

# Identification and Quantification of Plant Extracts Using Automated Nuclear Magnetic Resonance



Kimberly L. Colson<sup>1</sup>, Jimmy Yuk<sup>1</sup>, Michelle A. Markus<sup>1</sup> and Phuong Mai Le<sup>2</sup>

<sup>1</sup>Bruker BioSpin Corporation, Billerica, MA 01821 USA, <sup>2</sup>National Research Council, Ottawa, Canada

Valued for the perceived benefit derived from their natural compounds, botanical extracts are key ingredients in nutraceuticals and personal care products. Driven by consumers' desire to adopt healthier lifestyles, these billion dollar industries continue to grow, with nutraceuticals alone expected to reach \$204.9 billion by 2017.<sup>1</sup> Often the beneficial claims depend on specific compounds of the extract, thus there is great demand and need to ensure the purity and identity of extracts. NMR spectroscopy is a highly reproducible, automatable technique ideally suited to this task.

Botanical extracts are complex mixtures commonly prepared using extraction procedures to enhance the quantity of desired components thought to be responsible for the desired benefits. The quality of botanical extracts is often reflected by both the quantity of key components and absence of undesired materials such as adulterants, residuals solvents and unrelated botanical material.

NMR spectroscopy is a powerful technique for identifying and quantifying components in complex mixtures such as plant extracts and provides a fast and highly reproducible means for identity, purity, strength and composition verification; thereby providing product assessment and quality control analysis. Multiple compounds can be identified in the same spectrum. Typical 1D <sup>1</sup>H spectra require less than 10 minutes for data acquisition and analysis.

In this report, we demonstrate the procedure and the ability to automate the NMR analysis of goldenseal extract for use in botanical identity, evaluating identity and quantity of components and for quality control. Most previous researchers have used LC-UV methods to quantify berberine, canadine and hydrastine in goldenseal and lack highly compound specific information. NMR provides an information rich, fully-automated, rapid assessment tool that enhances assurances of product composition.

## Goldenseal: An Important Botanical

Goldenseal (*Hydrastis canadensis*; family *Ranunculaceae*) is a plant native to southeastern Canada and eastern US and has long been used in traditional medicine for its pharmacological activities as antibacterial [2, 3], immunostimulant [4], antimicrobial [5] and anticancer properties [6]. Today, this plant has emerged as one of the 20 most popular herbal supplements used worldwide. The combination of goldenseal and echinacea is one of the 15 top-selling botanical dietary supplements in the US with 2008 sales totaling about US 7 million dollars [5b]. The pharmacological effects of goldenseal are mainly attributed to the major alkaloids found in the plant berberine, hydrastine, and canadine [5, 7-9]. Due to the slow growing nature and the popularity of goldenseal, several other plants containing berberine have been substituted for the plant. The adulterants include goldthread (*Coptis japonica*), Oregon grape root (*Berberis aquifolium Pursh*), Barberry (*Berberis spp.*), and

Yellow root (*Xanthorhiza simplicissima*). Most of these adulterants contain berberine, but hydrastine and canadine are only known from goldenseal [5b; 10].

## Materials and Methods

Identification of material: The goldenseal root was identified morphologically by Dr. John Arnason at Ottawa University in 2005 -2006.

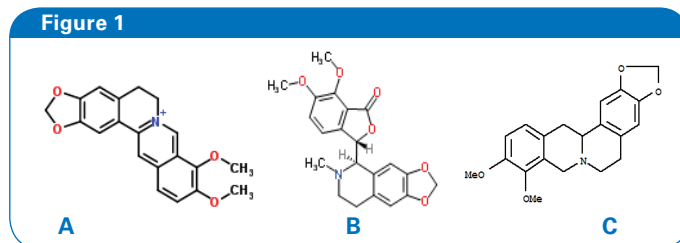
Goldenseal extraction: Dried goldenseal roots were sourced from a Hamilton (ON, Canada) goldenseal farm in May 2005 and milled to a powder (particle size less than 250  $\mu\text{m}$ ), thoroughly homogenized using a rotary mixing drum and thereafter stored at  $-80^{\circ}\text{C}$ . Approximately 200mg of this material samples were extracted by ultrasonic bath, at  $35^{\circ}\text{C}$  for 20min.

Extract Optimization: Various solvent systems were evaluated for the maximum recoveries of 3 major alkaloids (Figure 1, berberine, canadine and hydrastine) in goldenseal root powder. The recoveries of the major alkaloids were found to be highest (>97%) with a 90 % methanol/water/0.1% formic acid (v/v/v) solvent system. The material was sonicated for 20 min and extracted six times. The extract solutions were combined and then filtered. The supernatant liquid was evaporated to dryness with a speed-vac at room temperature. NMR of this dry crude extract of goldenseal root powder was used for detection and quantification of key metabolites known to exist in goldenseal including berberine, canadine and hydrastine. NRC certificate reference material under the names: Berb-1; Cana-1 and Hydras-1 with purity 93.35%; 99.36% and 99.32% (respectfully) were used as reference standards for these three components (berberine, canadine and hydrastine)

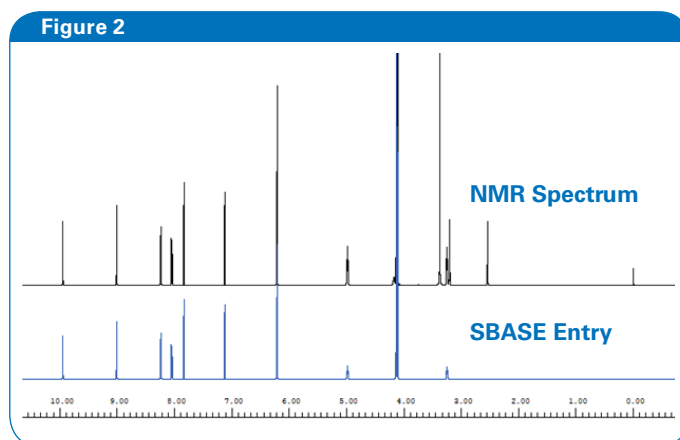
NMR Conditions: All samples were dissolved in 600ul DMSO- $d_6$ , vortexed (1 min), sonicated (5 min), vortexed (1 min) again and centrifuged (13,200 rpm). The supernatant was transferred to a 5mm NMR tube. Material weights for the extract, berberine, canadine and hydrastine were 16.4mg, 11.5 mg, 10.8 mg and 12.3 mg, respectively. NMR spectra were recorded at 400 MHz and 600 MHz on Bruker Avance III spectrometer using the  $^1\text{H}$  noesyigld1d pulse sequence. Spectra were acquired in 8 minutes per sample (32 scans). NMR analysis was done in automation using Bruker's Assure-RMS 1.5 pl2 software package that utilizes an external quantification standard and the PULCON[9] equation for absolute quantification results.

## Generation of a NMR Spectra Database

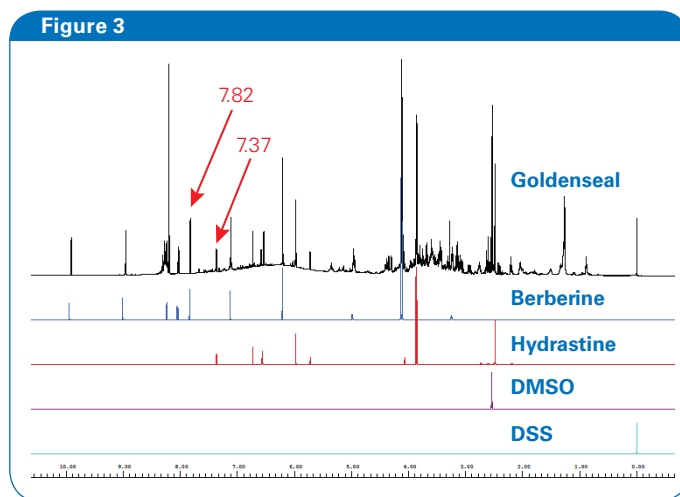
The  $^1\text{H}$  NMR spectra of certified reference material for berberine, canadine and hydrastine were used to develop a spectral database (SBASE) that served as a chemical shift reference library for the automated identification of the key



Molecular structures of berberine(A), hydrastine(B) and canadine(C).

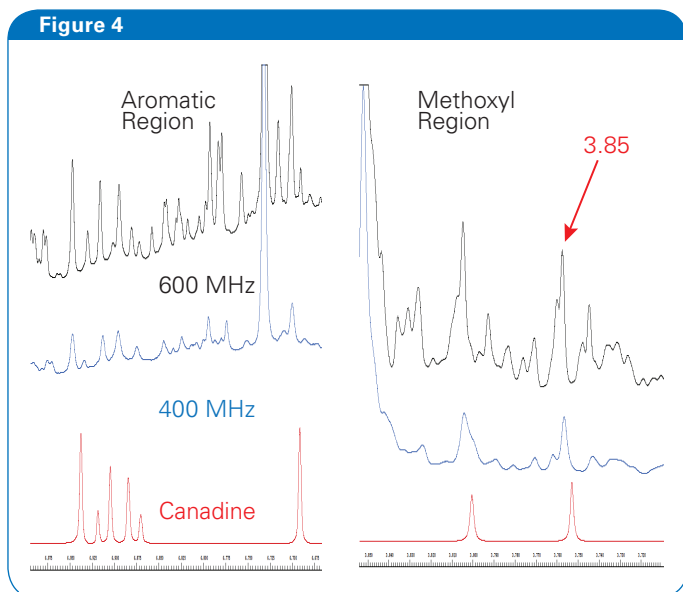


Example of generation of a Spectral Database (SBASE) entry.  $^1\text{H}$  NMR spectrum of berberine (top) and resulting SBASE entry (bottom) after DSS, DMSO- $D_6$ ,  $\text{H}_2\text{O}$ , broad exchangeable signals and noise were removed.



400 MHz Goldenseal  $^1\text{H}$  NMR spectrum (black) as compared to SBASE entries for berberine (blue), hydrastine (red), DMSO (violet) and DSS (turquoise). The signal-to-noise of berberine in the goldenseal spectrum for the signal at 7.82 ppm (single proton) at 400 MHz was 547. For hydrastine the S/N was 173 for the proton at 7.37 ppm (single proton).

metabolites of goldenseal. SBASE entries were created using Assure-RMS by removing noise, solvent and impurity peaks, and broad exchangeable signals from the  $^1\text{H}$  NMR spectrum as shown in Figure 2. The resulting SBASE entry for each compound included peak position, coupling, peak shape and peak intensity information. Figure 3 shows the berberine,



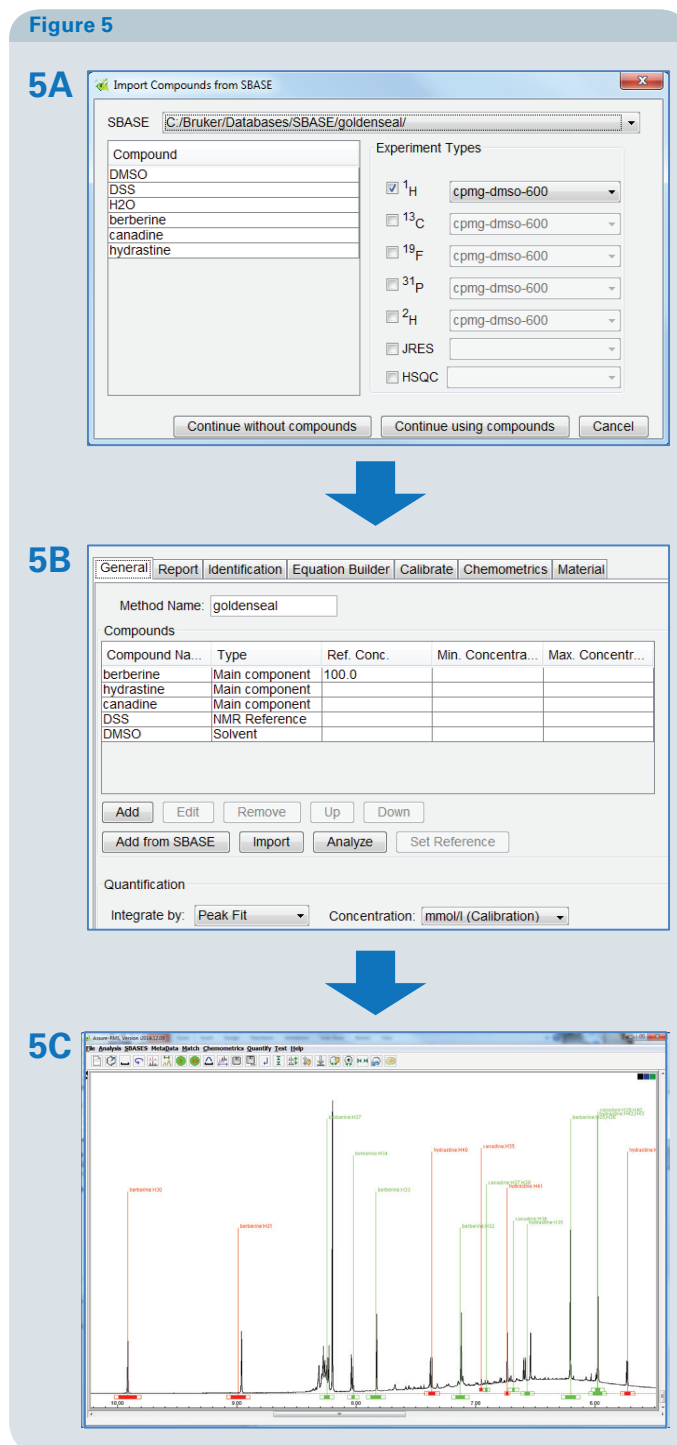
Expansions of the aromatic (left) and methoxyl (right) regions of the  $^1\text{H}$  NMR spectra of goldenseal at 600 MHz (black) and 400 MHz (blue) as compared to the SBASE entry for canadine (red). The signal-to-noise of the methoxyl signal (3 equivalent protons) at 3.85 ppm was 494 at 400 MHz and 891 at 600 MHz. Slight shifts in peak positions were observed.

hydrastine, DMSO (solvent) and DSS (NMR reference standard) SBASE entries as compared to the goldenseal extract. Two compounds of interest, berberine and hydrastine, are readily identified as being present in the extract spectrum by comparison of peak shift, intensity and coupling. The canadine SBASE entry was compared to the goldenseal and was also detectable (Figure 4).

## Detection and Quantification of Key Components

To establish the experimental conditions for automated analysis of goldenseal, the signal-to-noise of the three key components was considered from the crude extract spectrum. At 400 MHz it was determined that an 8 minute (32 scans) experiment provided adequate S/N for canadine, the component of least concentration of the three considered, as shown in Figure 4. The S/N, measured from the isolated methoxyl signal (3 equivalent protons) at 3.85 ppm, was 494 at 400 MHz and 891 at 600 MHz. Detection of components in significantly less concentration at 400 MHz would require a longer experiment time (more scans). The S/N increases by the square root of two for NMR and therefore the needed number of scans may be calculated based on the predicted concentration of components.

The automated analysis method was established using Assure-RMS as shown in Figure 5. Components from the SBASE were imported automatically by selecting all components from the SBASE (Fig. 5A). This populated a search list where the component type was entered (Fig. 5B). Minimum and maximum concentrations may also be set if the material is desired to conform to specific product specifications. The



The Assure-RMS procedure used for generation of the goldenseal automated analysis method included: (A) importation of components from the SBASE, (B) assignment of the component type (main component, solvent, NMR reference, possible adulterant, etc.), allowable minimum and maximum concentrations and quantification reporting criteria (mmol/L), and (C) identification of peaks selected for quantification (red) that are typically well resolved or easily identifiable signals.

program used this table along with the SBASE information to populate the search regions in the NMR spectrum (Fig. 5C) for automatic evaluation. Then, the peaks for quantification were reviewed for ability to quantify the peak based on other nearby or overlapping signals.

The Assure-RMS method was then saved and used for the automated analysis and reporting of goldenseal extract.

## Results

Automated analysis of goldenseal extract identified the presence of the 3 key components and reported quantification results as shown in Table 1. Of the three main components, berberine was present in the greatest quantity followed by hydrastine and canadine with values of 75.8mg/g, 54.1mg/g and 7.1mg/g respectfully. These results are consistent with the manual analysis of the goldenseal extract spectrum.

**Table 1**

Comopound	Category	Concentration (mg/g)	Std Dev
Berberine	Main Component	75.77	1.90
Hydrastine	Main Component	54.14	0.82
Canadine	Main Component	70.7	5.17
DSS	NMR Reference	NA <sup>1</sup>	NA <sup>1</sup>
DMSO	Solvent	NA <sup>1</sup>	NA <sup>1</sup>

Automated quantification results of components in goldenseal extract sample for data acquired at 400 MHz. <sup>1</sup>Entry used for Identity Match only. The concentration for DSS and DMSO was not determined.

## Conclusions

NMR accurately and simultaneously identified the presence and quantity of berberine, hydrastine and canadine from the crude sample extract without additional separation or purification. An NMR spectral database was generated and utilized for the automated analysis and may be expanded to search for additional components in goldenseal. This automated procedure is an efficient and effective method to identify the components of extracts and can be extended to other complex mixtures where verification of components is necessary for product assessment and quality control.

## References

1. "Nutraceuticals Market to Reach 2048 Billion by 2017." Nutraceuticals Market to Reach \$204.8 Billion by 2017 - See More At: [Http://www.nutraceuticalsworld.com/issues/2013-09/view\\_indus%ADtry-news/nutraceuticals-market-to-reach-2048-billion-by-2017/#sthash.yXYbT2Ig.dpuf](http://www.nutraceuticalsworld.com/issues/2013-09/view_indus%ADtry-news/nutraceuticals-market-to-reach-2048-billion-by-2017/#sthash.yXYbT2Ig.dpuf). Nutraceutical Word, 8 Sept. 2013. Web. 5 Jan. 2015.
2. Sczozoc F, Cometa MF, Tomassini L, Palmery M (2001). Antibacterial activity of Hydrastis canadensis extract and its major isolated alkaloids. *Planta Med* 67:561–564
3. Ettetfagh KA, Burns JT, Junio HA, Kaatz GW, Cech NB (2011). Goldenseal (*Hydrastis canadensis* L.) extracts synergistically enhance the antibacterial activity of berberine vial efflux pump inhibition. *Planta Med* 77(8):835–840
4. Jalees R, Dillow JM, Carter SM, Chou J, Le B, Maisel AS (1999). Increased production of antigenspecific immunoglobulins G and M following in vivo treatment with the medicinal plants *Echinacea angustifolia*, *Hydrastis canadensis*. *Immunol Lett* 68:391–395
5. Hwang BY, Roberts SK, Chadwick LR, Wu CD, Kinghorn AD (2003). Antimicrobial constituents from goldenseal (the rhizomes of *Hydrastis canadensis*) against selected oral pathogens. *Planta Med* 69:623–627
6. Karmakar SR, Biswas SJ, Khuda-Bukhsh AR (2010). Anticarcinogenic potentials of a plant extract (*Hydrastis canadensis*): I Evidence from in vivo studies in mice (*Mus musculus*). *Asian Pac J Cancer Prev* 11:545–551
- 6b. C. Cavaliere, P. Rea, M.E. Lynch and M. Blumenthal, Herbal supplement sales experience slight rise in 2008, *Herbal Gram* 82 (2009) 58-61.
7. Gentry EJ, Jampani HB, Kesharvarz-Shokri A, Morton MD, VanderVelde D, Telikepalli H, Mitscher LA (1998). Antitubercular natural products: Berberine from the roots of commercial *Hydrastis canadensis* powder. Isolation of inactive 8-oxotetrahydrothalifendine, canadine,  $\beta$ -hydrastine, and two new quinic acid esters, hydrocandinic acid esters-1 and 2. *J Nat Prod* 61:1187–1193
8. Bolle P, Cometa MF, Palmery M, Tucci P (1998). Response of rabbit detrusor muscle to total extract and major alkaloids of *Hydrastis canadensis*. *Phytother Res* 12:S86–S88
9. R. Upton *Goldenseal Root (Hydrastis canadensis): Standard of Analysis, Quality Control, and Therapeutics.*, (2001) 10-18.
10. Abdel-Haq H, CometaMF, Palmery M, LeoneMG, Silvestrini B, Saso L (2000). Relaxant effects of *Hydrastis canadensis* L. and its major alkaloids on guinea pig isolated trachea. *Pharmacol Toxicol* 87:218–222