietary supplements under current Good Manufacturing Practice (cGMP) regulations. The dietary supplement industry is now under the same type of regulation as the pharmaceutical industry. All companies, regardless of size, must be compliant with the cGMP's found in 21 CFR 111 by June 2010. Dietary supplement manufacturers must implement a quality control system and have all aspects of their process documented in order to pass an FDA audit of their facility. A key requirement of the cGMP regulation is to perform 100% identity testing of ingredients used in the production of dietary supplements.

Fourier transform near-infrared (FT-NIR) spectroscopy has been successfully used for ingredient identification in the pharmaceutical industry for many years. Near-infrared (NIR) is a United States, European and Japanese Pharmacopeia accepted technique for qualitative and quantitative analysis. Most dietary supplement manufacturers have over 100 ingredients that need to be positively identified prior to release to production in order to meet cGMP requirements. Performing identification testing by HP-TLC, HPLC and/or microscopy creates significant time, cost and labor burdens on a manufacturing facility. Often more than one of these techniques must be used to conclusively identify a received ingredient. These techniques require sample preparation and, in the case of microscopy and thin layer chromatography, can be subjective and involve multiple steps which can result in operator error influencing the outcome of the analysis. NIR technology is rapid, non-destructive and requires no sample preparation, consumables or solvents. Using NIR saves money and hands-on analysis time, and increases the facilities testing capacity (Table 1). The benefits of using the Thermo Scientific Antaris FT-NIR analyzer for ingredient identification include: the ability to perform analysis in the receiving warehouse and the ease of instrument and software operation which allows non-technical personnel to perform identification testing with only one keystroke.

Figure 1: Antaris II FT-NIR with SabIR probe optimized for ingredient identification testing

Key Metric	HPLC	HP-TLC/TLC	Microscopy	FT-NIR
Sample Preparation	Yes	Yes	Some	No
Actual Analysis Time	10-30 minutes	10-30 minutes	5 minutes	1 minute
Consumable Cost per Test	Medium	High	Low	None
Location of Analysis	Laboratory	Laboratory	Laboratory	Receiving Area or Laboratory

Table 1: FT-NIR advantages over other technologies for ingredient identification testing

Experiment

Before identity testing by FT-NIR can be implemented, a library must be developed that contains the compounds to be identified. There are three main steps to developing an ingredient identification library – planning, developing, and implementing. The planning step involves creating a list of all compounds to include in the library and collecting multiple standard samples for each compound. Libraries that are robust and offer good discriminating power require standards from multiple lots for each compound. It is critical to capture all relevant and unique spectral information for each compound in the library. The development step involves collecting the standard spectra and building the library method. The implementation step validates the method with independent samples not in the method to test that the method will positively identify compounds in the library and give a failure indication for samples that do not match a compound in the library. Other important parts of the implementation process include writing SOP's for the routine operator performing the identification analysis and an ongoing method maintenance.

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A nutraceutical ingredient library, containing 65 compounds, was developed using the Antaris™ II FT-NIR and the Thermo Scientific SabIR raw material probe (Figure 1). The SabIR™ probe allows for easy sampling of solid or liquid ingredients directly inside the original container, through packaging materials or in sampling containers. Near-infrared spectroscopy is a technique that looks at how light interacts with a sample. The near-infrared light can be absorbed, transmitted, or reflected. Source light from the Antaris FT-NIR analyzer travels through multiple fiber optic lines to the tip of the probe where the light interacts with the sample that is in contact with the probe's sapphire window. Light that is diffusely reflected back into the probe travels back through another set of fiber optic lines to a detector. A near-infrared spectrum is produced which shows the amount of source light that has been absorbed at each wavelength.

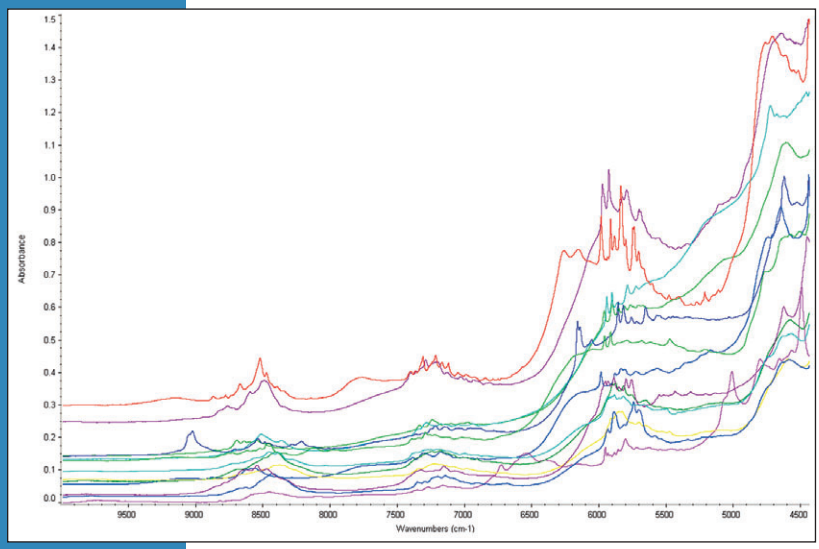

Figure 2: Amino Acid standards without any processing (raw spectra)

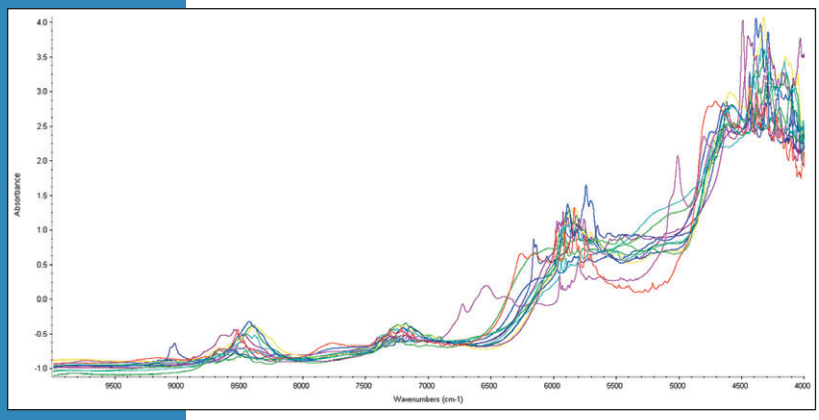

Figure 3: Example of baseline shifts being removed by standard normal variate (SNV) processing of Amino Acid standards

One of the challenges when developing an ingredient identification method is to remove the operator influence on the identification test. Sample presentation to the fiber optic probe is the biggest source of error in the identification test since different operators will orientate the probe differently to the sample. Operator-to-operator variation due to sample presentation often shows up as baselines shifts in the spectra. The nutraceutical library developed for this application contains standard spectra collected with variation in probe positioning for each compound. This built-in variation will result in a more robust library method that can be more easily and quickly implemented. Differences in particle size and the physical state within and between compounds in the library also results in baseline shifts in the spectra as can be seen for the amino acid standards in the library (Figure 2). One way to negate these baseline shifts is to apply spectra preprocessing that normalizes the spectral baselines before method development. For this application, the standard normal variate (SNV) pathlength treatment was used. SNV compensates for variations in sample thicknesses that are caused by differences in particle size, varying density of material analyzed, and scattering in solid samples. SNV normalizes the method standards to the same baseline scale as can be seen for the amino acid standards in Figure 3.

The nutraceutical ingredient library method developed for this application contains 65 compounds with multiple standards for each compound. The multiple standards for each compound were collected by varying the pressure and orientation of the SabIR probe to the sample. The compounds in the library can be grouped into several sub-categories including amino acids, vitamins, minerals and herbals. The spectral collection parameters were 32 co-averaged scans, 4 cm^{-1} resolution and spectral collection from 10,000-4000 cm^{-1}. The SNV pathlength treatment was applied to all standards in the library before method development.

Results and Discussion

A Discriminant Analysis method for ingredient identification was developed using the Thermo Scientific TQ Analyst chemometric software package. Discriminant Analysis is a commonly employed chemometric algorithm for identifying compounds. It can be used when only a few standards per compound are available and can properly identify chemically similar compounds in the same library. The Discriminant Analysis method uses principal components (PC's) to explain the spectral variation in the library. Principal components are orthogonal vectors that explain or condense spectral information in order to create a Discriminant Analysis method. For the nutraceutical library, 17 principal components were used to explain 99.4% of the spectral variation in the library standards. The 1st Principal Component explains the most spectral variation in the library and each remaining principal component explains the residual variation. The output from a Discriminant Analysis method is a Mahalanobis distance value showing how closely a sample is to the closest (identified) class center. For the amino acid

standards, we can see definitive separation based on amino acid compound in a scores plot of PC1 vs. PC2 (Figure 4). The plot also shows that the intra-class separation is very small and that there is no overlap between the different classes. This is the ideal separation for an identification method because when this method is used to identify unknown samples, it runs less risk of misclassifying samples. Only three amino acid compounds do not show significant separation in the PC1 vs. PC2 scores plot. These three amino acids, leucine, isoleucine and methionine, are chemically very similar to each other which makes for a more challenging discrimination. Leucine and isoleucine are isomers of each other while methionine has one less carbon in its chain and a sulfur group that leucine and isoleucine do not. Looking at PC3 vs. PC4 scores plot (Figure 5), we can see that these three amino acids discriminate very well.

The results provided by the Discriminant Analysis method include class identification and the distance from the center of the identified (closest) class to the unknown sample in Mahalanobis distance units. Other information often displayed is the next closest class along with the Mahalanobis distance to that class. It is advantageous to show the distance to the next closest class to gauge the quality of discrimination of the method. If the Mahalanobis distances are very similar between identified and next closest class, then the confidence level in the class identification is low. Mahalanobis distances are analogous to sigmas used for standard deviation in that they are a metric for describing distance from a mean value. A good Discriminant Analysis method shows next class distance values that are at least 1 Mahalanobis distance unit greater than the identified class value. During method development this threshold was used to determine if all the compounds could be accurately classified using one model. If the model would have shown any misclassified standards for a class, then more spectra would need to be added to the method for that class or refinement of the chemometric model would need to be performed. The method developed for the 65 nutraceutical ingredients shows good distinguishing power between the different classes as can be seen by the separation in Mahalanobis distances between the identified and next class (Table 2).

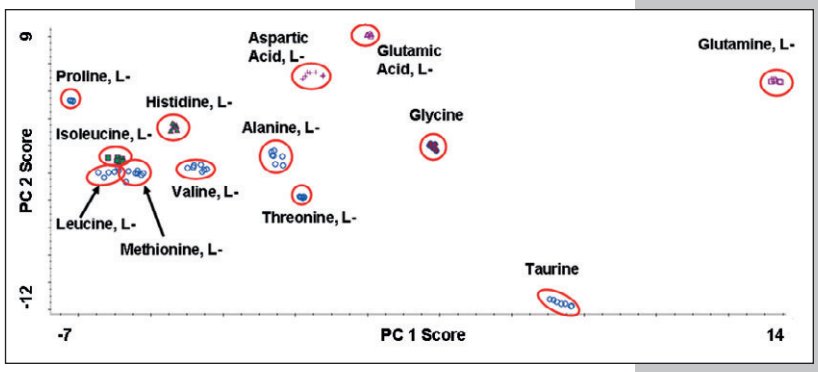

Figure 4: Principal Component 1 vs. 2 scores plot showing distinct separation of amino acids in the nutraceutical ingredient library

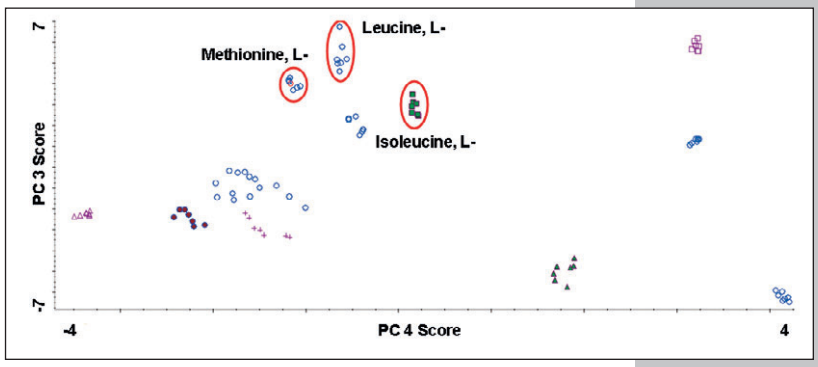

Figure 5: Principal Component (PC) 3 vs. 4 scores plot showing clear discrimination of the 3 amino acids that were the least separated in the PC 1 vs. PC 2 scores plot

Class ID	Distance	Next Class	Next Distance
Alpha Lipoic Acid	0.66	Flax Seed	6.83
Artichoke Extract	0.57	Ginko Biloba Extract	2.46
Ascorbic Acid	0.41	Manganese Gluconate	6.04
B-1 Thiamine Hydrochloride	0.37	Citric Acid	7.54
B-12 Cyanocobalamin	0.36	Dong Quai 4:1 Extract	1.92
B-2 Riboflavin	0.81	Lycopene	7.26
B-3 Niacinamide	0.33	Glutamine, L-	13.97
B-6 Pyridoxine HCL	0.92	Threonine, L-	11.12
Bilberry Extract	0.73	Cinnamon Extract	4.08
Black Cohosh	0.58	B-12 Cyanocobalamin	2.34
Carnuba Wax	0.16	Magnesium Stearate	5.53
Cellulose	0.36	Dong Quai 4:1 Extract	3.73
Chasteberry	1.09	Licorice Root	5.55
Cinnamon Extract	0.42	Ginko Biloba Extract	2.13
Citric Acid	0.20	Dong Quai 4:1 Extract	5.44
Crystalline Fructose	0.26	Dong Quai 4:1 Extract	4.34
Dong Quai 4:1 Extract	0.85	B-12 Cyanocobalamin	2.23
Dry Mixed Tocopherols	1.13	Beta Carotene	4.63
E-Succinate	0.89	Rosemary Extract	4.88
Ginko Biloba Extract	0.25	Cinnamon Extract	2.14
Glucosamine	0.46	Lutein FloraGlo	8.92
Gum Arabic	0.45	Croscarmelose Sodium	2.61
Lecithin	0.61	Dry Mixed Tocopherols	6.35
Licorice Root	0.47	Black Cohosh	2.58
L-Selenomethionine	0.18	Glutamic Acid, L-	3.31
Lutein FloraGlo	0.35	Dong Quai 4:1 Extract	3.61
Manganese Gluconate	0.43	Bilberry Extract	5.09
Milk Thistle Extract	1.14	Green Tea Extract	5.89
Rosemary Extract	1.12	E-Succinate	5.60
Soy Protein Isloate	1.39	Lycopene	6.98

Table 2: Summary of average Mahalanobis distances of each identified and the next closest class. A comparison of the identified class to next closest class Mahalanobis distances indicates how well the model can distinguish the different classes. Larger differences indicate that the identified class is spectrally dissimilar to the next closest class. For this library, all samples were correctly identified.

Ingredient identification is often performed on more than one near-infrared instrument within a company. This makes method transfer from one instrument to another instrument an important aspect of many near-infrared ingredient testing programs. It is unreasonable and a very undesirable task to develop instrument specific identification methods due to the time and personnel resources required in building multiple libraries. Being able to transfer identification methods to other near-infrared instruments without loss in method performance is very important. Several sources of variation must be overcome to successfully transfer a method to another near-infrared instrument. When transferring a method between two Antaris FT-NIR instruments, the main variable to account for is differences in sample presentation to the two instruments. The Antaris FT-NIR was designed, engineered and built for method transferability through strict adherence to specifications on components used to build the instruments and probes as well as rigorous test protocols to certified standards during manufacturing. The combination of precision engineering and testing to certified standards results in each Antaris instrument being identical to every other Antaris manufactured which facilitates method transfer.

Method transfer between two Antaris II FT-NIR analyzers with SabIR probes was successfully accomplished for this nutraceutical ingredient identification method. The amino acid standards collected as part of this application highlight the successful method transfer. One standard for each class of amino acid was analyzed as an independent validation sample on both the master and host Antaris. These standards were not included in the method developed on the master Antaris. For all 13 amino acid validation samples, proper classification was achieved on both instruments. A comparison of class ID distances and the next closest class distances between the master and host Antaris reveals that for all 13 amino acids, the difference in distance values are very small (Table 3). If the method transfer was suspect, we would have seen the class ID distance values that were much greater on the host Antaris compared to the master Antaris. Also the next distance values for the host Antaris would have been much lower than on the master Antaris. These would be two indicators of poor method transfer since the independent validation samples would be further away from the identified class and closer to the next closest class for the host Antaris when compared to the master Antaris. If the method transfer was very poor, we would have seen misclassification of validation samples on the host Antaris revealing significant loss in distinguishing power of the method.

	Antaris Master		Antaris Host		
Class ID	Distance	Next Distance	Class ID	Distance	Next Distance
Glycine	0.18	3.93	Glycine	0.21	3.83
Alanine, L-	0.65	4.34	Alanine, L-	0.58	4.57
Aspartic Acid, L-	0.28	2.35	Aspartic Acid, L-	0.37	2.63
Glutamic Acid, L-	0.07	2.33	Glutamic Acid, L-	0.09	2.31
Glutamine, L-	0.11	7.00	Glutamine, L-	0.18	6.92
Histidine, L-	0.30	3.53	Histidine, L-	0.31	3.40
Isoleucine, L-	0.28	1.43	Isoleucine, L-	0.20	1.38
Leucine, L-	0.48	2.05	Leucine, L-	0.18	1.94
Methionine, L-	0.15	2.00	Methionine, L-	0.20	2.03
Proline, L-	0.08	5.30	Proline, L-	0.12	5.20
Threonine, L-	0.11	3.97	Threonine, L-	0.12	3.96
Valine, L-	0.28	1.43	Valine, L-	0.38	1.55
Taurine	0.41	6.89	Taurine	0.95	6.99

Table 3: Class identifications for 13 independent validation samples analyzed on a master Antaris FT-NIR used to build the method and a host Antaris FT-NIR used to test method transferability. All 13 amino acids are identified correctly by both the master and host Antaris with very similar class ID Mahalanobis distances and distances to next closest class between instruments proving successful method transfer.

An ingredient identification testing program involves more than just creating a library that can determine whether the supplied ingredient is what the supplier says it is. There is an implementation phase for getting the near-infrared method into routine operation. Implementation includes writing and maintaining SOP's for performing the identity test and setting up a system for electronic storage of method results. To meet cGMP requirements and to pass an FDA audit, companies must have a fully documented process to show how the results from identification testing were generated. There also needs to be a system in place for validating the near-infrared instrument's performance on a routine basis. A significant amount of time and personnel resources are needed for implementing ingredient identification testing and the supporting quality system needed for cGMP compliance. Thermo Scientific RESULT software with Thermo Scientific ValPro qualification package relieves this time and personnel burden (Table 4). RESULT™ software with ValPro™ is a validation-ready package that includes Installation Qualification (IQ) and Operation Qualification (OQ) to ensure that the Antaris FT-NIR is installed and performs consistently throughout its lifecycle to meet both cGMP and USP requirements. RESULT supports electronic signature of identification results, customizable workflows with embedded SOP's, electronic export of data to LIMS and contains an electronic audit trail database. RESULT forces compliance with method SOP's, digital signatures and automates electronic archiving of result reports from identification tests. Operators simply start the workflow via one keystroke and all other required tasks occur automatically.

RESULT with ValPro Feature	Advantage
Operation via a keystroke or mouse click to run workflow	An operator can run the instrument with a minimum of training
RESULT workflow forces compliance with method SOP	Protection against operator error – software forces review of SOP as part of sample analysis
Electronic signature of results	Automatic traceability of who performed the identification test – lowers risk of FDA finding
Built in audit trail in RESULT	Changes to key attributes in RESULT software are recorded and can easily be retrieved for FDA audit
Automated OPC communication, delimited file export and direct export to Excel	Allows seamless transfer of electronic records to LIMS or other QC systems which reduces possibility of transcription errors
ValPro Qualification package – standards wheel and documentation system for IQ and OQ	Allows automated/scheduled instrument performance checking and standard templates for system lifecycle qualification

Table 4: RESULT with ValPro software package features and advantages for easy implementation and compliance with cGMP requirements for ingredient testing

Conclusion

Recent FDA regulations require dietary supplement manufacturers, labelers and packagers of all sizes to meet cGMP requirements. One key aspect of the cGMP requirements is 100% identity testing of ingredients used in the production of dietary supplements. For this study, a single Discriminant Analysis method was developed that conclusively distinguished 65 nutraceutical compounds in a library without a single sample being misclassified. Successful method transfer between two Antaris FT-NIR instruments was demonstrated through proper identification of independent validation samples on both a master and host Antaris along with class distance values on a host Antaris that matched the master Antaris. Method transfer is one of the most important aspects of any ingredient identification program since developing instrument specific methods is not an acceptable option from a resource stand point. Fourier transform near-infrared (FT-NIR) spectroscopy is a proven technology for ingredient identification based on its many years of use in the pharmaceutical industry. Near-infrared offers many advantages over other techniques for ingredient identification including speed of analysis, ability to analyze directly through packaging materials, and no sample preparation, consumables, or solvents. Near-infrared testing requires less operator time and fewer supplies which is a direct cost savings to the facility. Antaris FT-NIR brings its own unique set of benefits to ingredient testing including a robust analyzer designed to be in production environments and easy method transfer amongst Antaris instruments. RESULT with ValPro is a validation-ready software package that is easy for an operator to use. A single keystroke is all that is needed to perform an ingredient identification or to qualify the instrument for use. This study demonstrates many of the key benefits of implementing Antaris FT-NIR for ingredient identity testing required for meeting the current Good Manufacturing Practice (cGMP) regulations for companies in the dietary supplement industry. This study has shown the successful development of a nutraceutical ingredient identification library containing multiple types of compounds including amino acids, vitamins, minerals, and herbals.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

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AN51819_E 10/09M