Solid-State ^{1}H, ^{13}C and ^{11}B qNMR Analysis of Fruitex-B® – A Calcium Fructoborate Complex: Chemical Structure, Identification, Quantitative Analysis and Stability Study

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Overview of Study

Fruitex-B® (FrxB) is a patented plant mineral complex that is marketed as a nutritional supplement with potential health benefits for conditions linked to inflammation such as bone, joint and cardiovascular conditions. The product is a calcium fructoborate complex formed by the reaction of boric acid with fructose and calcium carbonate. Liquid and solid-state ^{13}C and ^{11}B NMR was utilized to establish a baseline for product quality and to establish a robust testing method for both identification and quantification of the mono-complex and di-complex present in the product, as well as free borate and free fructose that is present in the finished product. A quantitative ^{11}B NMR method was developed to quantify the amount of FrxB present in products where it was tableted or capsuled with magnesium stearate or maltodextrin. A quantitative ^{13}C NMR method was developed to quantify free fructose content in the complex. Finally, an NMR based product stability study was performed to monitor molecular level stability of the complex at temperature ranging from 35-70°C with exposure lasting from 2-18 hours.

Background

Boron is naturally occurring and essential element for plant and animal life. There are many different biological compounds that can form complexes with boron. Compounds capable of complexing with boric acid include sugar alcohols, pyranose and furanose sugars or their derivatives, organic acids. Boric acid forms esters and complexes with a wide variety of mono-, di-, and polyhydroxy compounds (Woods, 1996). One of the most stable esters of boric acid are complexes where boric acid is a bridge between two carbohydrate molecules, e.g. fructose-boron-fructose. The examination of boron complexation in plants and plant extracts by ^{11}B NMR demonstrated the majority of the boron was associated with a diester complexes of diols and hydroxycarboxylic acids in radish and apple respectively (Matsunaga & Nagata, 1995). The authors made conclusion that fructose is the most significant boron complexing molecule. Later these hypotheses were verified (Brown & Shelp, 1997 and Hu, et al., 1997) after successful isolation and full characterization of soluble boron complexes from higher plans. Calcium fructoborate (CF) is most commonly found in fresh fruits and vegetables. As a dietary supplement it is manufacture by VDF FutureCeuticals, Inc under the commercial name Fruitex-B®(FrxB) based on the US patent 5.962.049 (Miljkovic, 1999). The characterization of this complex has been reported previously (Rotaru et al., 2010) using thermal analysis, X-rays diffraction, ICP-MS, Raman spectrometry techniques.

In this study we investigate molecular composition, stability and identification of FrxB used as a dietary supplement for human nutrition (Dinca & Scorei, 2013, and Reyes-Izquierdo et al., 2012) using liquid- and solid-state ^{11}B and ^{13}C NMR.

Materials and Method

Materials

Fruitex-B ® calcium fructobotrate (FrxB) was manufactured and provided by Futureceuticals, Momence IL, USA according to the Miljkovic patent (US 5, 962,049).

All NMR analysis was performed in D_2O or H_2O/D_2O. D_2O (99.9%D) was obtained from Cambridge Isotopes Laboratories, Tewksbury MA, USA.

Samples were observed directly after they were received, after they had been thermally treated in a Duratech TCON dry bath system (capable of holding temperatures to +/- 0.1 °C), or as calibration standards which were made by mixing accurately weighed samples of FrxB with magnesium stearate or maltodextrin. Samples for solid-state NMR were weighed to the nearest 0.1 mg on a Sartorius GD-503-NTEP microbalance after they were packed into the MAS rotor.

NMR Spectroscopy

Liquid-state ^{11}B, ^{13}C, and ^{1}H NMR was performed on a Varian Mercury 300MVX NMR spectrometer equipped with a 5mm Varian ATB Probe at a resonance frequencies of 96.14 MHz (^{11}B), 75.36 MHz (^{13}C) and 299.67 MHz (^{1}H), respectively. ^{11}B spectra were acquired with a 45 degree tip angle pulse width, a relaxation delays of 0.2 seconds, an acquisition time of 80 ms with 8K points acquired with a spectral width of 100 kHz, and 1024 pulses were averaged. The data was zero filled to 65K points. The ^{13}C NMR was acquired with a 30 degree tip angle pulse width, a 5 seconds relaxation delay, 0.96 second acquisition time, with 24K points acquired with a spectral width of 25 kHz, and 10-12,000 pulses were averaged. The data was zero filled to 131K points. The ^{1}H NMR spectra were obtained with a 30 degree pulse angle, a 2 second relaxation delay, a 4.448 second acquisition time, with 32K points acquired over a spectral width of 7.2 kHz, 128 pulses were averaged. The data was zero-filled to 131K points. The data was acquired in a quantitative manner with inverse gated decoupling of protons during the acquisition of the ^{11}B and ^{13}C experiments. All samples were dissolved in D_2O (Cambridge Isotope Laboratories). No pH adjustments were performed on the samples after dissolution.

Solid-State ^{13}C (50.30 MHz) and ^{11}B (64.17 MHz) NMR spectra were obtained on a Varian UnityPlus-200 NMR spectrometer equipped with a Doty Scientific 7mm Supersonic CP-MAS probe. Magic angle spinning (MAS) speeds of around 6 kHz were employed. The ^{13}C NMR data was acquired using cross polarization which prepares the magnetization on the protons initially and then transfers the spin locked magnetization to the ^{13}C nuclei. The advantage of this experiment is the fact that the experiment is performed at the spin-lattice relaxation rate (T_1) of protons in the sample which is considerably shorter than the T_1 of ^{13}C nuclei in the same sample. Thus, one obtains a significant enhancement of the ^{13}C signal from the polarization transfer and can pulse at a shorter pulse-repetition rate. The ^{13}C experiment on calcium fructoborate complex were acquired with an 8 second relaxation rate, and acquisition time of 25.6 ms, with 1K points being acquired over a spectral with of 40 kHz, and 4096 pulses were averaged. The exception to these acquisition parameters were those used for pure crystalline fructose. The ^{11}B NMR spectra were acquired with MAS and with the sample remaining static in the NMR probe. The experiments were acquired with a central transition selective pulse width, a 0.2 second relaxation time, with 1K points being acquired in an acquisition time of 10.2 ms, and with a spectral width of 100 kHz.

Structure of Fruitex-B Fructoborate Complex

^{1}H NMR

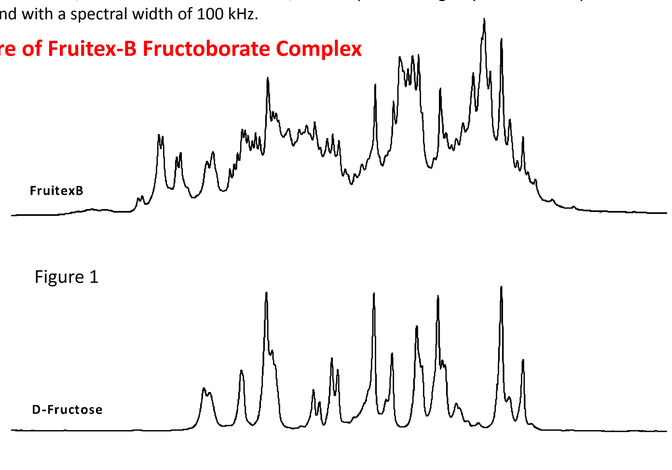

Figure 1

The spectrum above shows the comparison of the ^{1}H NMR spectra of pure D-fructose and Fruitex-B fructoborate complex (FrxB). Free fructose is observed as well as the mono-ester/di-ester complex in the FrxB sample, but the overall spectrum is complicated and no assignments have been made due to its complexity. However, the ^{1}H NMR spectrum can be used to quantify the presence of FrxB in maltodextrin and other soluble adulterants.

^{13}C NMR

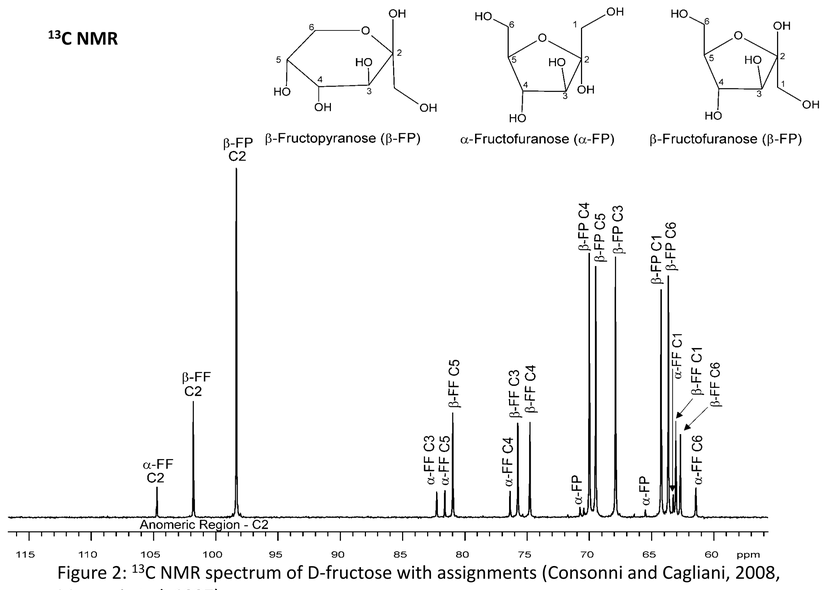

Figure 2: ^{13}C NMR spectrum of D-fructose with assignments (Consonni and Cagliani, 2008, Mazzoni et al, 1997).

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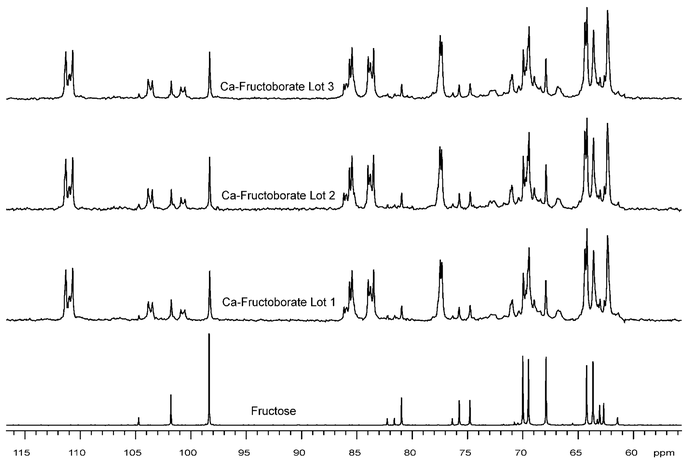


Figure 3: ^{13}C NMR of D-Fructose compared to several production lots of Fruitex-B

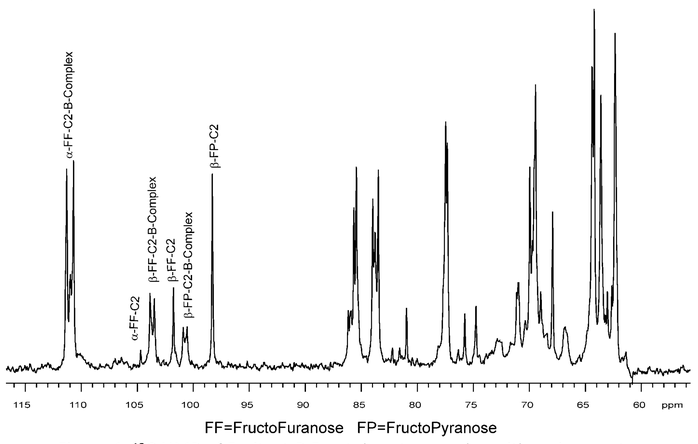


Figure 4: ^{13}C NMR of Fruitext-B Fructoborate complex with assignments

D-Fructose Tautomer Type ^{13}C NMR - Anomeric Region

Fructose Tautomer	Mole%
β-FP	71.1
β-FF	22.4
α-FF	6.5

Relative Molar Concentrations found in Different Lots of Calcium Fructoborate Complex

Fructose Tautomer	Lot 1 (mole%)	Lot 2 (mole%)	Lot 3 (mole%)
β-FP (Free Fructose	15.5	13.9	15.7
β-FF (Free Fructose)	7.9	7.1	8.5
α-FF (Free Fructose)	1.6	2.0	1.8
α-FF-B-Complex	48.3	50.5	47.6
β-FF-B-Complex	17.0	16.3	16.3
β-FP-B-Complex	9.6	10.2	10.0

Unreacted fructose (free fructose) is observed in the complex mixture and a 3-7 ppm downfield shift of the fructose resonances is observed for the carbons coordinated to borate in the fructoborate complex. The change of relative signal intensities in the regions of the spectrum that are associated with furanose tautomers, indicates that the tautomer distribution of the complex strongly favors the reaction of borate with the fructofuranose (FF) form.

The ^{13}C NMR of the anomeric (C-2) region can also be utilized to determine the consistency of the FrxB product from one batch to the next (see Figure 4).

The fact that the FrxB complex peaks are multi-component in all cases leads to the conclusion that the borate reacts with multiple hydroxyls with OH condensation reactions occurring predominantly on the C-1/C-2 as well as on the C-3/C-4 of the FF forms. It is expected from the mole ratios utilized in the synthesis of the FrxB complex that the complex is predominantly the di-ester form BL_2^- form (one borate coordinated to two fructose molecules) with the minor constituent being the monoester form (BL^-), as well as some free/unreacted borate.

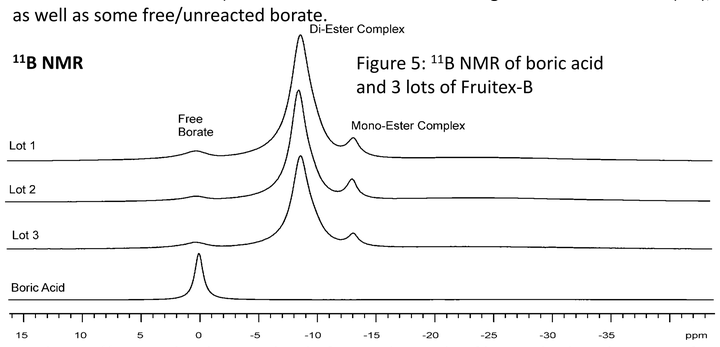
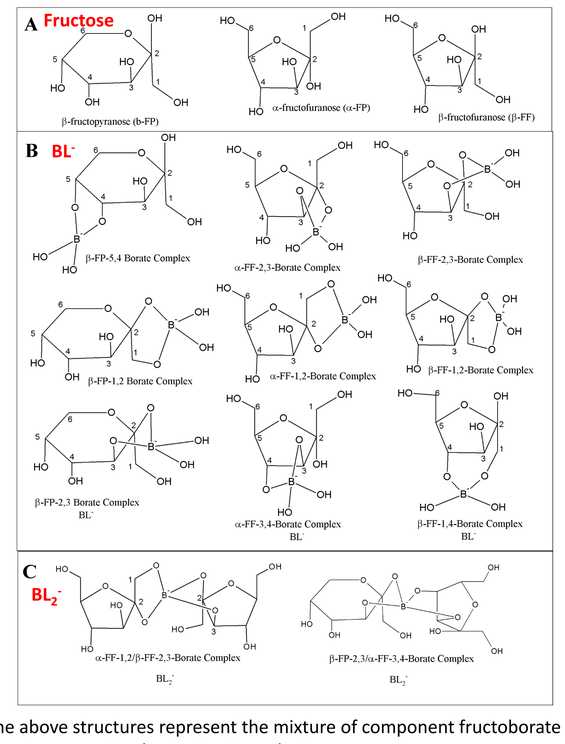


Figure 5: ^{11}B NMR of boric acid and 3 lots of Fruitex-B

Liquid-state ^{11}B NMR has been utilized often in the study of biomedical applications of boron (Bendel, 2005). In this study liquid-state ^{11}B NMR was obtained on order to observe the FrxB complex from the perspective of the boron chemistry. Previous research has identified that three basic types of boron are observed in aqueous solutions of FrxB. Free boric acid is observed at 0 ppm, the di-ester complex (BL_2^-) is observed at -9 ppm, and the mono-ester (BL^-) complex is observed at -13 ppm (Makkee et al., 1985, Reyes-Izquierdo et al., 2012, and Smith et al., 1998). The relative molar concentrations of these three types of boron were found to be approximately 5%, 85%, and 10%, respectively. Figure 5 shows the liquid-state ^{11}B NMR spectra of boric acid and 3 batches of FrxB.



The above structures represent the mixture of component fructoborate Species present in the Fruitex-B product

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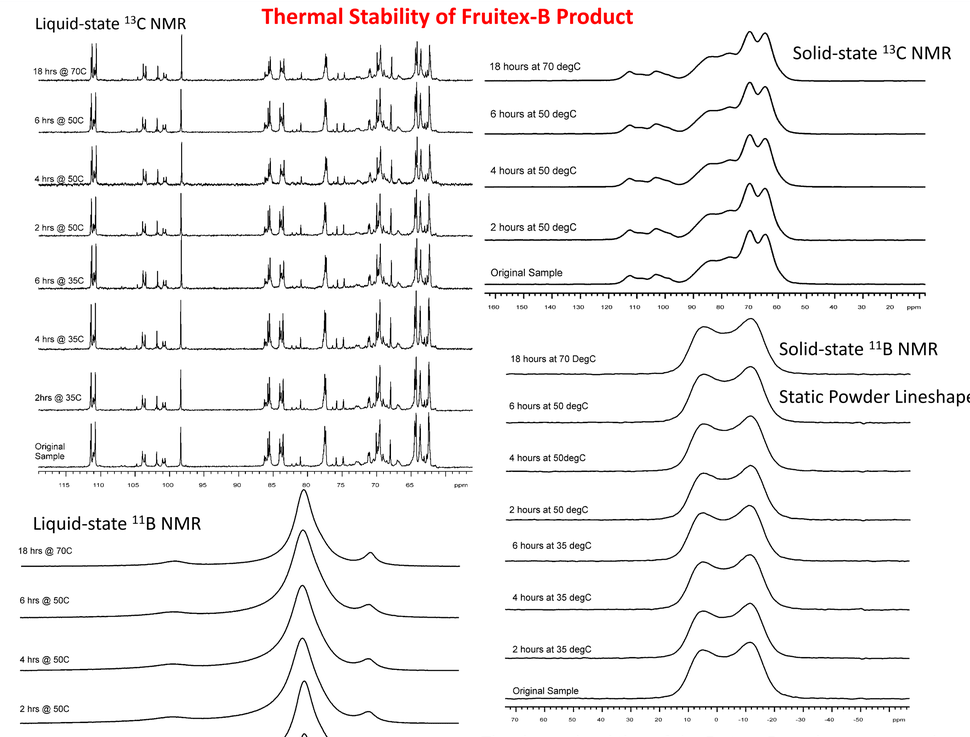
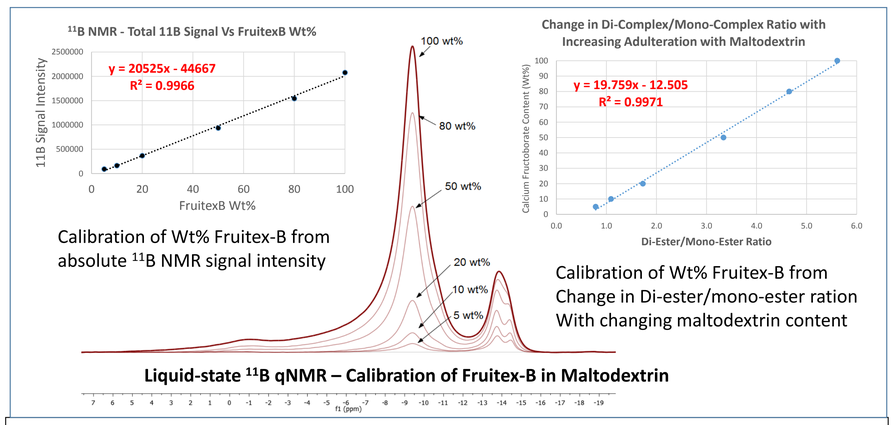
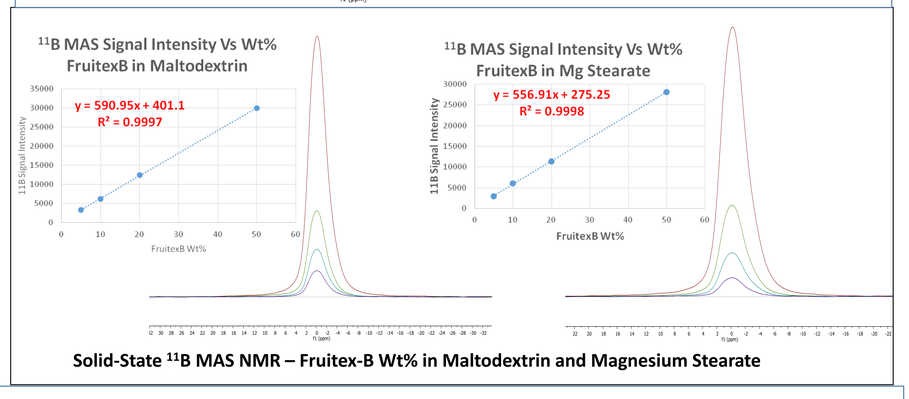
Thermal Stability of Fruitex-B Product

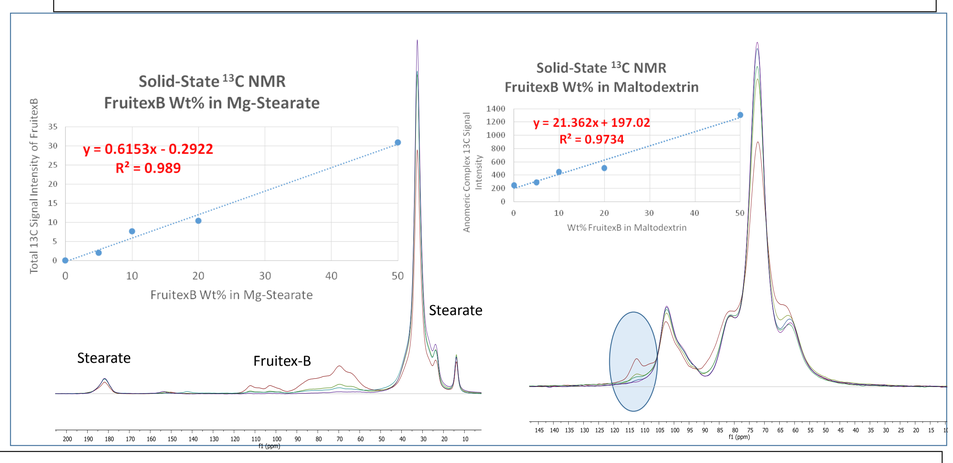


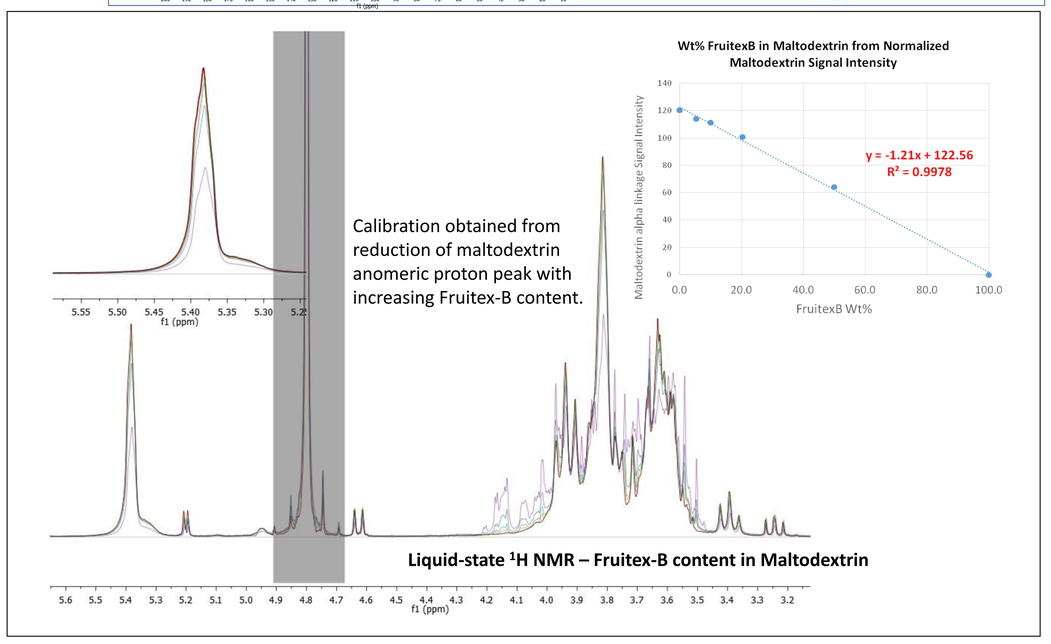
The thermal stability of the Fruitex-B product was tested by exposing the product to temperatures of 35, 50 and 70°C for between 2 and 18 hours. These figures show the results obtained by liquid and solid-state NMR experiments. The samples showed no observable changes over the course of the stability test. Free borate, BL- and BL2- were Calculated from the ^{11}B NMR and the ^{13}C NMR was utilized To calculate free fructose and the α-FF/β-ff/β-FP component complex concentrations.

Quantitation of Fruitex-B in the Presence of Excipients and Adulterants









Quantitation is possible by ^{1}H, ^{13}C, ^{11}B NMR with either liquid or solid-state experiments where appropriate.

Conclusion

Multinuclear liquid and solid-state NMR spectroscopy was capable of defining the fructoborate structure, and allowed identification of the pure complex as well as Quantitation of Fruitex-B product in the presence of excipient and adulterants.