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Quantitative ^{1}H qNMR Method for Complex Mixture Analysis: Determination of Acetylated Polysaccharides, Glucose, Maltodextrin, Isocitrate, Malic Acid in Aloe Vera Leaf Juice.

Also … Preservatives, Additives, and Degradation Products in Aloe Vera Raw Materials and Consumer Products

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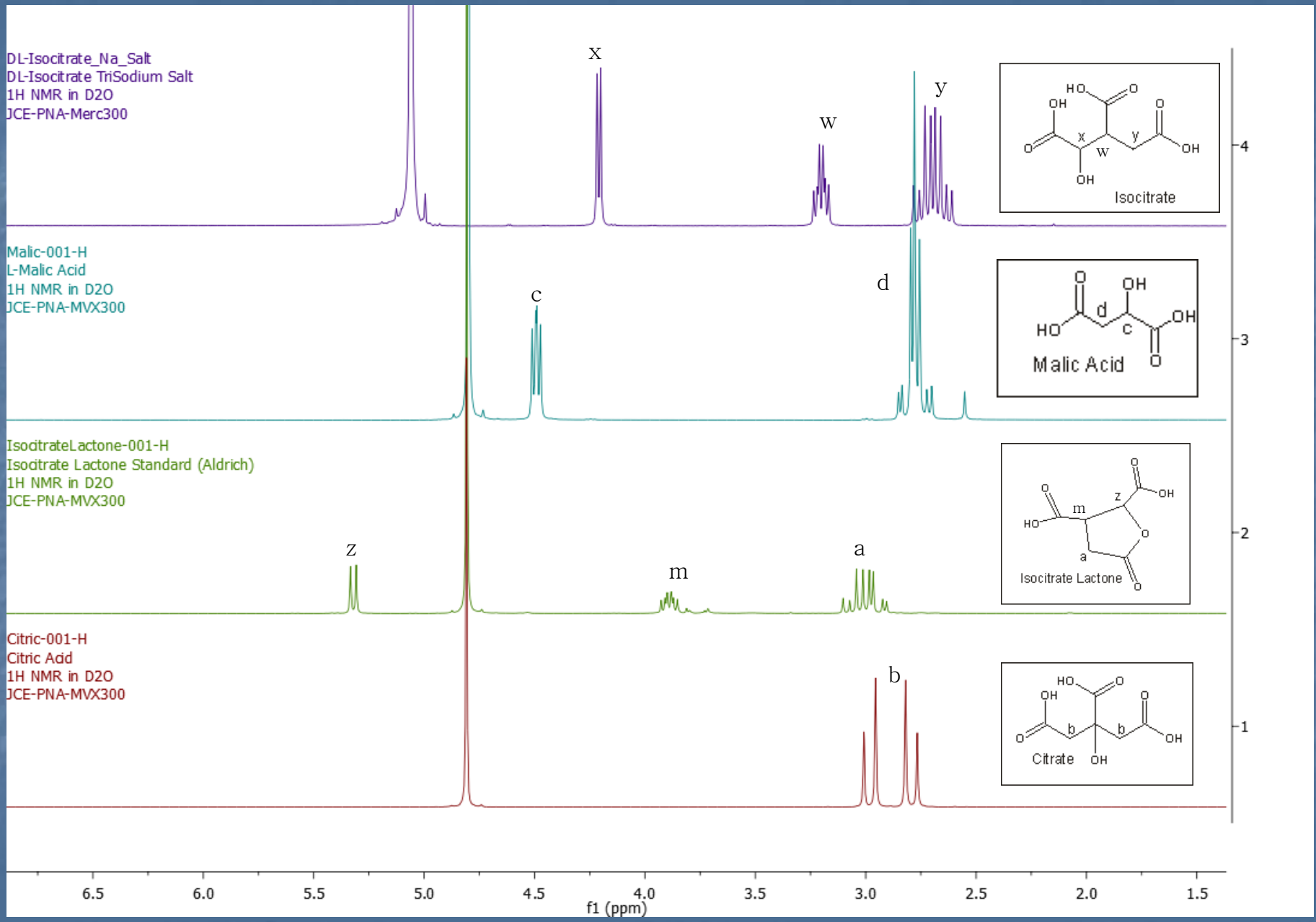
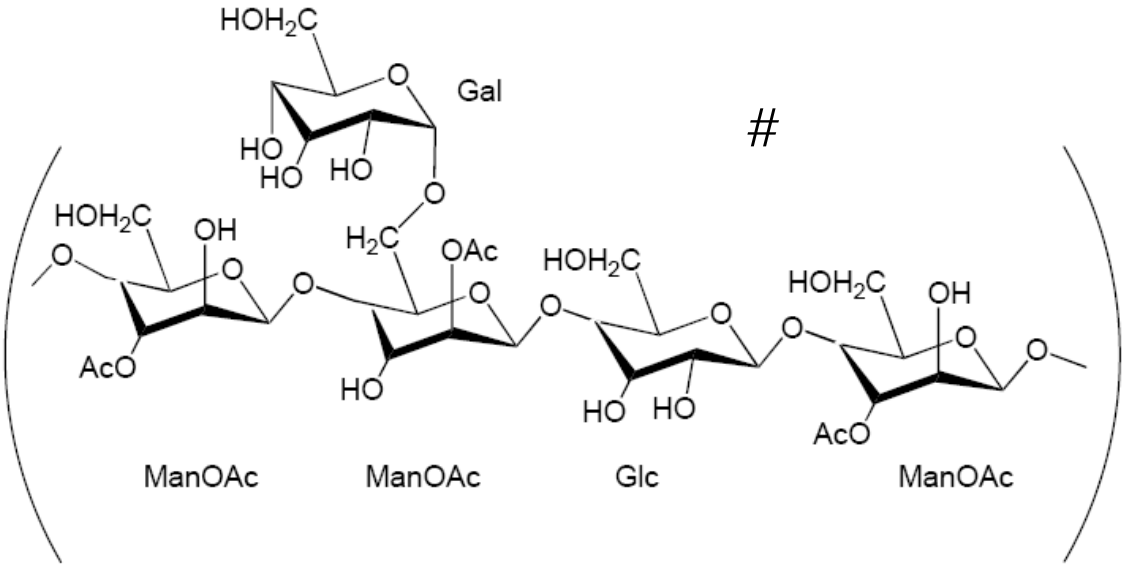
NMR Systems and Experimental Conditions

Acquisition Time	3-8 Seconds
Relaxation (Recycle) Delay	2-6 Seconds
Frequency, MHz	300-500 MHz
Nucleus	^{1}H
Number of Pulse Accumulations*	16-256
Original FID Points	16384-84000
Zero-filled Points	32768-262144
Pulse sequence	Single pulse
Solvent	D_2O
Sweep width, ppm	16
Temperature	Ambient (25 ºC)
Line Broadening	0.35 Hz
Steady State Pulses	8
Pre-Acquisition Delay	60 seconds

Advantages of Using NMR in Complex Mixture Analysis

- Minimal Sample Preparation
- Reproducible (site to site, country to country)
- Linear and Quantitative Spectral Response
- Targeted AND Non-Targeted
- Qualitative
- Quantitative
- Compound Specific
- Rapid – 10 Minutes
- Affordable


DL-Isocitrate_Na_Salt
DL-Isocitrate TriSodium Salt
1H NMR in D2O
JCE-PNA-Merc300
x
w
y
Isocitrate
Malic-001-H
L-Malic Acid
1H NMR in D2O
JCE-PNA-MVX300
c
d
Malic Acid
IsocitrateLactone-001-H
Isocitrate Lactone Standard (Aldrich)
1H NMR in D2O
JCE-PNA-MVX300
z
m
a
Isocitrate Lactone
Citric-001-H
Citric Acid
1H NMR in D2O
JCE-PNA-MVX300
b
Citrate
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)
4
3
2
1



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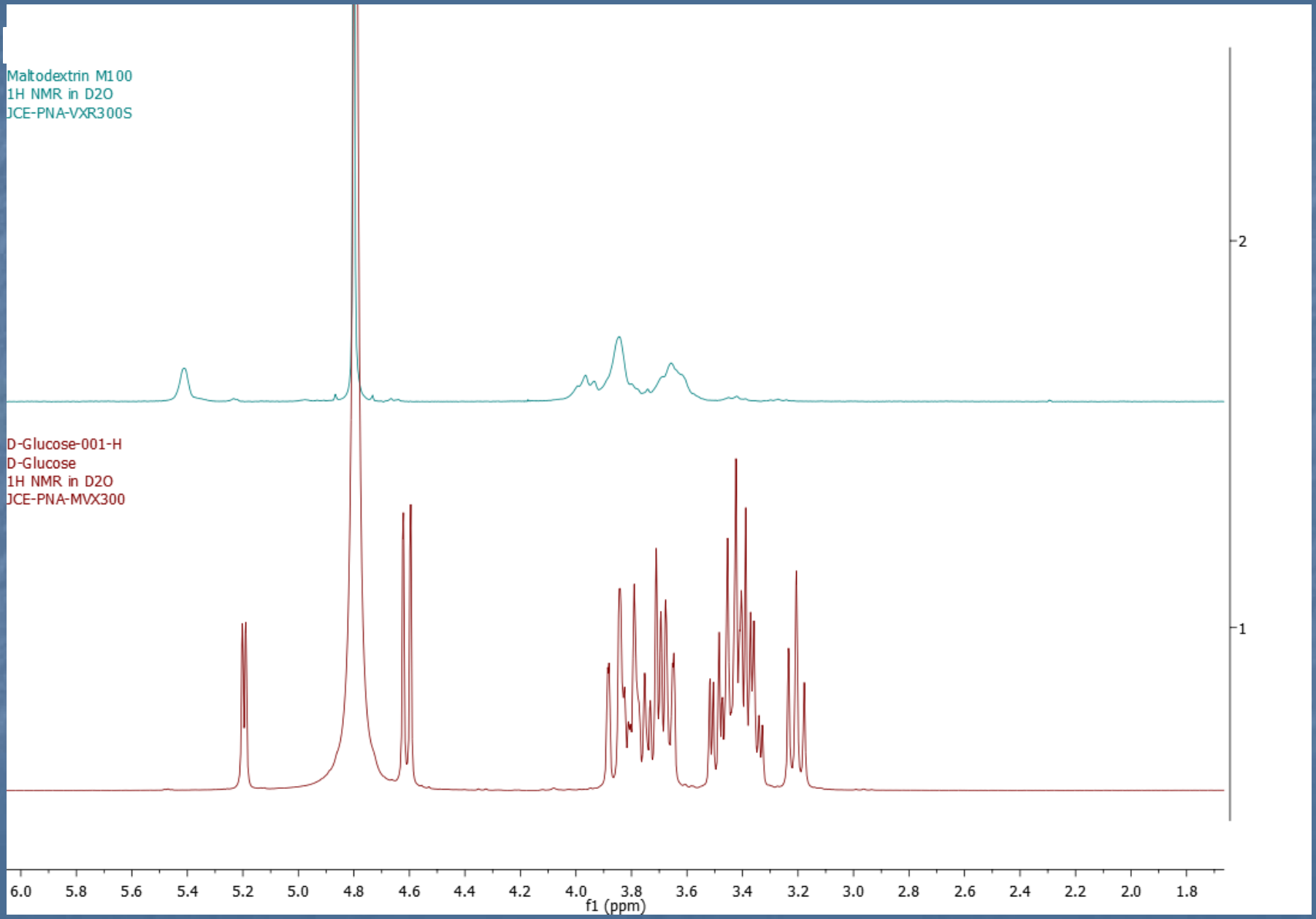
Scheme 1: Proposed structure for the major component of *Aloe vera* polysaccharide

Chemical Characterization of the Immunomodulating Polysaccharide of *Aloe Vera L., W.J.Goux, et al,*
http://www.iasc.org/AloeStructure080604.pdf

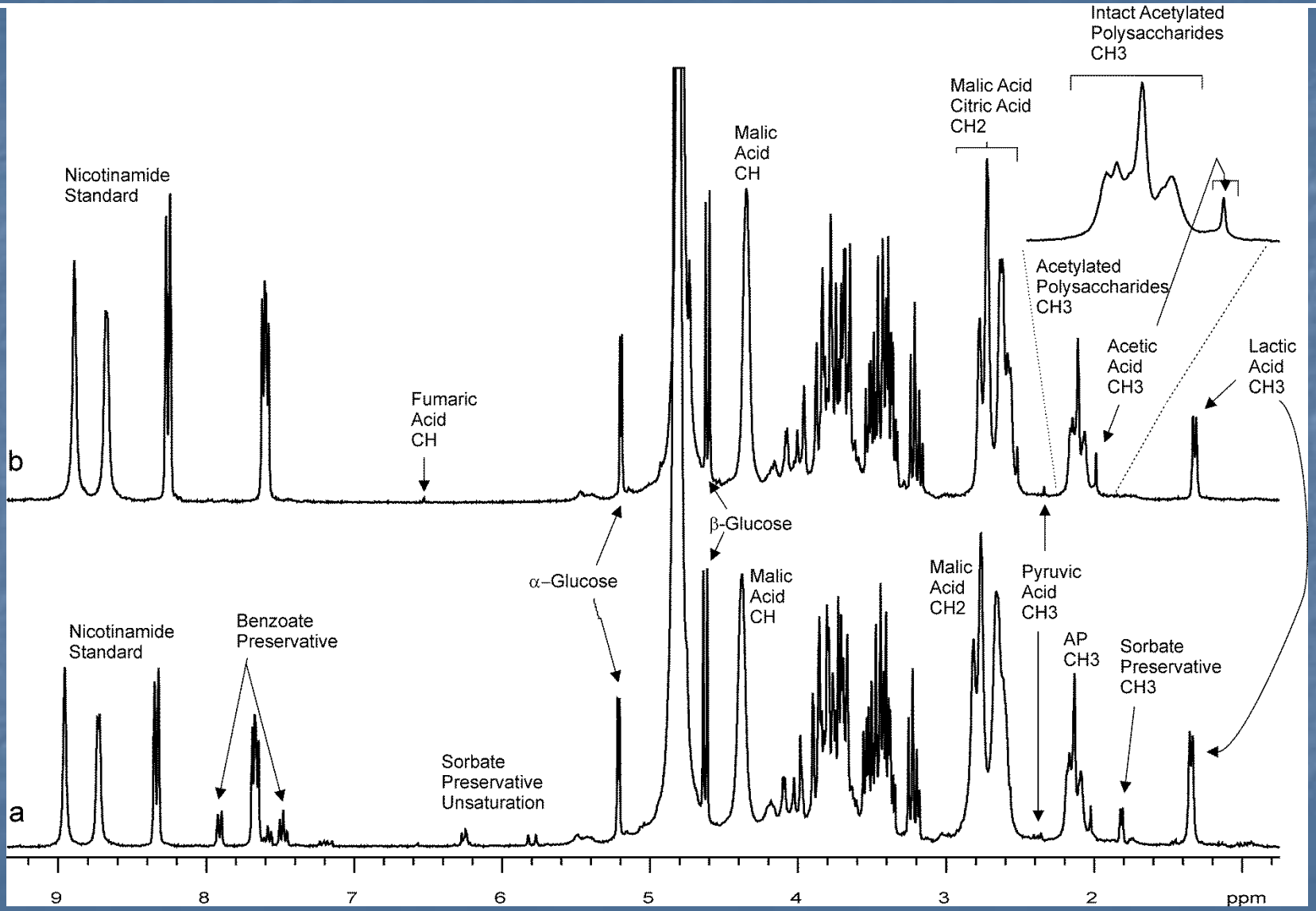

Maltodextrin M100
1H NMR in D2O
JCE-PNA-VXR300S
D-Glucose-001-H
D-Glucose
1H NMR in D2O
JCE-PNA-MVX300
2
1
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
f1 (ppm)

Aloe Vera Inner Leaf Juice Constituents and Additives that need to be Analyzed and Reported for IASC Certification

Compound	IASC Certification requirement
Acemannan	≥ 5% dry weight
Glucose	Present
Aloin	10 ppm or less in 0.5% aloe vera solids solution, analysed by HPLC or other fit for purpose methodology approved by IASC
Isocitrate	≤ 5% dry weight
Maltodextrin	Must be listed on label and analysis must meet label claims. If undeclared, is considered an adulterant.
Solids	≥ 0.46% in single-strength juice (for example, a 10x concentrate should have ≥ 4.6%)
Ash	≤ 40%

Characteristic chemical shift values, peak multiplicity, protonated carbon type and N values used for detection and quantitation of the major natural components of aloe vera leaf juice

Substance	Signal Type and N Parameter	Chemical shift, ppm
Acetylated Polysaccharides	Broad Group of CH_3 Singlets (N=3)	2.0-2.3
Isocitric acid	CH, Doublet (N=1)	4.25
Malic acid	CH, 4 peak multiplet (N=1)	4.45
α-Glucose	CH Doublet (N=1)	4.6
β-Glucose	CH Doublet (N=1)	5.2
Isocitric lactone	CH Doublet (N=1)	5.05



Freeze-Dried Inner Leaf Juices with (a) and without (b) preservatives

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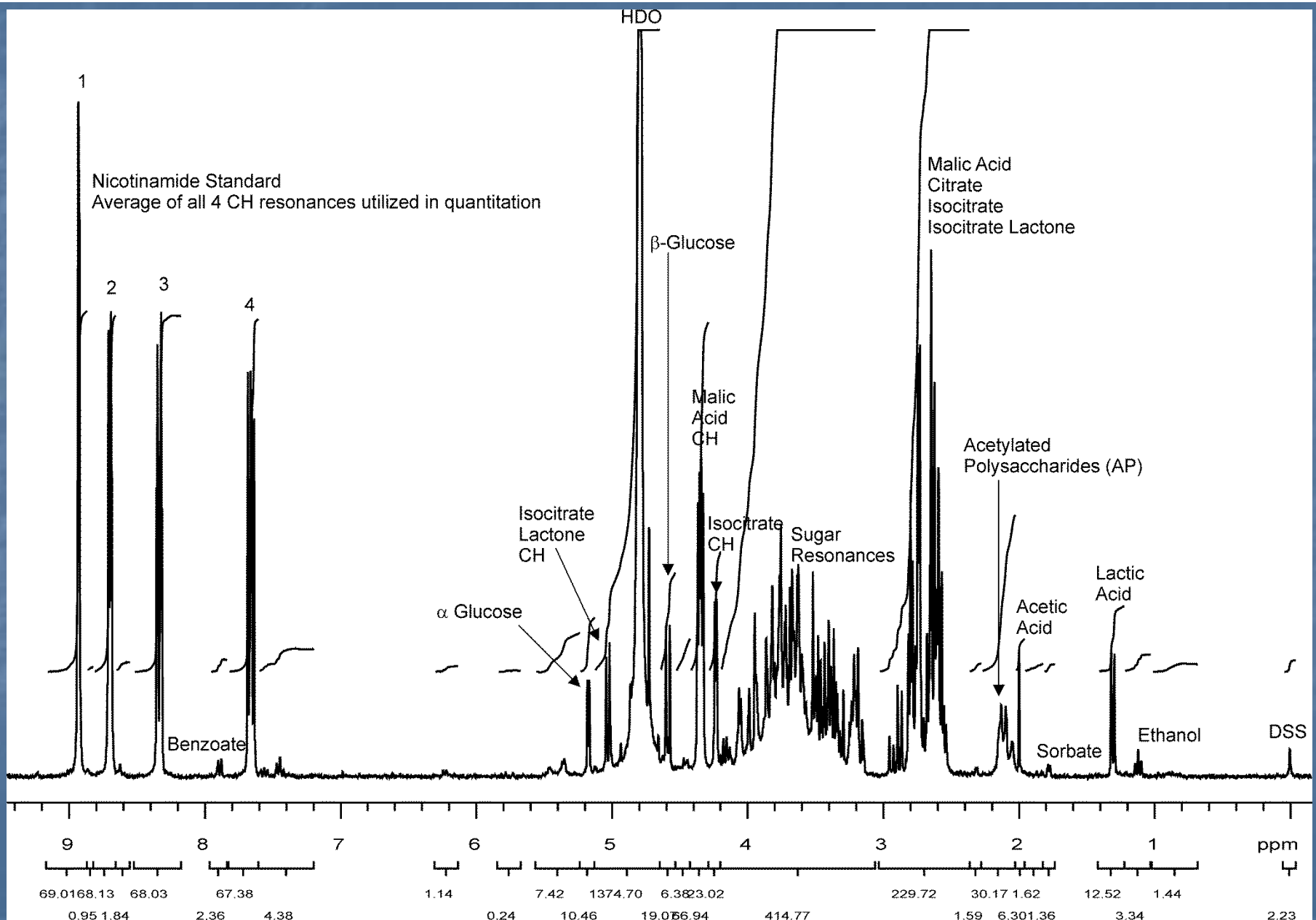
Commercial freeze-dried 200x aloe vera leaf juice powder



Chemical shift values, peak and chemistry descriptions, molar conversion factors that can be used for detection and quantitation of aloe vera leaf juice preservatives, additives, and degradation products

Compound	Type of compound	Signal type	Chemical shift, ppm
Propylene glycol	Additive	CH_3, doublet (N=3)	1.1
Ethanol	Degradation product or additive	CH_3, triplet (N=3)	1.15
Lactic acid	Degradation product	CH_3, doublet (N=3)	1.33
Potassium sorbate	Preservative	CH_3, doublet (N=3)	1.82
Acetic acid	Degradation product	CH_3, singlet (N=3)	1.96
Pyruvic acid	Degradation product	CH_3, singlet (N=3)	2.35
Citric acid	Naturally present or added as pH regulator or preservative	2 x CH_2, Multiplet (N=4)	2.5-3.0
Succinic acid	Degradation product	2 x CH_2, singlet (N=4)	2.6
Glycerol	Additive	CH_2 and CH, multiplet	3.5
Glycine	Additive	CH_2, singlet (N=2)	3.51
Sucrose	Additive	CH, doublet (N=1)	5.4
Fumaric acid	Degradation product	2 x CH, singlet (N=2)	6.5
Sodium benzoate	Preservative	2 x CH, doublet (N=2)	7.95
Formic acid	Degradation product	CH, singlet (N=1)	8.2-8.3

Freeze Dried Aloe Vera Leaf Juice – Acidification Required for Isocitrate Quantification.

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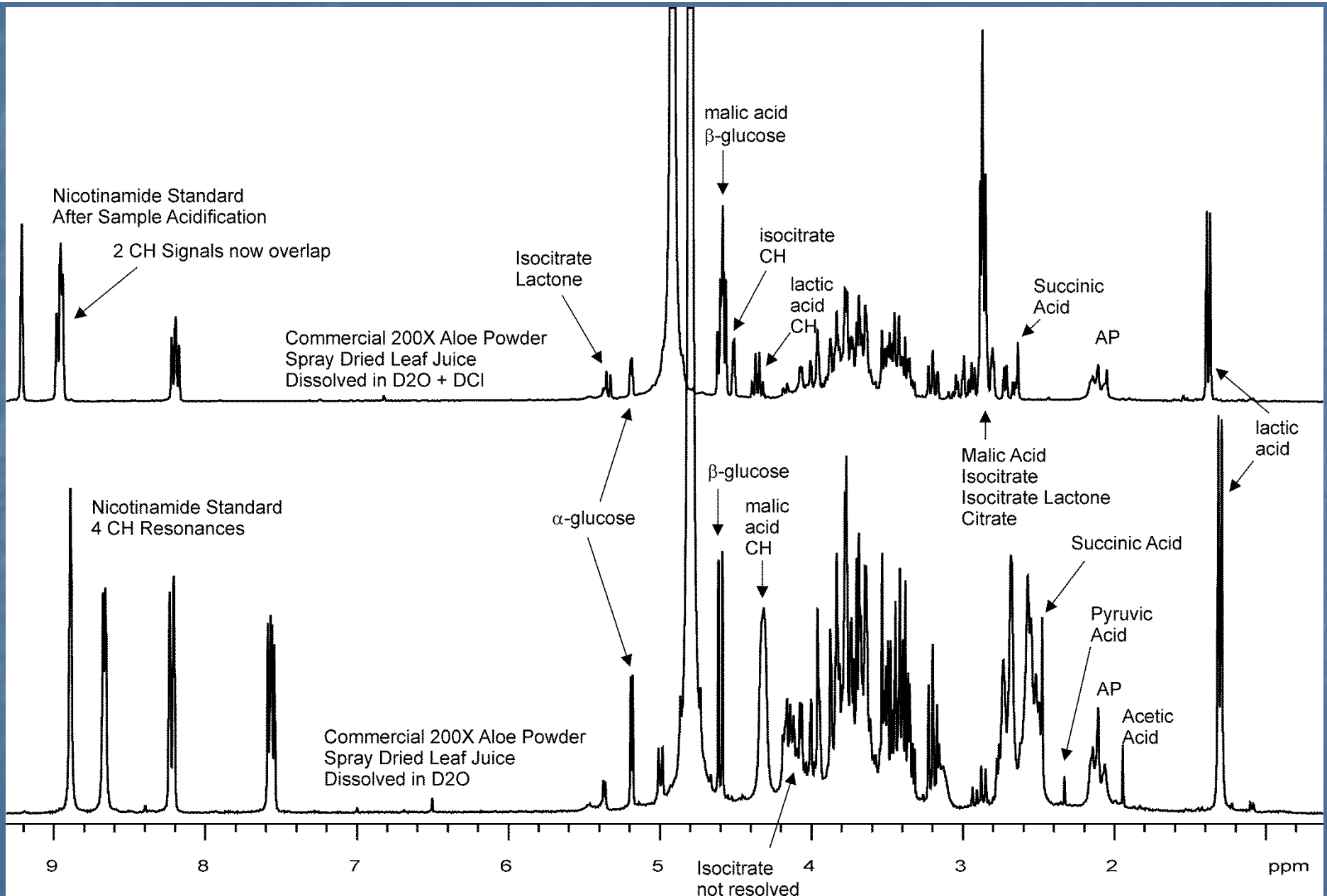
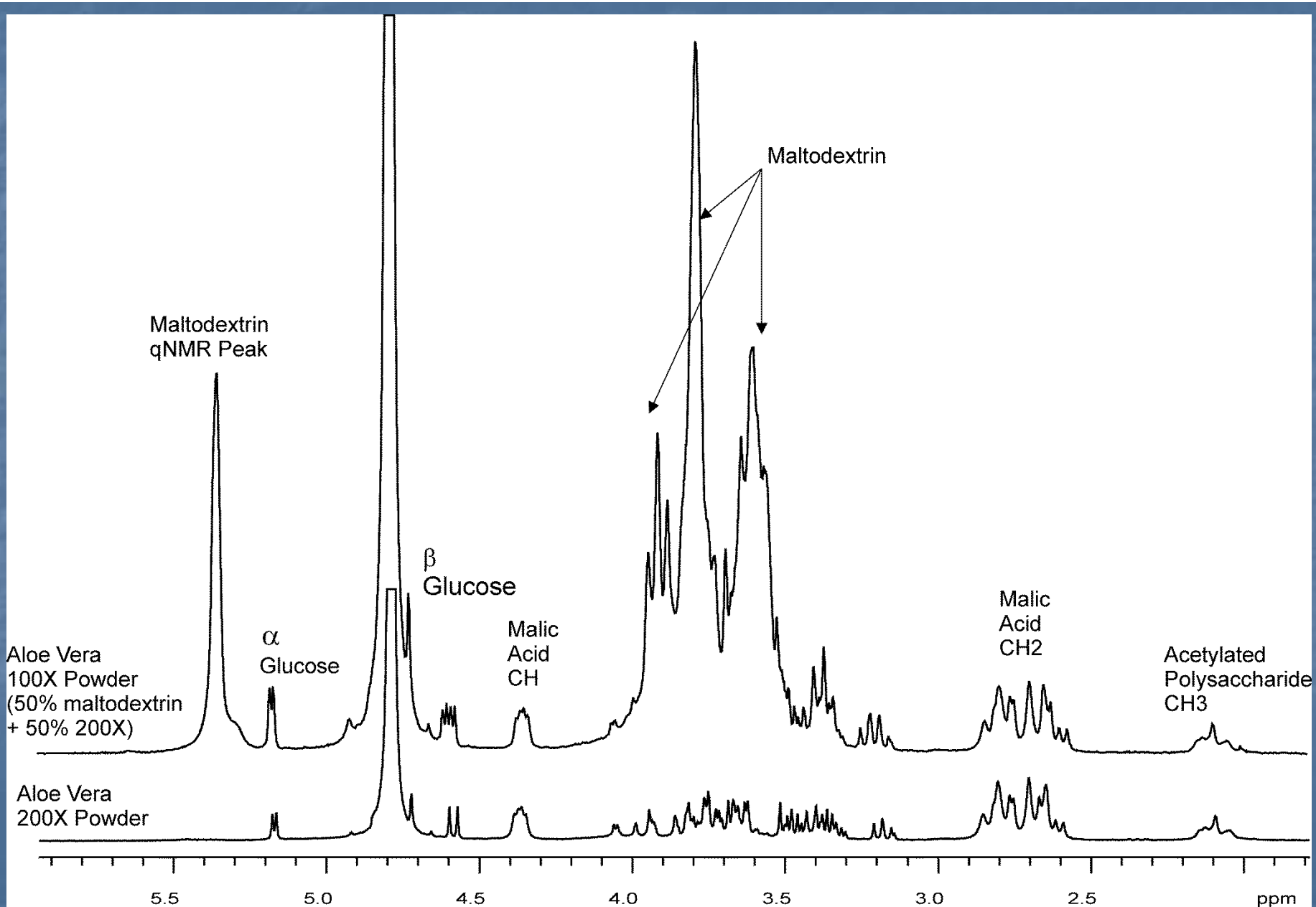


Commercial 200x inner leaf juice powder
Commercial 100x inner leaf juice powder containing 50% maltodextrin



Aloe Vera Leaf Juice Freeze Dried and Mixed 80% with Maltodextrin

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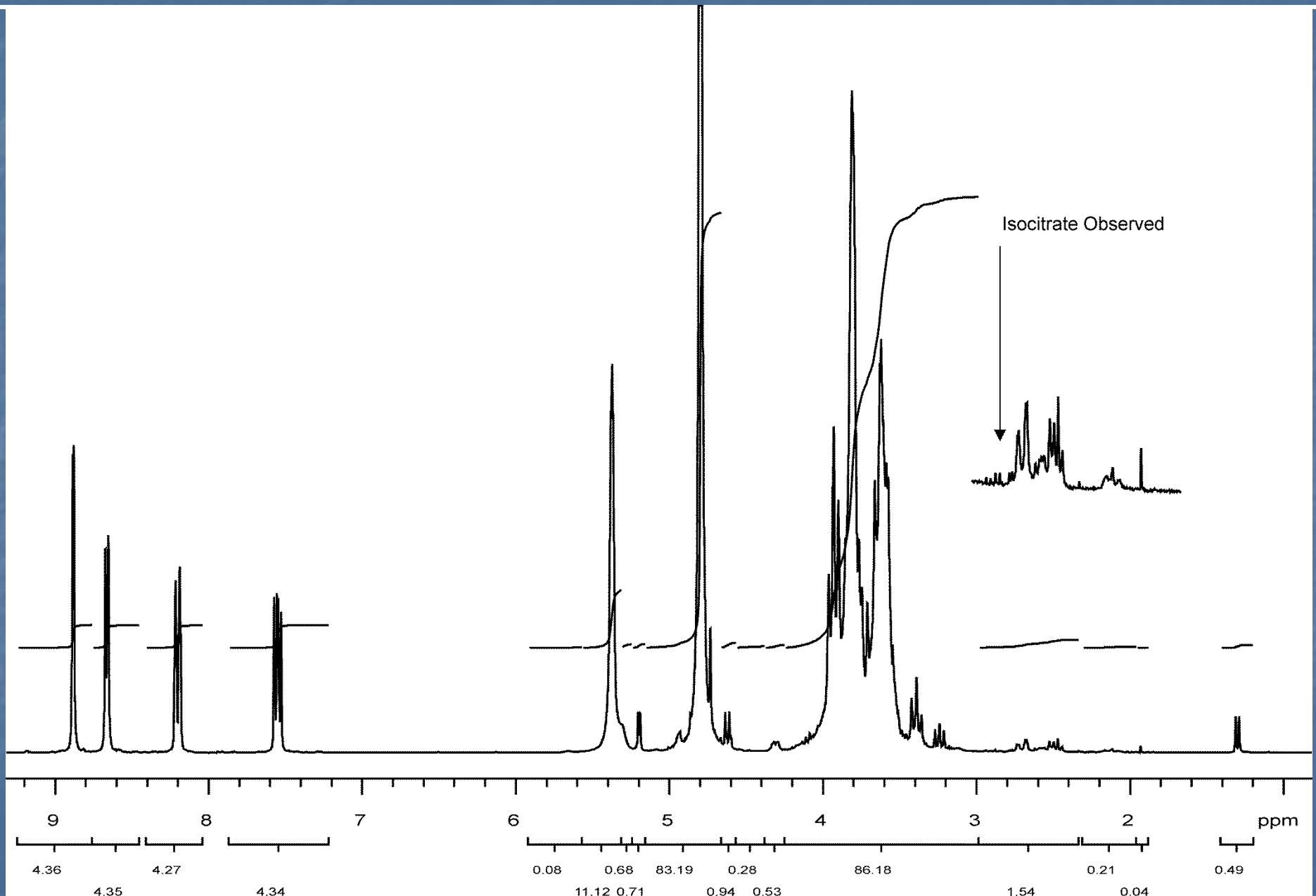


Acetylated Polysaccharide Quantitation

Demonstrated that the acetylation of the mannose monomer units is at 78% (Ref. 2, Manna et al., 1993).

Mannose represents 84% of the polysaccharide backbone with the remainder being composed of glucose, galactose, and a few other saccharides (Ref 3., Chow et al., 2005).

The acetylation content and the presence of 16% other saccharides must be taken into account so as not to underestimate the AP content.

The molecular weight of AP is calculated under the assumption that 1 water molecule is removed upon condensation of acetyl with mannosyl monomer, and each mannosyl unit shares in the loss of a single water molecule upon condensation form the predominantly mannose based polysaccharide.

Thus, $MW_{mannosyl} = Mw_{mannose} - (MW_{water})/2 = 180.2 - 18 = 162.2$ g/mol

$Mw_{AcMann} = Mw_{acetyl\ group} + MW_{mannosyl} - MW_{water} = 60 + 162.2 - 18 = 204.2$ g/mol

Now taking into account the 0.78/1 acetyl/mannosyl residue ratio as well as the presence of ~16% non-mannosysl saccharides in the AP we can calculate the concentration of AP (C_{AP}) by the following equation.

$$C_{AP} = \left(\frac{W_{Nic} * I_{AcMann} * N_{Nic} * MW_{AcMann}}{I_{Nic} * N_{AcMann} * MW_{Nic}} + \frac{W_{Nic} * I_{AcMann} * N_{Nic} * MW_{Mann} * (0.22/0.78)}{I_{Nic} * N_{AcMann} * MW_{Nic}} + \frac{W_{Nic} * I_{AcMann} * N_{Nic} * MW_{Glu} * (0.16/0.84)}{I_{Nic} * N_{AcMann} * MW_{Nic}}\right) * \frac{1}{W_{sample}} * 100\%$$

$$= \frac{W_{Nic} * I_{AcMann}}{I_{Nic}} * \frac{N_{Nic}}{N_{AcMann} * MW_{Nic}} * (MW_{AcMann} + MW_{Mann} * 0.28 + MW_{Glu} * 0.19) * \frac{1}{W_{sample}} * 100\%$$

$$= \frac{W_{Nic} * I_{AcMann}}{I_{Nic} * W_{sample}} * \frac{4}{3 * 122.1} * (204.2 + (162.2 * 0.47)) * 100\%$$

$$= \frac{W_{Nic} * I_{AcMann}}{I_{Nic} * W_{sample}} * 3.06 * 100\%$$

AcMann = acetylmannosyl, *Mann*=Mannosyl, *Glu*=glucosyl

N = number of protons in the group - molar conversion factor - $N_{Nic}=4$, $N_{AP}=3$

C_{AP} = content of acetylated polysaccharides (AP) in the sample, wt%

W_{Nic} = weight of added internal standard (mg), W_{Sample} = weight of sample (mg)

I_{AcMann} = integration area of acetylation methyls (multiple peaks in 2.0-2.3 ppm region)

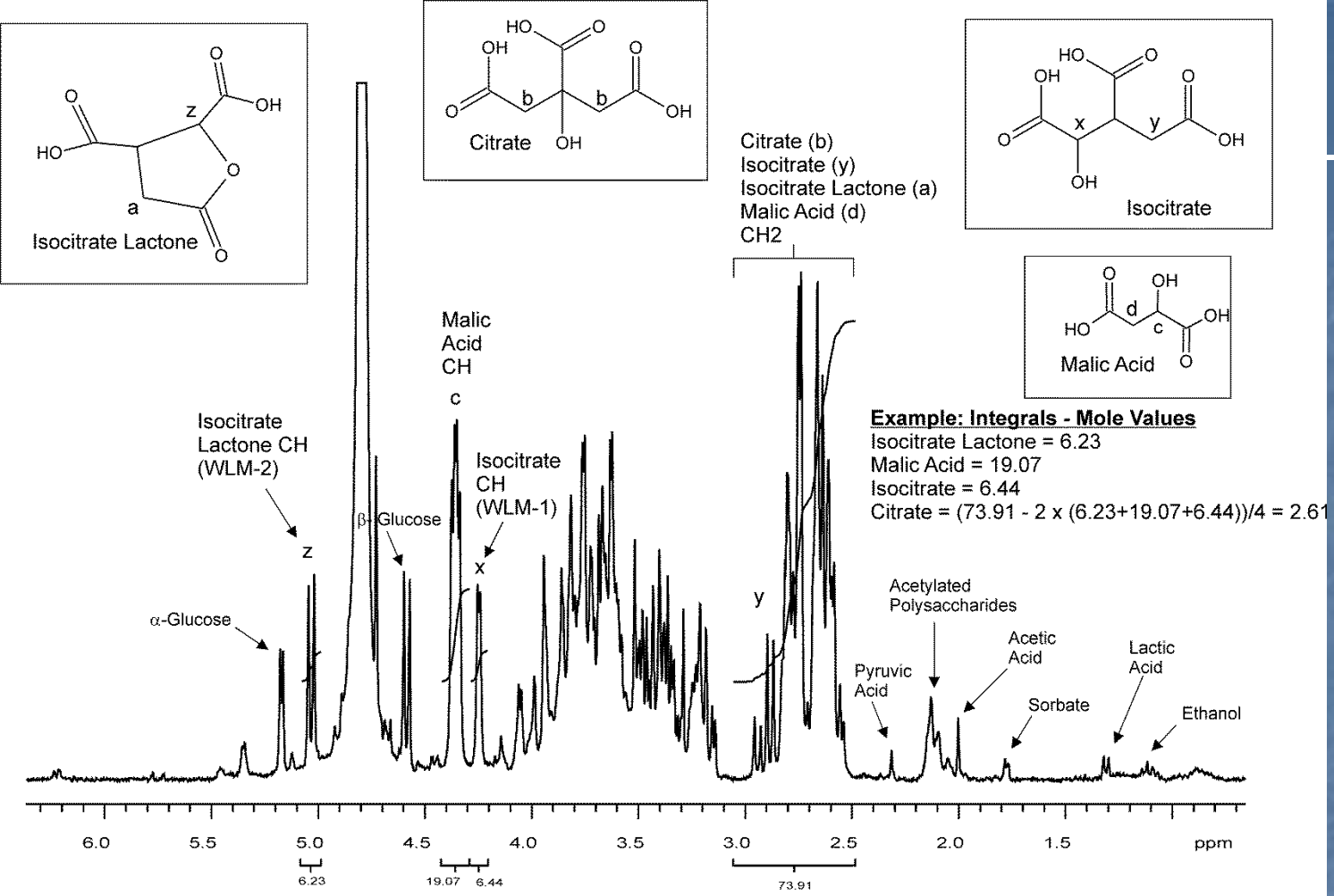
I_{Nic} = sum of integration areas of the 4 aromatic CH peaks of the nicotinamide standard

MW_{AcMann} = Molecular Weight of acetylated polysaccharides (204.2 g/mol)

MW_{Nic} = molecular weight of nicotinamide standard (122.1 g/mol)

$MW_{Mann}=MW_{Glu}=162.2$

Isocitrate Quantitation



Example: Integrals - Mole Values

Isocitrate Lactone = 6.23
Malic Acid = 19.07
Isocitrate = 6.44
Citrate = (73.91 - 2 x (6.23+19.07+6.44))/4 = 2.61

1H NMR of Freeze Dried Aloe Vera Leaf Juice: Assignments and Explantion of quantification of Isocitrate (WLM-1), Isocitrate Lactone (WLM-2), Citrate, and Malic Acid.
Note: peaks marked z, x, and y do not appear in aloe vera inner leaf materials.

C_{ICA} (wt%) = 100% * (W_{Nic} * I_{ICA} * N_{Nic} * MW_{ICA}) / (I_{Nic} * N_{ICA} * MW_{Nic} * W_{Sample})
= 100% * (W_{Nic} * I_{ICA} * 4 * 192.1) / (I_{Nic} * 1 * 122.1 * W_{Sample}) = 100% * (W_{Nic} *I_{ICA}/I_{Nic} *W_{Sample}) * 6.29

C_{ICA} = content of isocitrate in the sample, weight%
W_{Nic} = weight of added nicotinamide internal standard (mg), W_{Sample} = weight of sample (mg)
I_{ICA} = integration area of CH proton peak of isocitrate (doublet at 4.45 ppm (dissolved in D_2O and acidified with DCl)
I_{Nic} = sum of integration areas of the 4 aromatic CH peaks of the nicotinamide standard
MW_{ICA} = Molecular Weight of Isocitrate (192.1 g/mol), MW_{Nic} = molecular weight of nicotinamide standard (122.1 g/mol)
N = number of protons in the group - molar conversion factor, N_{Nic}=4, N_{ICA}=1

Quantitative Proton Nuclear Magnetic Resonance Spectrometry (^{1}H NMR) for Determination of Acetylated Polysaccharides, Glucose, Maltodextrin, and Isocitrate in Aloe Vera Leaf Juice

Included in the American Herbal Pharmacopoeia Monograph on Aloe Vera Leaf, Leaf Juice & Inner Leaf Juice