RESTEK
nutraceuticals

Applicationsnote

Analyzing Nutraceutical Products by Liquid and Gas Chromatography

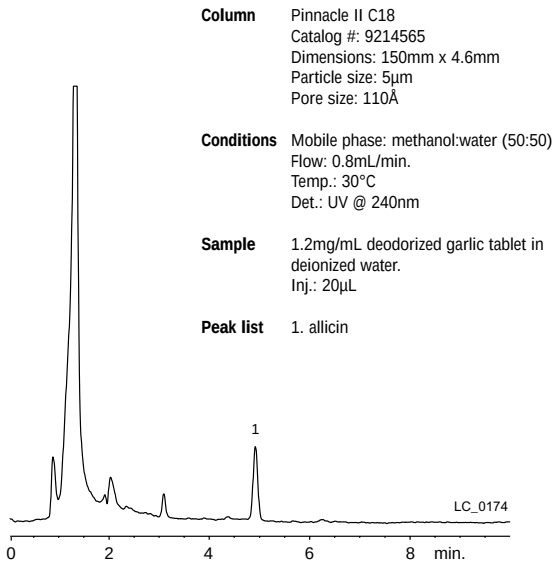
Introduction

The idea of using herbal compounds to enhance one's health has been around for thousands of years. Over the past decade, however, the nutraceutical industry has seen rapid growth as more people add flowers, leaves, roots, and fruits of botanicals to their diets in the hopes of gaining health benefits. The dietary supplement industry (including vitamins, minerals, herbals, and amino acids) was approximately $16 billion in 2000 with botanicals accounting for 25%.[1] Herbal ingredients that once were packaged primarily in pills and capsules and found in health food stores, now can be found in fruit juices, spreads, and snack foods.[2] This includes ingredients such as glucosamine, added to ease aches and pains; and kava or St. John's wort, added for calmness and a sense of well-being.

Herbal products are very complex, often containing hundreds of compounds, and it is not always clear which compounds are responsible for the beneficial properties. Marker compounds—phytochemicals that have been identified and are known to have some relationship to the reported health benefit—can be evaluated qualitatively to identify a raw material or to verify purity. To determine the concentration or strength of a material, quantitative analysis is necessary. Other testing typically performed on raw materials includes a physical exam, microscopy, and determination of ash, heavy metals, residual fumigants, and pesticide levels. Microbiological testing can be included depending on the herbal material.

Figure 1

Rapidly analyze allicin in garlic by HPLC using a Pinnacle II™ C18 column.



Dietary supplements are regulated under the Dietary Supplement Health Education Act (DSHEA) of 1994. Before DSHEA, nutraceuticals were regulated as either foods or drugs, depending on their intended purpose.[3] The Food and Drug Administration (FDA) has the authority under DSHEA to take action against unsafe products or improperly labeled products. For example, the FDA can stop the distribution of products that it finds to be toxic, unsanitary, that increase the risk of illness or injury, or that make unsubstantiated health claims. Under current regulations, however, FDA pre-market approval of dietary supplements is not required. This leaves the testing of dietary supplements to the discretion of the manufacturer.

Several organizations have recognized a need for standardizing procedures of herbal product analysis and are involved in programs that will assist the FDA in the regulation of the dietary supplement industry. The US Pharmacopoeia (USP) has launched a dietary supplement certification pilot program to address the issue of product quality. The USP program seeks to ensure that the product contains the ingredients declared on the label at the reported levels; that the product is within the required limits on contaminants; and that the general requirements for the manufacturing practices of dietary supplements are satisfied.[1] This program is meant to complement DSHEA and allows companies to add a USP certification mark on their label if the requirements are met. The pilot program includes post-market surveillance of the nutraceutical products and auditing of the manufacturing facilities. As of August 2001, the USP had over 20 official monographs for herbal materials, with many more in revision or draft form.[3]

Other organizations, such as the Institute for Nutraceutical Advancement (INA), are working to develop new methods for the quantitation of marker compounds. The goal of the INA Method Validation Program (MVP) is to submit these new methods to an organization (e.g., Association of Official Analytical Chemists [AOAC] International) for collaborative study and inclusion in their official methods program. AOAC International also has a Dietary Supplement Task Group that provides a standard set of procedures for the analysis of botanical compounds.

High performance liquid chromatography (HPLC) and gas chromatography (GC) are excellent tools for quantitative analysis of marker compounds in botanical samples. Thin layer chromatog-

raphy (TLC) also is used, primarily for the qualitative identification of herbals and for purity evaluations. HPLC is used in approximately 90% of the current methods for marker compounds and mainly relies on a reversed phase separation with UV-VIS, refractive index or light-scattering detection. GC, while not as widely applicable, is very useful for the analysis of volatile marker compounds and residual solvents. For both HPLC and GC botanical analyses, column reproducibility and robustness is very important. Restek HPLC and GC columns are able to meet the demands of analyzing these complex systems.

HPLC Analysis of Allicin in Garlic

Garlic, or Allium sativum, has the reported benefits of lowering cholesterol, reducing hypertension, and acting as an antimicrobial agent in living systems. Garlic often is added to the diet as garlic powder or garlic capsules. The active ingredients in garlic are thought to be the sulfur-containing compounds, or thiosulfinates, present in the volatile oil. Alliin, which converts to allicin (S-allyl-2-propenthiosulfinate) in the presence of the enzyme alliinase, is present at about 1% in garlic cloves.

INA Method 110.001—the analysis of allicin in garlic—is an isocratic reversed phase HPLC procedure using a C18 column, with UV detection at 240nm (Figure 1). Because allicin has limited stability in solution, samples should be analyzed soon after extracting into cold water. In addition, the allicin reference standard needs to be prepared by extracting the allicin from garlic powder by solid phase extraction (SPE) and calculating the concentration based on the UV absorbance of the standard solution.

HPLC Analysis of Hyperforin in St. John's Wort

St. John's wort, or Hypericum perforatum, is purported to ease the symptoms of mild depression, anxiety, and insomnia. The active ingredient is thought to be hyperforin, although other compounds might also contribute to the beneficial effects. St. John's wort can be added to the diet in either capsule or tablet form of the leaves, stems or flowers, or as an extract.

According to INA Method 112.001—the HPLC analysis of hyperforin and adhyperforin in St. John's wort—the samples are extracted with methanol in an ultrasonic bath. Chromatographic separation is performed on a C18 reversed phase column with acetonitrile and phosphate buffer as the mobile phase. After UV detection at 270nm, the hyperforin and adhyperforin are quantitated by comparison of the response of these compounds to the response of a standard solution of hyperforin. The analysis of a St. John's wort capsule using a Pinnacle II™ C18 column shows excellent peak shapes for the active ingredients in this herbal product (Figure 2).

HPLC Analysis of Phenolics in Echinacea

Echinacea, one of the most popular nutraceutical herbs, is thought to enhance the immune system and act as an immune stimulant against colds and flus. It can be taken in capsule or tablet form, or used in the preparation of an herbal tea. The active compounds in echinacea are thought to be caffeic acid derivatives such as caftaric acid, cichoric acid, chlorogenic acid, and echinacoside.

Figure 2

HPLC analysis of hyperforin and adhyperforin in St. John's Wort capsule using a Pinnacle II™ C18 column shows excellent peak shape for the marker compounds.

Column Pinnacle II C18
Catalog #: 9214565
Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 110Å

Conditions Mobile phase: acetonitrile:
0.01N phosphate buffer, pH 2.5 (85:15)
Flow: 1.2mL/min.
Temp.: 30°C, autosampler @ 15°C
Det.: UV @ 270nm

Sample St. John's Wort capsule
Inj.: 20µL
Conc.: 4.17mg/mL capsule,
contents in methanol

Peak list 1. hyperforin
2. adhyperforin

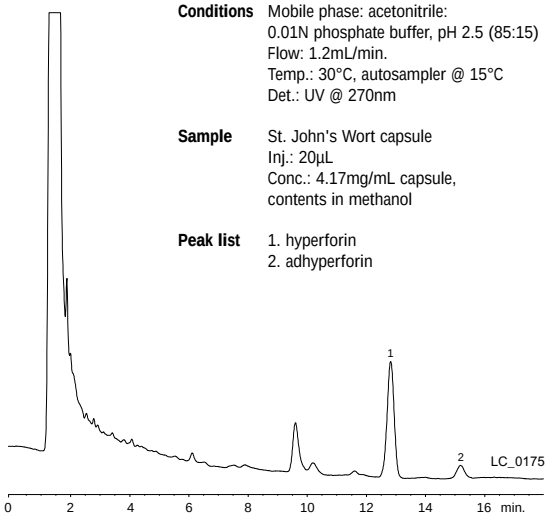


Figure 3

HPLC analysis of phenolic compounds in echinacea capsules using a Pinnacle II™ C18 column achieves resolution of four key marker compounds.

Column Pinnacle II C18
Catalog #: 9214565
Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 110Å

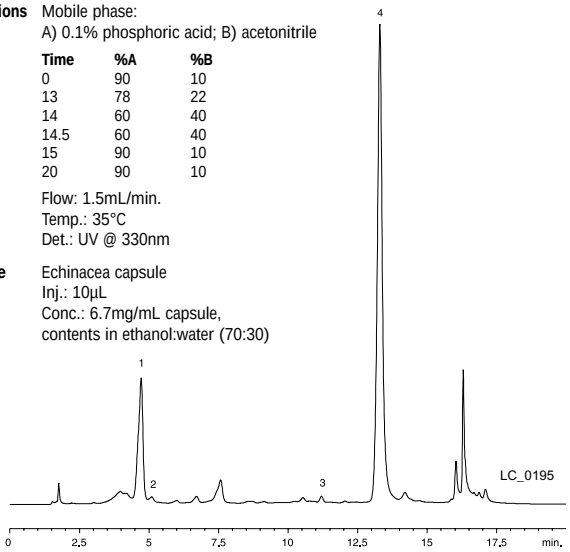
Peak list 1. caftaric acid (4.6 min.)
2. chlorogenic acid (5 min.)
3. echinacoside (11.2min.)
4. cichoric acid (13.3 min.)

Conditions Mobile phase:
A) 0.1% phosphoric acid; B) acetonitrile

Time	%A	%B
0	90	10
13	78	22
14	60	40
14.5	60	40
15	90	10
20	90	10

Flow: 1.5mL/min.
Temp.: 35°C
Det.: UV @ 330nm

Sample Echinacea capsule
Inj.: 10µL
Conc.: 6.7mg/mL capsule,
contents in ethanol:water (70:30)



According to INA Method 106.000—HPLC analysis to determine the levels of these phenolic compounds in echinacea—samples are extracted into an ethanol:water mixture with shaking. The analysis is performed on a C18 reversed phase column using a mobile phase of acetonitrile:dilute phosphoric acid. The caffeic acid derivatives are detected based on their UV absorbance at 330nm and quantitated by comparison to an external standard solution of chlorogenic acid. We used the Pinnacle II™ C18 column to analyze a capsule of echinacea and achieved resolution of four key marker compounds (Figure 3).

GC Analysis of Fatty Acids in Saw Palmetto

Saw palmetto, or Serenoa repens, has been used to treat prostrate enlargement and lower urinary tract symptoms. The partially dried, ripe fruits of this plant are typically extracted with ethanol, carbon dioxide or hexane to collect the active ingredients. Saw palmetto contains a wide range of compounds, including free fatty acids, free fatty alcohols, and monoglycerides. The free fatty acids, which make up 70-95% of the purified lipophilic extract, often are used as the marker compounds for this herbal product.

The USP monograph for saw palmetto includes a procedure for the quantitation of fatty acids by GC. To perform this analysis, the samples are pulverized to a powder, saponified, and derivatized with boron trifluoride in methanol. The test standard includes 11 fatty acid methyl esters (FAMEs) and an internal standard (IS), and the chromatographic separation is performed on a polar Carbowax® column. The analysis of free fatty acids as their methyl esters on an Rtx®-Wax capillary GC column shows excellent resolution of all of the FAMEs when analyzed according to the USP procedure (Figure 4).

Summary

An increasing number of nutraceutical ingredients and products are becoming a part of our diet. Because of this, we need accurate quantitation of the active ingredients or marker compounds in both raw materials and finished products. Restek has a wide range of HPLC and GC columns to analyze these complex systems for the nutraceutical and food and beverage industries. Restek columns offer excellent lot-to-lot reproducibility and must meet stringent quality assurance criteria before they are sent to customers. For HPLC procedures, Pinnacle II™ reversed phase columns offer excellent peak shapes for the active ingredients in a wide range of botanical compounds. The Rtx®-Wax capillary column is an excellent choice for the GC analysis of derivatized fatty acids. The Crossbond® technology used to create this column results in long column lifetimes and reproducible results.

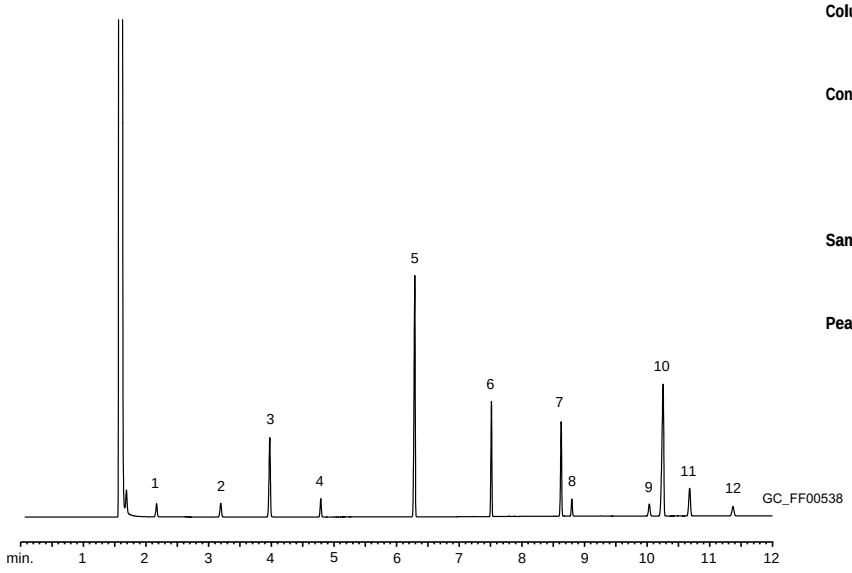
References

1. USP Quality Demonstration Program for Dietary Supplements, Draft 2.0 (2000), The US Pharmacopoeia, Rockville, MD.
2. Barnes, Julian G. and Winter, Greg. The New York Times, May 27, 2001.
3. The US Pharmacopoeia, Rockville, MD.

References not available from Restek.

Figure 4

An Rtx®-Wax capillary GC column achieves excellent resolution of the FAMEs in a saw palmetto standard.



Column Rtx®-Wax
Catalog #: 12423
Dimensions: 30m, 0.25mm, 0.25µm

Conditions Oven temp.: 120°C (hold 3 min.) to 220°C at 20°C/min. (hold 12 min.)
Inj./det. temp.: 250°C/300°C
Carrier gas: helium
Linear velocity: 1mL/min. 34cm/sec.
Split ratio: 100:1

Sample Saw palmetto standard
Inj.: 1µL
Conc.: see peak list

Peak list
1. methyl caproate (C6:0), 0.4mg/mL
2. methyl caprylate (C8:0), 0.4mg/mL
3. methyl nonanoate (C9:0), 2.0mg/mL
4. methyl caprate (C10:0), 0.4mg/mL
5. methyl laurate (C12:0), 5.0mg/mL
6. methyl myristate (C14:0), 2.0mg/mL
7. methyl palmitate (C16:0), 2.0mg/mL
8. methyl palmitoleate (C16:1), 0.4mg/mL
9. methyl stearate (C18:0), 0.4mg/mL
10. methyl oleate (C18:1), 5.0mg/mL
11. methyl linoleate (C18:2), 1.0mg/mL
12. methyl linolenate (C18:3), 0.4mg/mL

Pinnacle II™ 5µm C18 HPLC Columns

Length	1.0mm ID	2.1mm ID	3.2mm ID	4.6mm ID
50mm	9214551	9214552	9214553	9214555
100mm	9214511	9214512	9214513	9214515
150mm	9214561	9214562	9214563	9214565
250mm	9214571	9214572	9214573	9214575

Pinnacle II™ 5µm C8 HPLC Columns

Length	1.0mm ID	2.1mm ID	3.2mm ID	4.6mm ID
50mm	9213551	9213552	9213553	9213555
100mm	9213511	9213512	9213513	9213515
150mm	9213561	9213562	9213563	9213565
250mm	9213571	9213572	9213573	9213575

Pinnacle II™ 5µm Cyano HPLC Columns

Length	1.0mm ID	2.1mm ID	3.2mm ID	4.6mm ID
50mm	9216551	9216552	9216553	9216555
100mm	9216511	9216512	9216513	9216515
150mm	9216561	9216562	9216563	9216565
250mm	9216571	9216572	9216573	9216575

Pinnacle II™ 5µm Phenyl HPLC Columns

Length	1.0mm ID	2.1mm ID	3.2mm ID	4.6mm ID
50mm	9215551	9215552	9215553	9215555
100mm	9215511	9215512	9215513	9215515
150mm	9215561	9215562	9215563	9215565
250mm	9215571	9215572	9215573	9215575

Trident™ Direct Guard Column System*

Description	qty.	cat.#
High pressure filter	each	25082
1cm guard cartridge holder with filter	each	25084
2cm guard cartridge holder with filter	each	25086
PEEK® connection tip for Waters®-style end fittings	each	25088
Replacement cap frits: 4mm, 2.0µm	5-pack	25022
Replacement cap frits: 4mm, 0.5µm	5-pack	25023
Replacement cap frits: 2mm, 2.0µm	5-pack	25057

**The standard PEEK® tip in Trident™ Direct systems is compatible with Parker, Upchurch®, Valco®, and other CPI-style fittings. To use Trident™ Direct systems with Waters®-style end fittings, the tip must be replaced with cat.# 25088.*

Other Literature:

Food, Flavor, & Fragrances Catalog (lit. cat. #59260)

HPLC Catalog (lit. cat. #59241)

Pinnacle™ II New Product Flyer (lit. cat. #59281)

Selection Guide for Polar WAX GC Columns (lit. cat. #59890)

The Institute for Nutraceutical Advancement (INA) Validates GC Methods for Saw Palmetto Using Rtx®-5 and Stabilwax® Columns Applications Note (lit. cat. #59136)

Analyzing the Heat Levels of Hot Sauces Using an Ultra C18 HPLC Column Applications Note (lit. cat. #59199)

Monitoring Volatiles in Food Contact Packaging by Purge & Trap GC/MS Applications Note (lit. cat. #59348)

Analyzing Free Fatty Acids Using a Stabilwax® DA Column Applications Note (lit. cat. #59155B)

Rtx®-Wax Fused Silica Capillary Columns

ID	df	15-meter	30-meter	60-meter
0.25mm	0.10	12405	12408	—
	0.25	12420	12423	12426
	0.50	12435	12438	12441
0.32mm	0.10	12406	12409	—
	0.25	12421	12424	12427
	0.50	12436	12439	12442
	1.00	12451	12454	12457
0.53mm	0.25	12422	12425	—
	0.50	12437	12440	12443
	1.00	12452	12455	12458
ID	**df**	**10-meter**	**20-meter**	
0.10mm	0.10	41601	41602	
	0.20	41603	41604	

Resprep™ SPE Cartridge—Reversed Bonded Phase

Description	1mL 100mg 100-pk	3mL 200mg 50-pk	3mL 500mg 50-pk	6mL 500mg 30-pk	6mL 1000mg 30-pk	20mL 5g 20-pk	60mL 10g 16-pk
C18	26030	26031	24050	24052	24051	26034	26035
C8	26036	26037	26038	26039	26040	—	—