Cinnamon: an Important Natural Health Product with Quantification of Key Components using NMR Spectroscopy

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Cinnamon is one of the most important spices in the world. While being important for its culinary use, cinnamon is also highly valued for providing significant health benefits [1] including lowering blood sugar levels [2-5], reducing heart disease risk factors [5-7], and even addressing neurological disorders [8-10]. High levels of antioxidants are in present in cinnamon and likely contribute to these health benefits. Cinnamon is made from the dried inner bark of *Cinnamomum verum* (Ceylon cinnamon) and is referred to as "true" cinnamon. Barks of C. *cassia* (Chinese cassia), C. *burmannii* (Indonesian cassia), and C. *loureiroi* (Saigon cassia), collectively known as cassia cinnamon, are also marketed as cinnamon. Because of its lower cost, Cassia cinnamon and, in particular, Indonesian cassia has replaced Ceylon cinnamon as the species most widely used in the United States, Canada, and Europe. [11] Cassia cinnamon has been shown to contain high levels of the naturally occurring compound coumarin, which can be hepatotoxic to individuals sensitive to it. Ceylon cinnamon contains a significantly lower amount of coumarin. [12]

Addressing a Health Concern

The concern for the increased consumption of the lower cost cassia cinnamon, and hence the ingestion of more coumarin, resulted in the European Food Safety Authority (EFSA) establishing a tolerable daily intake (TDI) of 0.1 mg coumarin/kg body weight. [13] A number of clinical trials have shown cassia cinnamon to have modest antidiabetic activity. [12] As a result, there are currently many dietary supplements on the market containing both true and cassia cinnamon. Supplement labels contain suggested usage levels from 1 gram up to 4 grams of cinnamon per day. The amounts of coumarin and other marker compounds in cinnamon bark, powder, foods, and supplements have previously been analyzed with a validated UPLC-UV/MS method. [11] We have developed a rapid fully automated high throughput method to quantitate coumarin, the main flavor compound cinnamaldehyde, and other key components in cinnamon containing products utilizing ^{1}H-Nuclear Magnetic Resonance (NMR).

Materials and Methods

Three brands of ground cinnamon powder (P1-P3) were purchased from local grocery stores. P1 and P2 were labeled as cassia with no species specified. P3 was labeled as Ceylon cinnamon. Four brands of cinnamon supplements in capsule form were purchased, (S1-S4). S1 was labeled as certified organic Ceylon cinnamon. S2-S4 were all labeled as *Cinnamomum cassia* or just cassia.

NMR samples of cinnamon were prepared by weighing ~200 mg of powdered cinnamon and cinnamon supplement sample, adding 1.5 mL CDCl3, sonicating, and filtering 600 uL of the CDCl3 extract into a 5 mm NMR tube. NMR standard samples of expected cinnamon components including coumarin 1, cinnamyl alcohol 2, cinnamaldehyde 3, cinnamic acid 4,

eugenol 5, and cinnamyl acetate 6 were prepared by dissolving ~90-150 mg of each in 2 mL CDCl3 and transferring 600 uL of each to 5mm NMR tubes. A standard mixture (SM) of the expected components was made by combining each of the expected component solutions.

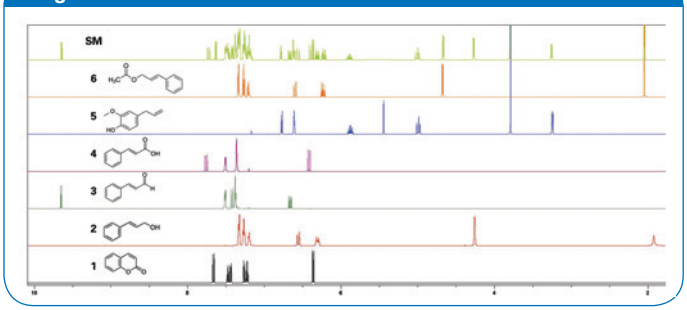
NMR spectra were recorded at 600 MHz on a Bruker AVANCE III spectrometer using the ^{1}H noesyigld1d pulse sequence. Spectra were acquired with 64 scans and a 10s relaxation delay using a sweep width of 20 ppm and 64 K points. Referencing was to the lock solvent. NMR analysis was done in automation using Bruker's AssureNMR version 2.0 software package that utilizes an external quantification standard and the PULCON[14] equation for absolute quantification results.

Serving size was used, along with the NMR data, to calculate the daily dose of coumarin. Recommended serving sizes for the supplements as described on product labels (Table 1) were as follows: S1 = 2*500 mg capsules/day; S2 = 2*500 mg capsules daily, S3 = 1*1000 mg capsules twice a day; and S4 =2*1000 mg capsules twice a day. Daily doses of coumarin were calculated from these recommendations. Note that the coumarin content of supplement S4 exceeds the TDI for an average 60 kg human (6 mg/day). As a comparison, the total coumarin content of 1.0 g of P1-P3 were also calculated.

Generation of an NMR Spectra Database

The ^{1}H NMR spectra of standard reference material for coumarin 1, cinnamyl alcohol 2, cinnamaldehyde 3, cinnamic acid 4, eugenol 5, and cinnamyl acetate 6 were used to develop a NMR spectral database (SBASE) that served as a chemical shift reference library for the automated identification of the key metabolites of cinnamon. SBASE entries were created using AssureNMR by removing noise, solvent and impurity peaks, and broad exchangeable signals from the ^{1}H NMR spectrum as shown in Figure 1. The resulting SBASE entry included peak position, coupling, peak shape and peak intensity information for each compound to enable accurate identification match.

Figure 1

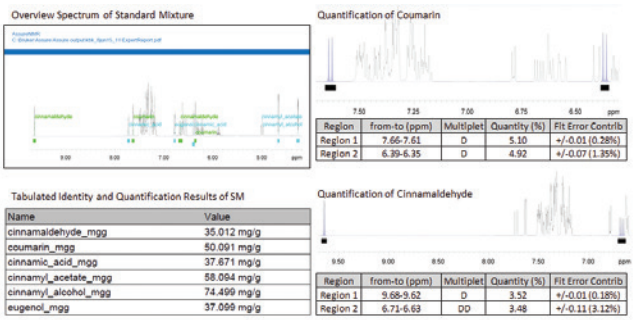


^{1}H-NMR spectra of coumarin (1), cinnamyl alcohol (2), cinnamaldehyde (3), cinnamic acid (4), eugenol (5), cinnamyl acetate (6), and Standard Mixture (SM).

Detection and Quantification of Key Components

An AssureNMR method was developed using AssureNMR for automated identification and quantitation of coumarin and five (5) other key components of cinnamon. [11] The AssureNMR method was generated by (1) importing the spectra from the NMR SBASE,(2) identifying peaks for quantification, selecting lineshape fitting for the resolved compound resonances, and (3) selecting reporting criteria. In cases in which quantitation was done on more than one resonance of a compound, the average was reported along with the standard deviation. The AssureNMR method was instructed to automatically use an external quantitation standard which utilizes an external sample of known concentration and the PULCON method to establish quantitative results. Upon executing the automated AssureNMR method, quantitative ^{1}H NMR spectra were obtained using a 1D-NOESY pulse sequence (Figure 1) and sample preparation information (weight of each dry component and volume of $CDCl_3$) was collected by the software. All concentration calculations were performed automatically within AssureNMR. The quantification calibration file was automatically stored to each cinnamon sample and the software used this file to calculate the concentration of the metabolites in each sample using PULCON [14]. Results for the components of cinnamon were reported by the software as mg compound/g cinnamon in a detailed ExpertReport file that is automatically generated and contains results and quantitation detail, excerpts of which are shown in Figure 2.

Figure 2



AssureNMR quantitation of the Standard Mixture (SM), results and quantitative details from the ExpertReport.

Results

Automated analysis showed the presence of some of the metabolites possibly present in the cinnamon samples. The quantification results taken from the ExpertReports are tabulated in Table 1. Visual inspection of the spectra confirmed the absence of detectable amounts of the components listed as "nd".

As expected, results show that coumarin 1 is detected in Cassia samples at significantly higher amounts than samples

originating form C. *verum*. Considerable variation of coumarin concentration occurs in the Cassia samples ranging from 0.498 to 3.082 mg/g of cinnamon. Concentrations of cinnamaldehyde 3, the major flavor component, are also highly variable in the samples ranging from 0.840 to 23.882 mg/g of cinnamon.

Conclusions

NMR provides a valuable tool for identification and quantification of components in the natural health product, cinnamon, without the need for additional separation or purification. The high reproducibility of NMR allows for fully automated NMR acquisition, analysis and reporting and uses an NMR spectral database which may be expanded to search for additional components in cinnamon. The NMR SBASE and AssureNMR method may also be transferred to other instruments and sites to be used for product assessment without the need to regenerate reference spectra.

NMR spectroscopy can be extended to other complex mixtures where verification of components is necessary for product assessment and quality control.

Table 1

		Concentration measured for each components (mg/g cinnamon)							
Sample	Source	1 coumarin	2	3	4	5	6	Recommended Serving (g/day)	mg coumarin per day*
S1	C. verum	0.009	nd	0.840	nd	nd	nd	1	0.009
S2	Cassia	0.498	nd	2.071	nd	nd	nd	1	0.498
S3	Cassia	0.972	nd	6.091	nd	nd	nd	2	1.944
S4	Cassia	1.519	0.257	13.575	nd	nd	nd	4	6.076
P1	Cassia	0.885	0.177	6.343	nd	nd	nd	1	0.885
P2	Cassia	3.082	0.413	23.882	nd	nd	nd	1	3.082
P3	C. verum	nd	0.265	3.041	nd	0.195	nd	1	~0.000

*mg of coumarin that would be consumed based on the daily recommended serving and the actual amount of coumarin detected. The TDI for a 60 kg human is 6.0 mg/day. "nd" denotes "not detected"

Quantitation of coumarin (1), cinnamyl alcohol (2), cinnamaldehyde (3), cinnamic acid (4), eugenol (5), and cinnamyl acetate (6) in cinnamon supplements S1-S4 and powders P1-P3.

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