

The Institute for Nutraceutical Advancement (INA) Validates GC Methods for Saw Palmetto Using Rtx®-5 and Stabilwax® Columns

History of the INA Methods Validation Program

Consumer demand for natural products and dietary supplements has grown exponentially, with increasing amounts of botanical materials being used in the manufacture of a large variety of products. As the supplies and number of suppliers multiply, the consistency of raw materials has become an issue for virtually every major player in the natural products industry. Even companies with conscientious and responsible quality control procedures have found it difficult to ensure consistency in their products due to the lack of any published standards for analysis.

These issues, along with other science and market-based factors, led 29 companies to come together in an international effort to validate and make available analytical methods that will meet the demand for global consistency in the testing of botanicals.

The effort is called the Methods Validation Program, or MVP, and it is the first project for the newly formed Institute for Nutraceutical Advancement (INA). INA is a non-corporate division of Denver-based Industrial Laboratories, an independent laboratory that provides analytical and consulting services to the natural products industry.

The INA MVP is being developed under the direction of a broad range of representatives from within the natural products industry, including suppliers, manufacturers, retailers, marketing companies, a grower and an independent laboratory. Companies from both the United States and Europe are represented. In addition, ten major natural products organizations, including the Food and Drug Administration (FDA), have accepted seats on the INA MVP Advisory Committee as a way of ensuring that the process is inclusive. (Additional information is available at <http://www.nutraceuticalinstitute.com/whoweare.>)

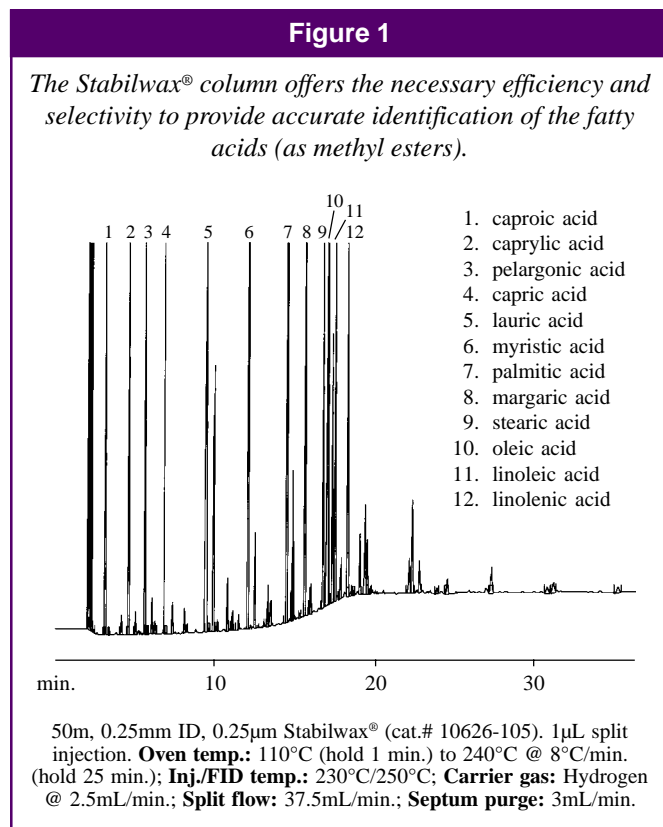
All currently validated methods from the INA can be viewed at their website: <http://www.nutraceuticalinstitute.com/methods>. Although many use high performance liquid chromatography (HPLC), two methods involve gas chromatography (GC) for the analysis of fatty acids and sterols in saw palmetto. This fruit contains several principles thought to have physiological activity, including fatty acids such as caproic, capric, lauric, myristic, oleic, palmitic, stearic, and 1 to 2% essential oils. Furthermore, saw palmetto contains phytosterols and high molecular weight polysaccharides such as β -sitosterol, β -sitosterol 3-O- β -D-glucoside, campesterol, stigmasterol. Purified ethanolic or CO₂ extracts of saw palmetto usually contain 70 to 80% free fatty acids. The fatty acids present are in several forms: free fatty

acids, fatty acid esters of the fatty alcohols, and fatty acid esters of the phytosterols. This oil is commonly blended with excipients to form a dry powder at 30% free fatty acids concentration.

Determination of Fatty Acids in *Serenoa Repens* (Saw Palmetto or Sabel) by GC

This assay can be used to determine fatty acid distribution in saw palmetto fruit, oil extract, and blended powders. Determination is performed using GC, after transesterification of the triglycerides into the methyl esters occurs. For more specific information on the method itself and all procedures involved, please refer to <http://www.nsfina.org/methods/sterolsset.html>

The fatty acids from saw palmetto are separated in Figure 1, which was obtained using a Restek Stabilwax® column and a Shimadzu GC-14A GC, with split injection and a flame ionization detector (FID). The Stabilwax® column offers the necessary efficiency and selectivity to provide accurate identification of the fatty acids (as methyl esters).



Determination of Sterols in *Serenoa Repens* (Saw Palmetto or Sabel) by GC

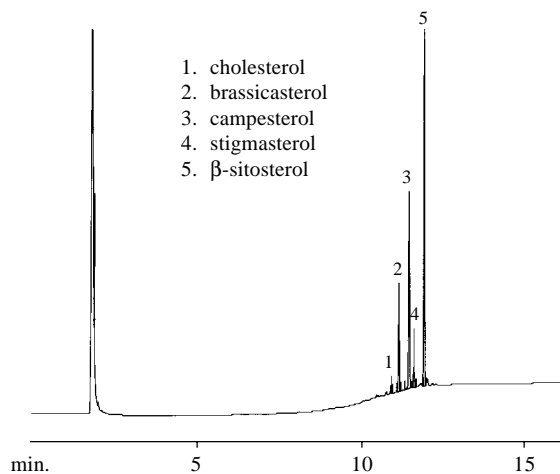
This assay can be used to determine stigmasterol, campesterol, brassicasterol, and β -sitosterol in saw palmetto fruit, oil extract, and blended powders. Determination is performed using GC after hydrolysis, saponification, and derivatization. For more specific information on the method itself and all procedures involved, please refer to <http://www.nutraceuticalinstitute.com/methods/sterols.html>

The sterols from saw palmetto are shown in Figure 2, which was obtained by using a Restek Rtx[®]-5 column and a Hewlett Packard 5890 Series II GC equipped with an FID and an autosampler. The Restek Rtx[®]-5 column contains a 5% diphenyl/95% dimethyl polysiloxane phase, and has the thermal stability to provide elution and accurate quantitation of the phytosterols, even up to 340°C.

Special thanks to Dr. Mark Lange, Director, and to Kathryn Bass, Marketing Director, of MVP for allowing us to print this material. Much of this text has been directly downloaded from the INA website.

Figure 2

The Rtx[®]-5 column has the thermal stability to provide elution and accurate quantitation of the phytosterols, even up to 340°C.



60m, 0.25mm ID, 0.25 μ m Rtx[®]-5 (cat.# 10226). 1 μ L splitless injection*.
Oven temp.: 200°C (hold 1 min.) to 340°C @ 15°C/min. (hold 10 min.);
Inj./FID temp.: 345°C/355°C.

*Split injection may be used. A split flow of 112mL/min. is suggested. However, split injection may result in higher variability of results.

Product Listing

Stabilwax[®] Columns

ID	df (μ m)	Stable to	50m
0.25mm	0.25	250°C	10626-105

Rtx[®]-5 Columns

ID	df (μ m)	Stable to	60m
0.25mm	0.25	360°C	10226

Inlet Sleeves for HP GCs

Description	ID/OD/Length	ea.	5-pk.	25-pk.
2mm Splitless	2.0/6.5/78.5mm	20712	20713	20714
4mm Splitless	4.0/6.5/78.5mm	20772	20773	20774

Inlet Sleeves for Shimadzu GCs

Description	ID/OD/Length	ea.	5-pk.	25-pk.
99mm Split	3.5/5.0/99mm	20860	20861	20862
Cyclosplitter [®]	3.5/5.0/99mm	20870	20871	—

For more information on Restek's Stabilwax[®] and Rtx[®]-5 GC columns, please request our informative Fast Facts Flyers (Lit. cat.# 59316 for Stabilwax[®] and Lit. cat.# 59310 for Rtx[®]-5).

Restek Trademarks: Cyclosplitter, Rtx, Stabilwax.

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