PROCESS NMR ASSOCIATES

Quantitative Small Mixture Analysis (SMA): Quality of Aloe Vera, Acid Profiles of Sour Beers, PDE5i Adulteration of Male Enhancement Formulations

Presented By

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Process NMR Associates, LLC
Danbury, Connecticut

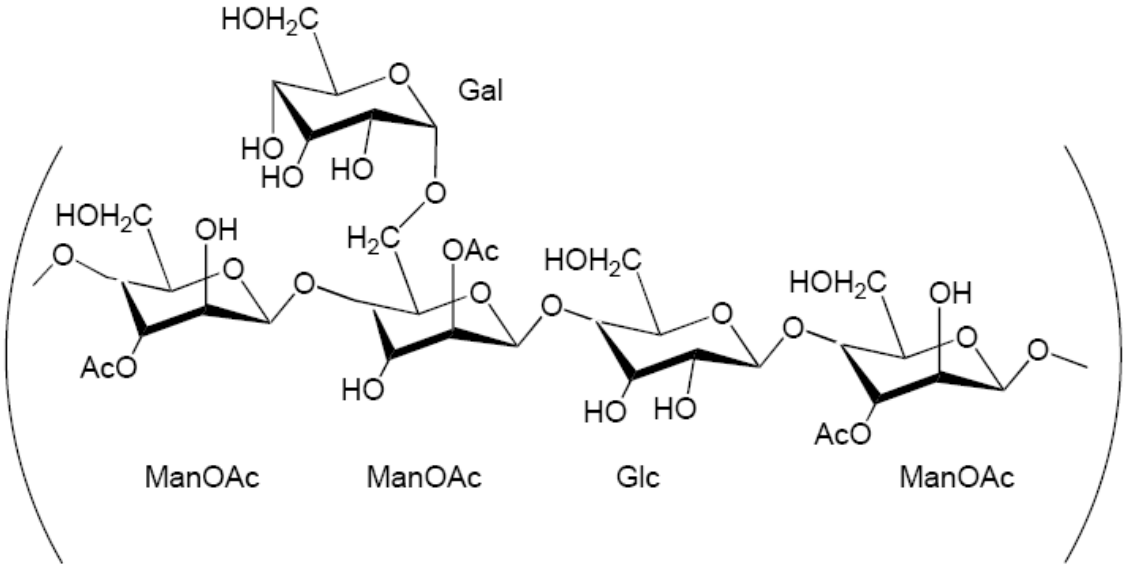
March 23, 2014
Mnova UM at 55th ENC, Boston MA

PROCESS NMR ASSOCIATES

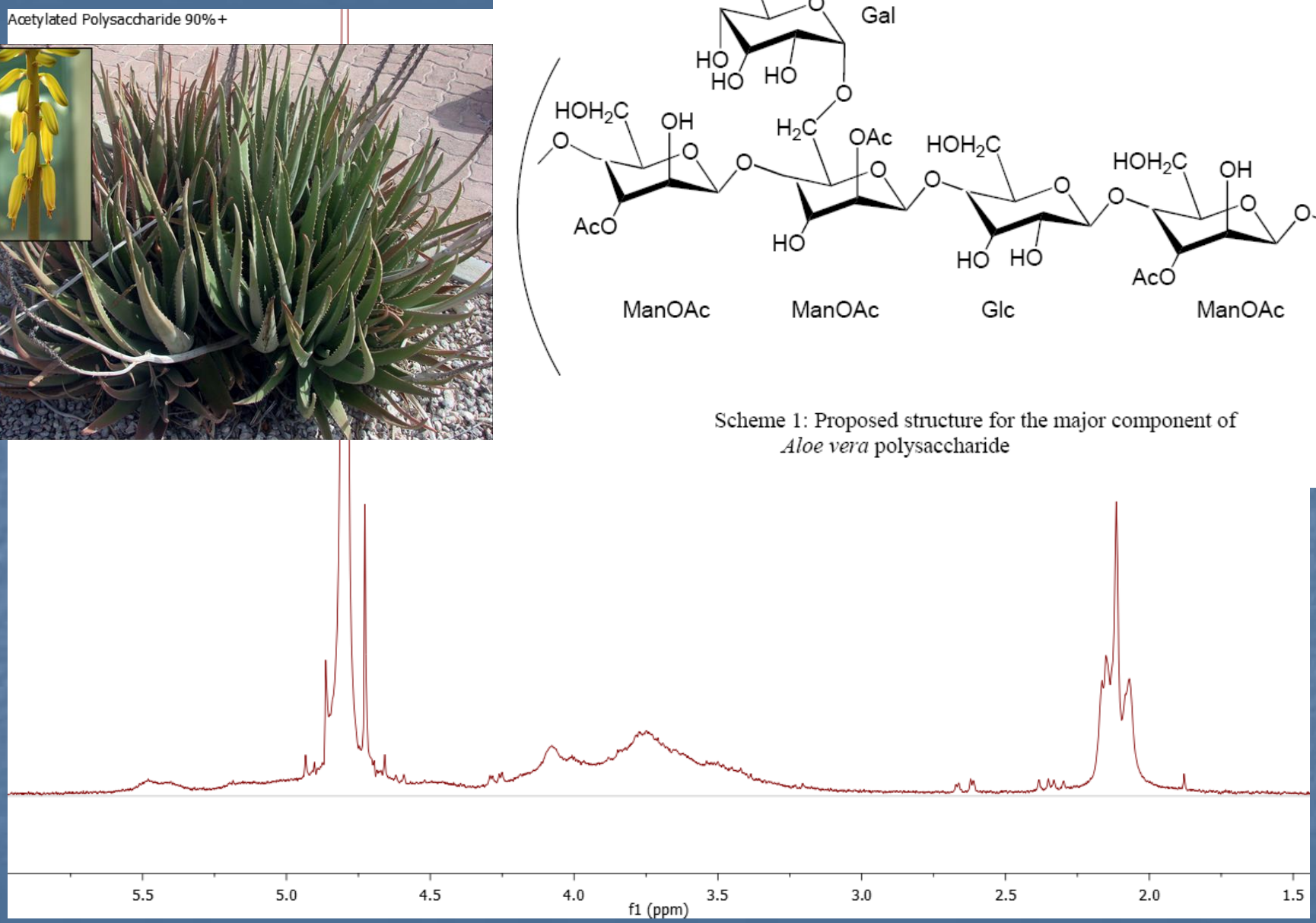
Contract NMR Analysis

NMR Application Development

Aloe Vera

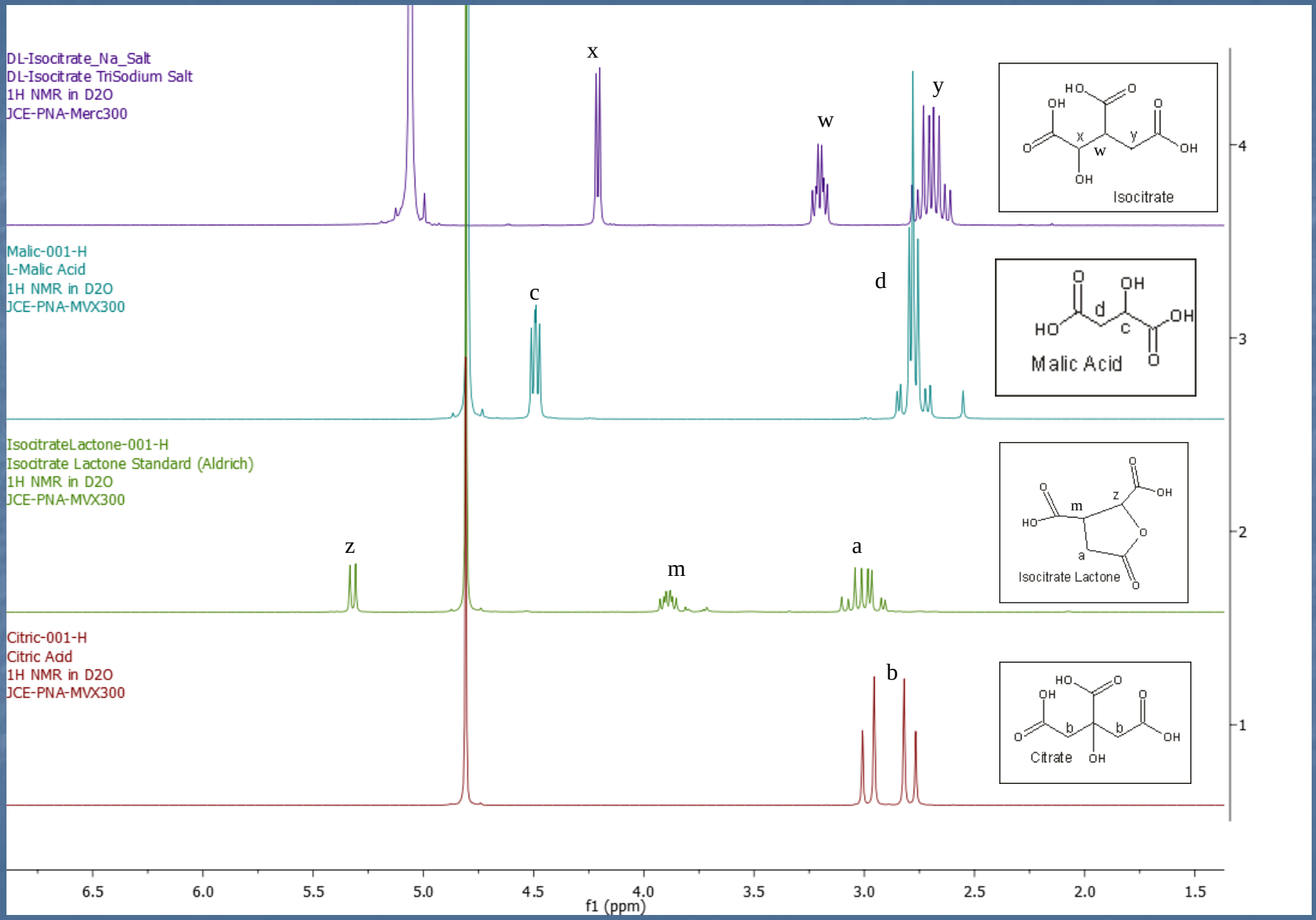


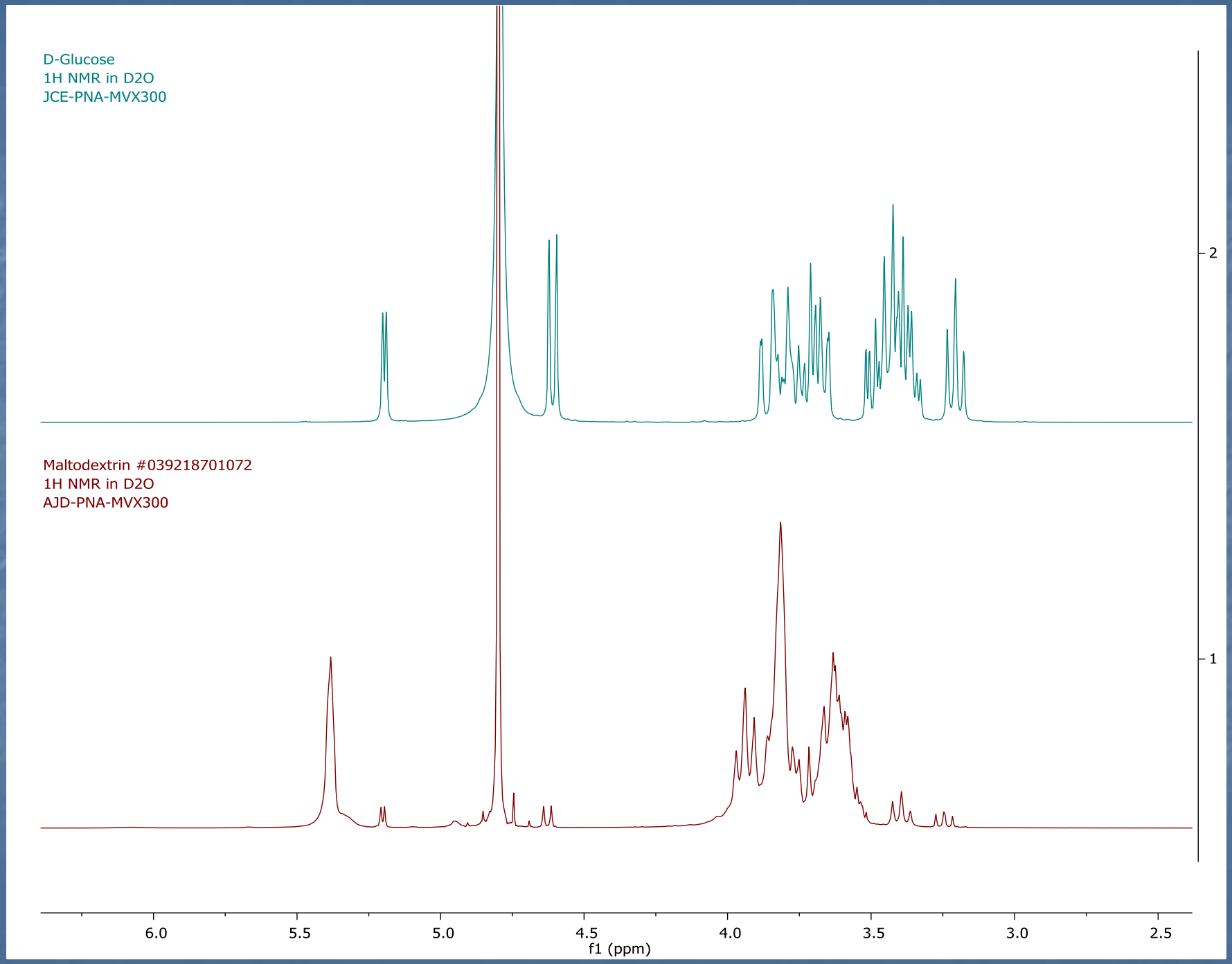
Scheme 1: Proposed structure for the major component of *Aloe vera* polysaccharide

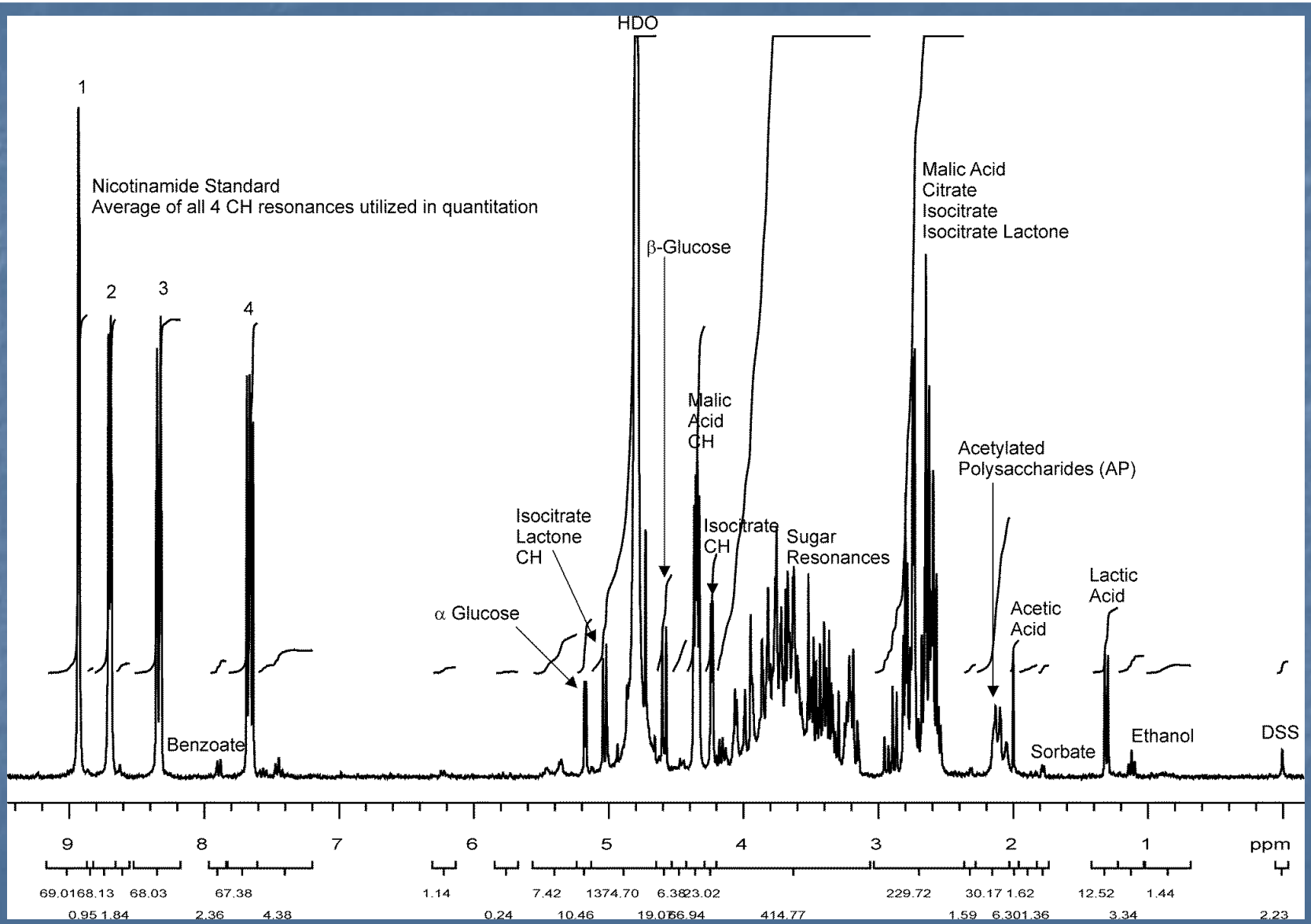


Chemical Characterization of the Immunomodulating Polysaccharide of *Aloe Vera L., W.J.Goux, et al,*

http://www.iasc.org/AloeStructure080604.pdf


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


D-Glucose
1H NMR in D2O
JCE-PNA-MVX300
Maltodextrin #039218701072
1H NMR in D2O
AJD-PNA-MVX300
2
1
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f1 (ppm)



Commercial freeze-dried 200x aloe vera leaf juice powder

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%

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

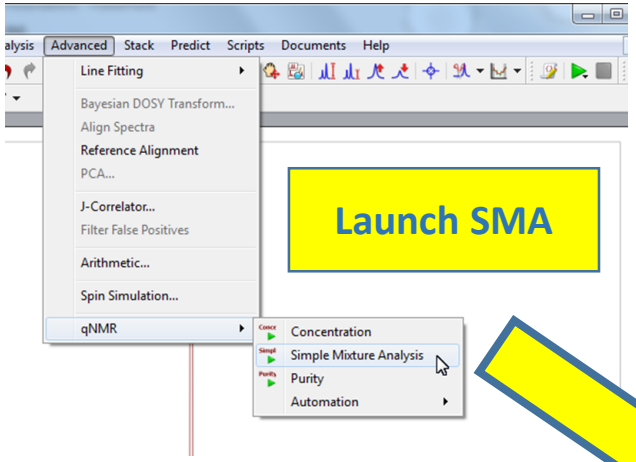
Characteristic chemical shift values, peak multiplicity, protonated carbon type and N values used for detection and quantitation of the major natural components of aloe vera leaf juice

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

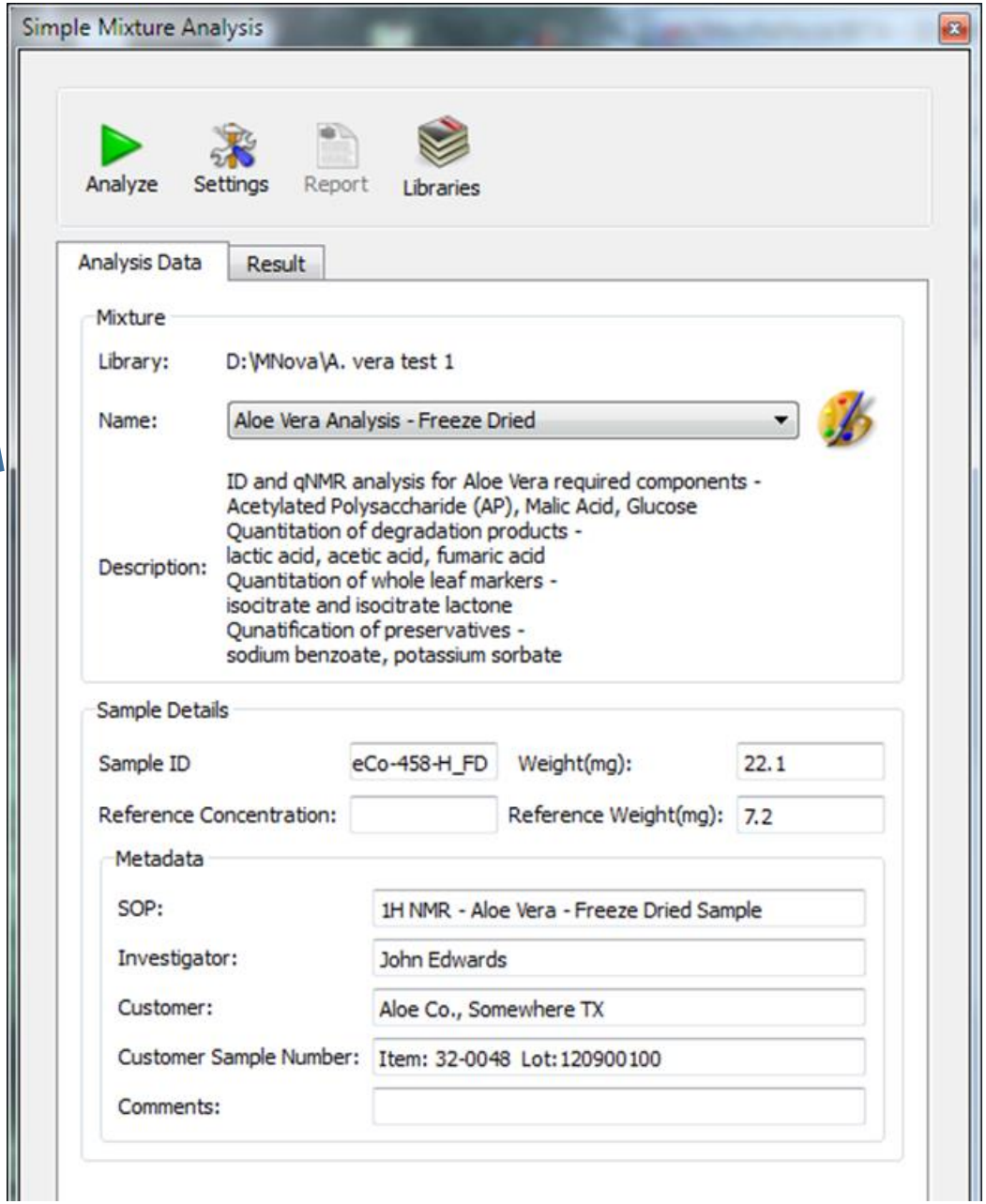
Chemical shift values, peak and chemistry descriptions, molar conversion factors that can be used for detection and quantitation of aloe vera leaf juice preservatives, additives, and degradation products

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

Analysis of Aloe Sample 458 – Whole Leaf Aloe Powder

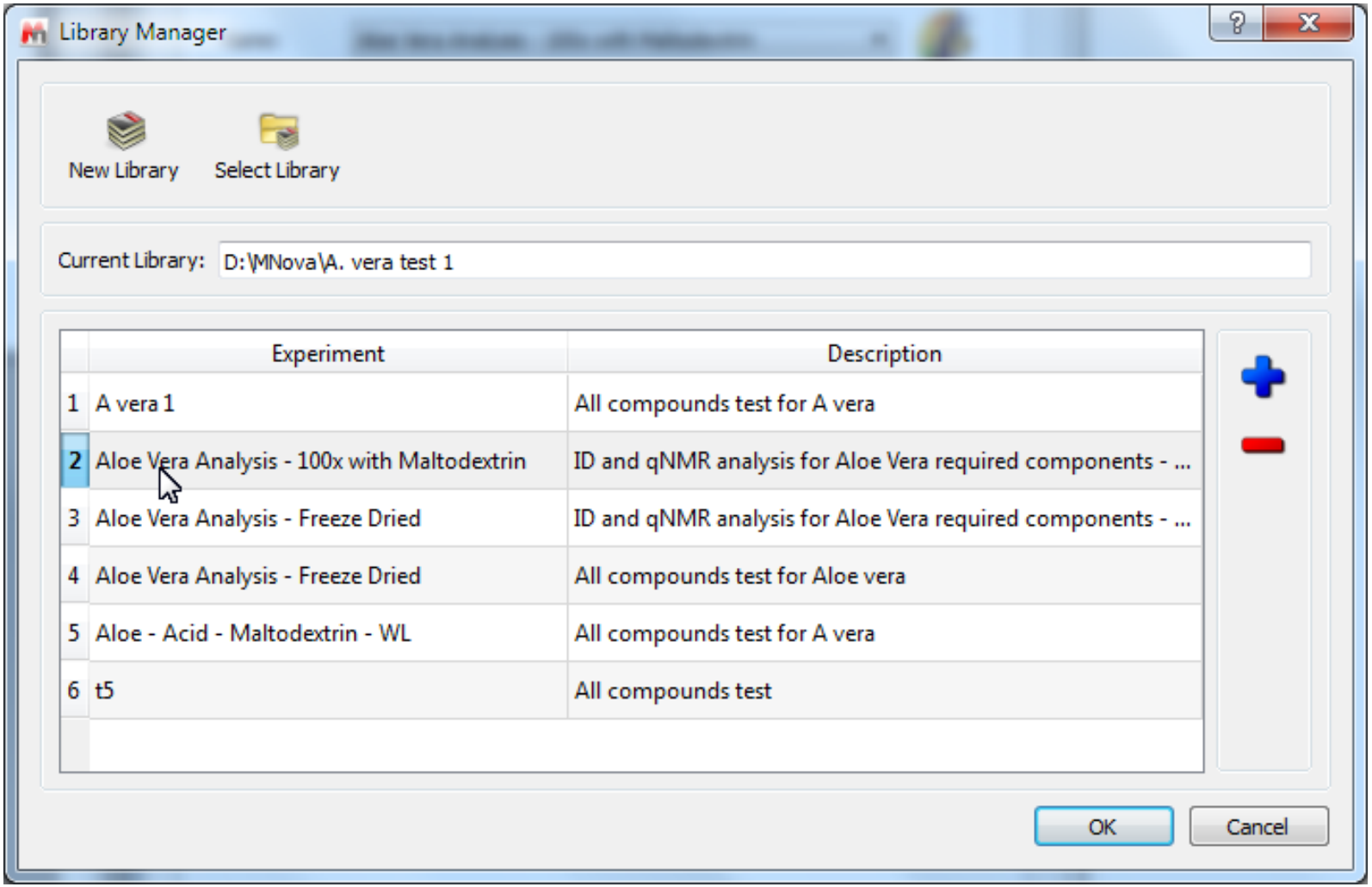


Launch SMA



Starting Screen

- ✓ **Add sample weight**
- ✓ **Add reference weights**
- ✓ **Add Metadata**
- ✓ **Choose Previously SavedLibrary and Experiments**


Library Manager
New Library
Select Library
Current Library: D:\MNova\A. vera test 1
Experiment
Description
1 A vera 1
All compounds test for A vera
2 Aloe Vera Analysis - 100x with Maltodextrin
ID and qNMR analysis for Aloe Vera required components - ...
3 Aloe Vera Analysis - Freeze Dried
ID and qNMR analysis for Aloe Vera required components - ...
4 Aloe Vera Analysis - Freeze Dried
All compounds test for Aloe vera
5 Aloe - Acid - Maltodextrin - WL
All compounds test for A vera
6 t5
All compounds test
OK
Cancel

Experiment Setup

Add Component Information Name, Molecular Wt, # protons, Shift Ranges of Component Peaks

Acceptable results can be specified, and outliers will be flagged

Setup Calculation Formulas

Experiment

Experiment

Name: Aloe Vera Analysis - Freeze Dried

Description:
ID and qNMR analysis for Aloe Vera required components -
Acetylated Polysaccharide (AP), Malic Acid, Glucose
Quantitation of degradation products -
lactic acid, acetic acid, fumaric acid
Quantitation of whole leaf markers -
isocitrate and isocitrate lactone
Qunatification of preservatives -
sodium benzoate, potassium sorbate

Units: wt%

Compound	Type	Mol. Weight	Color	Spectrum
acetic acid	Compound	60.05	Red	1
beta-glucose	Compound	180.16	Green	1
Isocitrate	Compound	192.12	Orange	1
isocitrate lactone	Compound	174.11	Orange	1

Compound Editor

Name: Isocitrate

Color: Orange Spectrum Nº: 1 Molecular Weight: 192.12

Type: Compound Acceptable Limit From: To:

Formula

100*(RW*I1*NNR1*MW)/(IR1*NN1*MWR*SW)

Formula Editor

Ranges

	From	To	NN
1	4.21	4.27	1

OK Cancel

Formula Setup

Enter qNMR calculations

Formula Editor

```
100*(RW*I1*NNR1*MW)/(IR1*NN1*MWR*SW)
```

Check Formula

LEGEND:

CONCENTRATIONS OF OTHER COMPOUNDS:
C1: Concentration of: isocitric lactone
C2: Concentration of: AP
C3: Concentration of: Ethanol
CCF : Concentration Factor of Reference
C5: Concentration of: glucose
C6: Concentration of: alpha-glucose
C7: Concentration of: malic acid
C8: Concentration of: acetic acid
C9: Concentration of: beta-glucose
C10: Concentration of: Isocitrate

INTEGRALS:
IR1: Reference Integral of range [7.54 , 7.68]
I1: Integral of range [1.34 , 1.27]

NUMBER OF NUCLIDES:
NNR1: Reference Nuclides of range [7.54 , 7.68]
NN1: Nuclides of range [1.34 , 1.27]

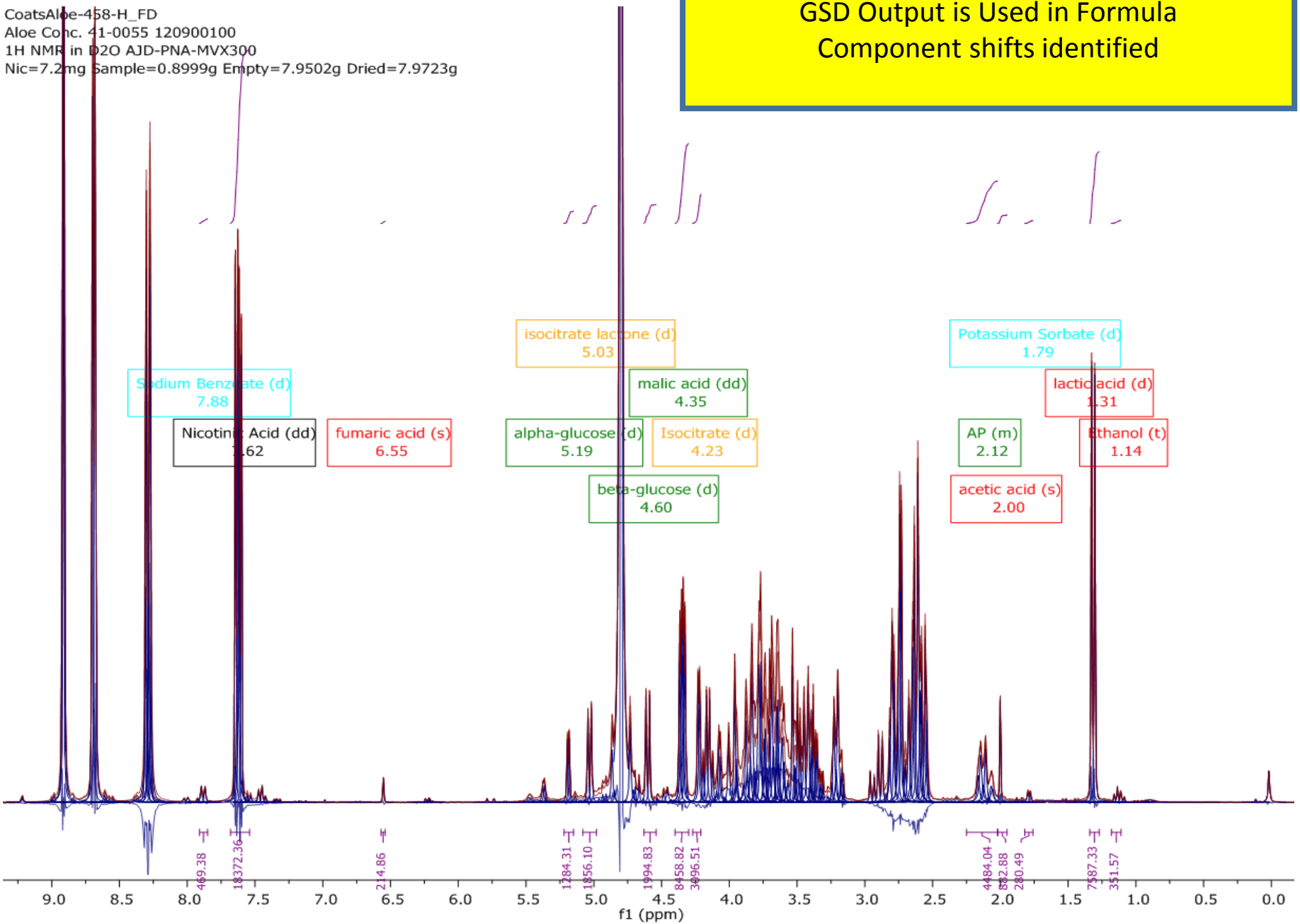
OTHERS:
MW: Molecular Weight
SW: Sample Weight
RW: Reference Weight
RC: Reference Concentration
MWR : Molecular Weight of Reference

OK Cancel

Note: The Experimental and Formula Setup are performed only once and are then saved in a library for further use

Spectral Result - Sample 458

GSD Output is Used in Formula
Component shifts identified



Opera | Google | SNA | Local | localhost/D:/Sparc5/MestreLab | Search with (

Simple Mixtures Analysis

Compound	Result
AP	6.08 wt%
Ethanol	0.08 wt%
alpha-glucose	3.36 wt%
lactic acid	3.31 wt%
malic acid	16.49 wt%
acetic acid	0.26 wt%
beta-glucose	5.22 wt%
Isocitrate	8.65 wt%
isocitrate lactone	4.70 wt%
fumaric acid	0.18 wt%
Sodium Benzoate	0.49 wt%
Potassium Sorbate	0.20 wt%

Sample Details:

Sample ID: AloeCo-458-H_FD
Sample Weight: 22.1
Reference Weight: 7.2
SOP: 1H NMR - Aloe Vera - Freeze Dried Sample
Investigator: John Edwards
Customer: Aloe Co., Somewhere TX
Customer Sample Number: Item: 32-0048 Lot:120900100

XHTML Results Output

AloeCo-458 - Aloe Vera Whole Leaf Product - Freeze Dried Powder

Component	MNova Mixture Analysis	Manual Integration
AP - Acetylated Polysaccharide	6.08	6.47
Ethanol	0.08	0.08
glucose	8.58	8.72
lactic acid	3.31	3.6
malic acid	16.49	16.52
acetic acid	0.26	0.32
Isocitrate	8.65	8.33
isocitrate lactone	4.70	4.91
fumaric acid	0.18	0.14
Sodium Benzoate	0.49	0.52
Potassium Sorbate	0.20	0.25

Comparison of ^{1}H qNMR calculations of component wt% obtained from :

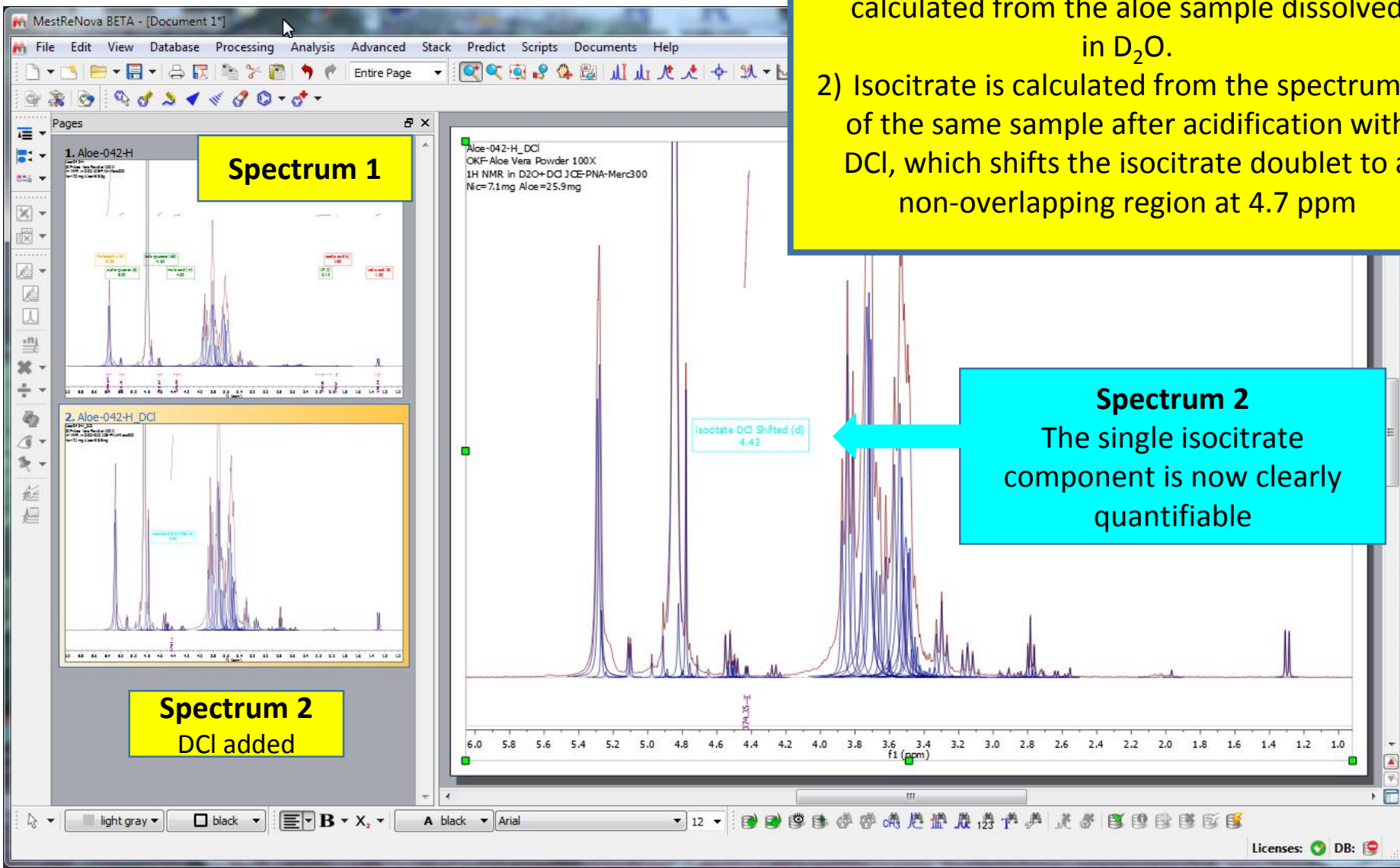
1) Mnova SMA

2) Manual Integration + Excel Method (validated)

Aloe Sample 42 :
Whole Leaf Powder with Maltodextrin Adulterant
Two Experiments Analyzed Simultaneously

For Aloe sample 42 the full analysis required 2 related samples, but the final analysis was achieved in a single step.

1) All parameters except isocitrate were calculated from the aloe sample dissolved in D_2O.
2) Isocitrate is calculated from the spectrum of the same sample after acidification with DCl, which shifts the isocitrate doublet to a non-overlapping region at 4.7 ppm



Experimental Setup

Note reference to Spectrum 1 andSpectrum 2 in the component listing. This allows the majority of the component concentrations to be calculated from Spectrum 1 and the isocitrate concentration from Spectrum 2 which is of the acidified aloe sample.

Simple Mixture Analysis

Analyze | Settings | Report | Libraries

Analysis Data | Result

Mixture

Library: D:\Mnova\A. vera test 1

Name: Aloe Vera Analysis - 100x with Maltodextrin

Description: ID and qNMR analysis for Aloe Vera required components - Acetylated Polysaccharide (AP), Malic Acid, Glucose Quantitation of degradation products - lactic acid, acetic acid, fumaric acid Quantitation of whole leaf markers - isocitrate Quantitattion of Adulterant - Maltodextrin

Sample Details

Sample ID: AloeCo-042 | Weight(mg): 39.3

Reference Concentration: | Reference Weight(mg): 7

Metadata

SOP: 1H NMR - Aloe +Maltodextrin - Powder

Investigator: John Edwards

Customer: Aloe Co - Somewhere TX

Customer Sample Number: OKF - 100x

Comments:

Experiment

Experiment

Name: Aloe Vera Analysis - 100x with Maltodextrin

Description: ID and qNMR analysis for Aloe Vera required components Acetylated Polysaccharide (AP), Malic Acid, Glucose Quantitation of degradation products - lactic acid, acetic acid, fumaric acid Quantitation of whole leaf markers - isocitrate Quantitattion of Adulterant - Maltodextrin

Units: wt%

Compound	Type	Mol. Weight	Color	Spectrum
acetic acid	Compound	60.05	Red	1
beta-glucose	Compound	180.16	Green	1
isocitate DCI Shift...	Compound	192.12	Cyan	2
Maltodextrin	Compound	180	Orange	1

Compound Editor

Name:

Color: Red | Spectrum Nº: 1 | Molecular Weight:

Type: Ref | Acceptable Limit From: | To:

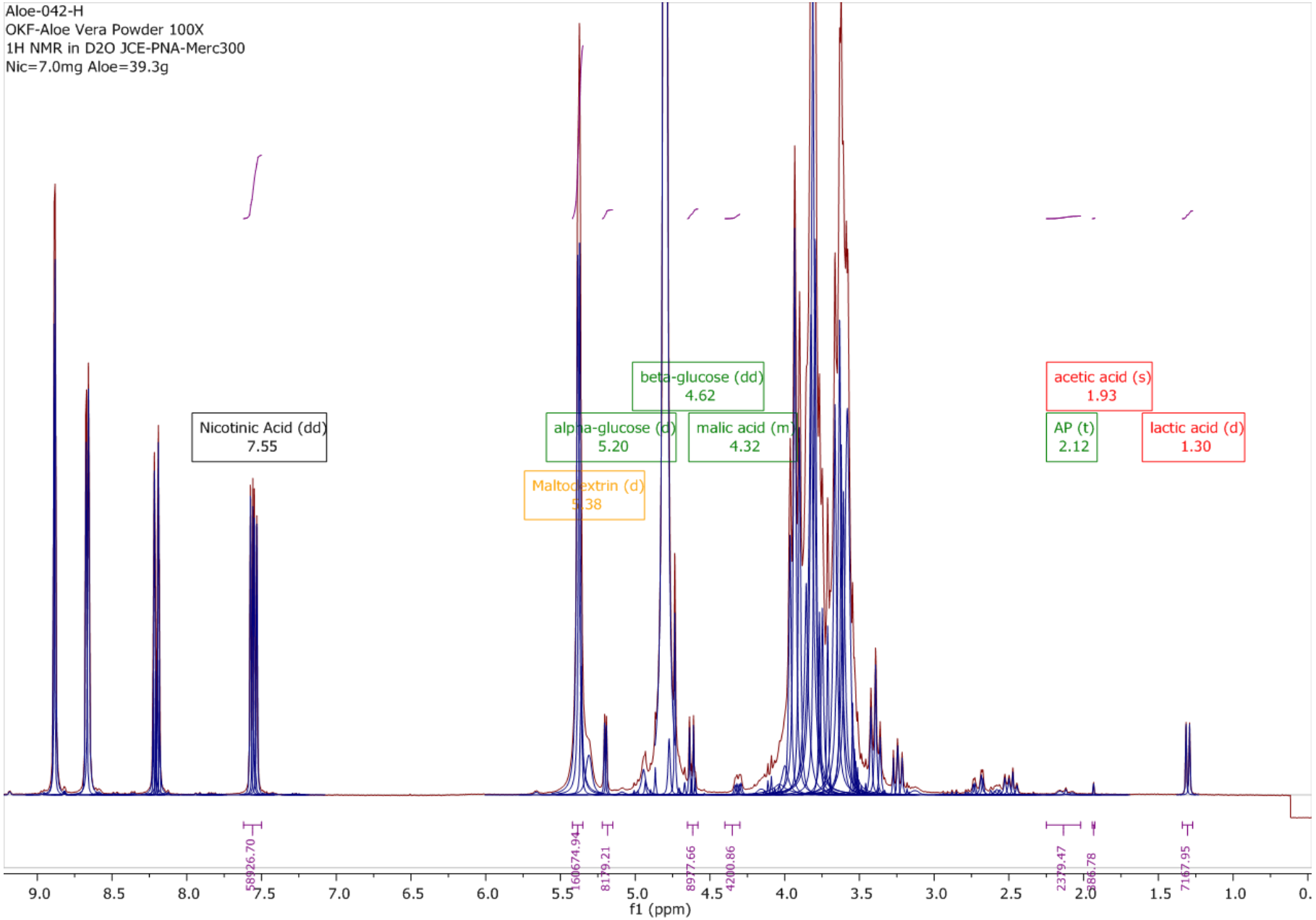
Formula

Formula Editor

Ranges

	From	To	NN
1			

OK | Cancel


Aloe-042-H
OKF-Aloe Vera Powder 100X
1H NMR in D2O JCE-PNA-Merc300
Nic=7.0mg Aloe=39.3g
Nicotinic Acid (dd)
7.55
alpha-glucose (d)
5.20
Maltodextrin (d)
5.38
beta-glucose (dd)
4.62
malic acid (m)
4.32
acetic acid (s)
1.93
AP (t)
2.12
lactic acid (d)
1.30
58926.70
160674.94
8179.21
8977.66
4200.86
2379.47
886.78
7167.95
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.
f1 (ppm)

Opera

SNA

Local localhost/D:/Spa Search

Simple Mixtures Analysis

Compound	Result
AP	0.55 wt%
Ethanol	0.00 wt%
alpha-glucose	3.65 wt%
lactic acid	0.53 wt%
malic acid	1.40 wt%
acetic acid	0.02 wt%
beta-glucose	4.01 wt%
isocitate DCl Shifted	0.19 wt%
Maltodextrin	71.66 wt%

Sample Details:

Sample ID: AloeCo-042
Sample Weight: 39.3
Reference Weight: 7
SOP: 1H NMR - Aloe+Maltodextrin - Powder
Investigator: John Edwards
Customer: Aloe Co - Somewhere TX
Customer Sample Number: OKF - 100x

AloeCo-42 - Aloe Vera Whole Leaf Powder - Mixed with Maltodextrin

Component	MNova Mixture Analysis	Manual Integration
AP - Acetylated Polysaccharide	0.55	0.47
Ethanol	0.00	0.00
glucose	7.66	8.08
lactic acid	0.53	0.50
malic acid	1.40	1.42
acetic acid	0.02	0.02
isocitate DCl Shifted	0.19	0.30
Maltodextrin	71.66	72.37

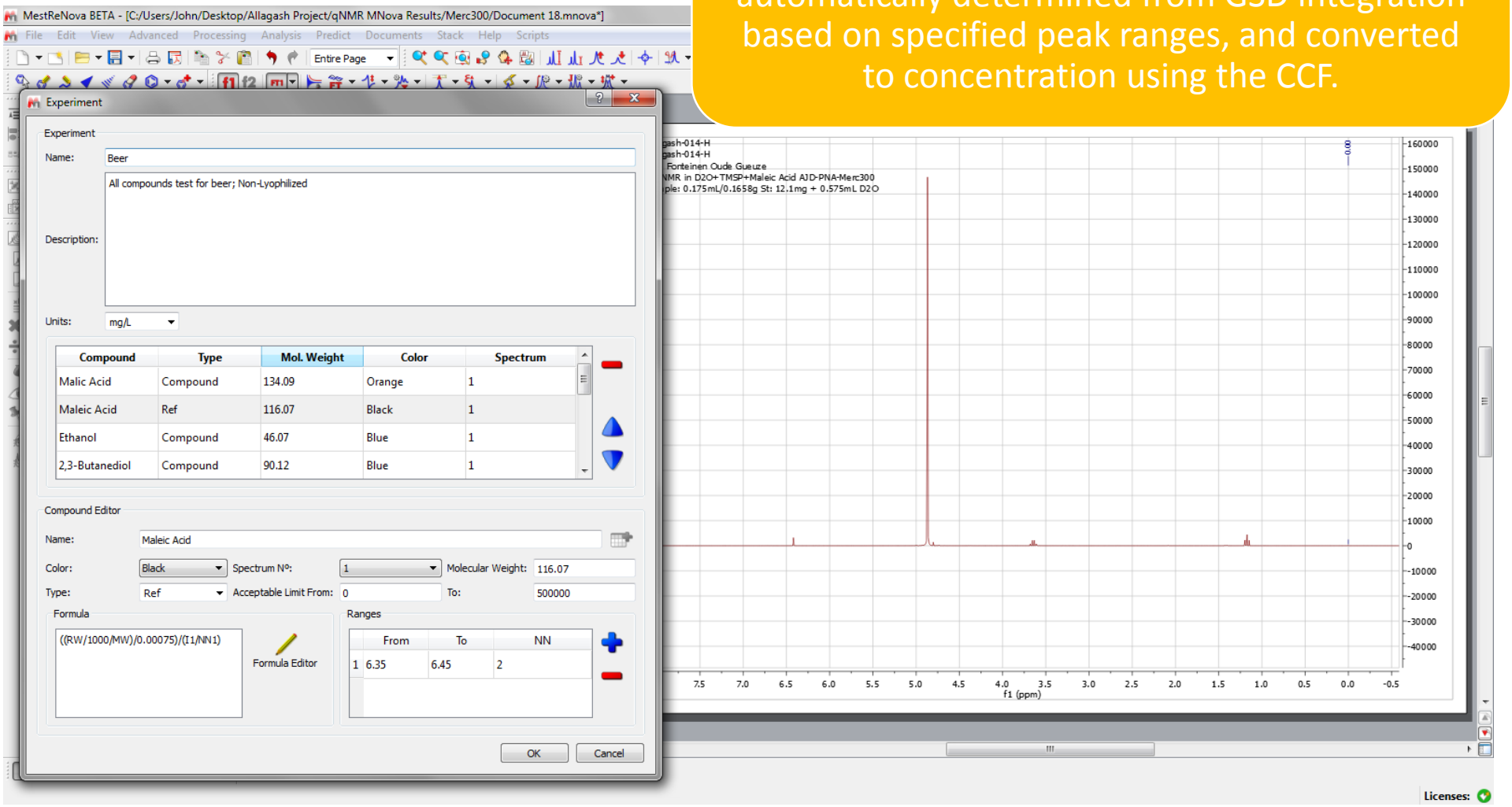
Validation

Comparison of qNMR calculations of component wt% obtained from

1) Automated Mixture Analysis
2) Manual Integration + Excel Method

qNMR standard Parameters: concentration reference mass (RW, in grams), sample volume (in L), Molecular Weight (MW), and Hydrogen # (NN1) are entered to determine the *Concentration Conversion Factor* (CCF).

For each component, Integral (I#) is automatically determined from GSD integration based on specified peak ranges, and converted to concentration using the CCF.



Concentrations of target metabolites are calculated as a function of CCF, NN1, MW & I#. The *terminal coefficient* (4285.71) is a correction factor for sample dilution and conversion to mg/L.

MestReNova BETA - [C:/Users/John/Desktop/Allaga

File Edit View Advanced Processing Analysis Predict Documents Stack Help Scripts

Entire Page

Experiment

Experiment

Name: Beer - Non-lyophilized

Description: All compounds test for beer; Non-Lyophilized

Units: mg/L

Compound	Type	Mol. Weight	Color	Spectrum
Citric Acid	Compound	192.12	Orange	1
Lactic Acid	Compound	90.08	Orange	1
Succinic Acid	Compound	118.09	Orange	1
Ethyl Acetate	Compound	88.11	Blue	1

Compound Editor

Name: Lactic Acid

Color: Orange Spectrum Nº: 1 Molecular Weight: 90.08

Type: Compound Acceptable Limit From: 0 To: 500000

Formula

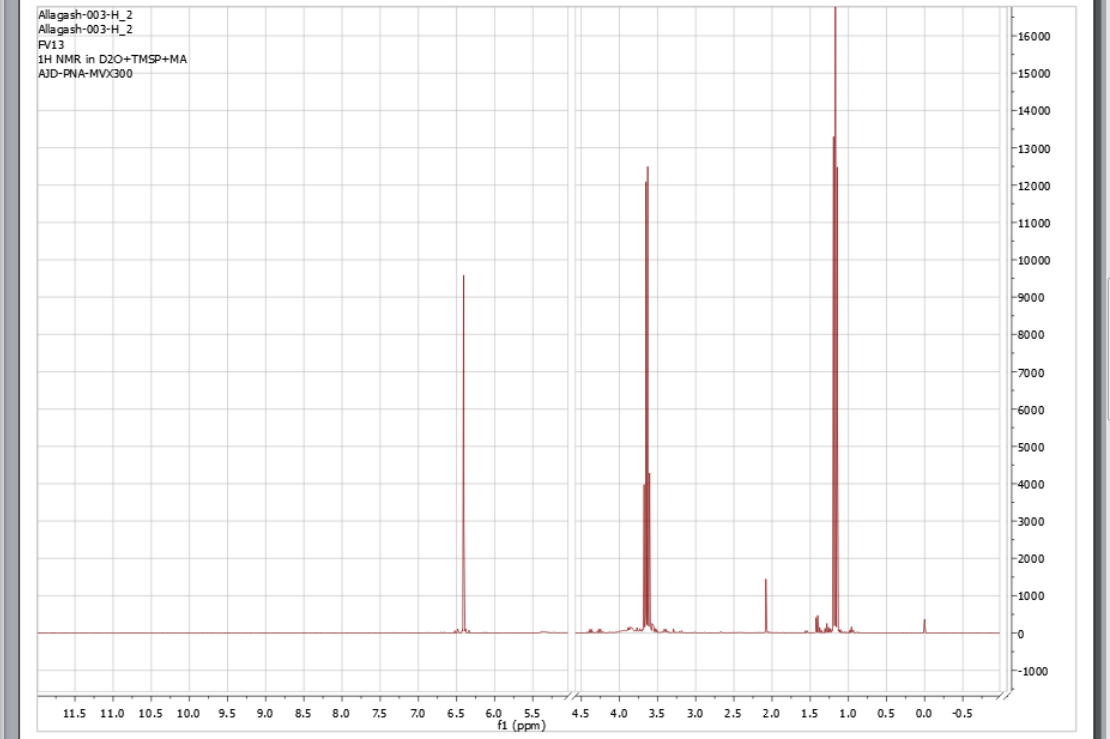
CCF*(I1/NN1)*MW*4285.71

Formula Editor

Ranges

	From	To	NN
1	1.395	1.43	3

OK Cancel



Licenses:

Global Spectral Deconvolution & Peak Picking automatically identifies and integrates peaks based on entered peak ranges. Quantitation results are immediately derived can be exported as XML & XHTML files, copied to clipboard, or pasted onto the spectrum in Mnova.

MestReNova BETA - [C:/Users/John/Desktop/Allagash Project/qNMR MNova Results/Merc300/Document 18.mnova*]

File Edit View Advanced Processing Analysis Predict Documents Stack Help Scripts

Simple Mixture Analysis

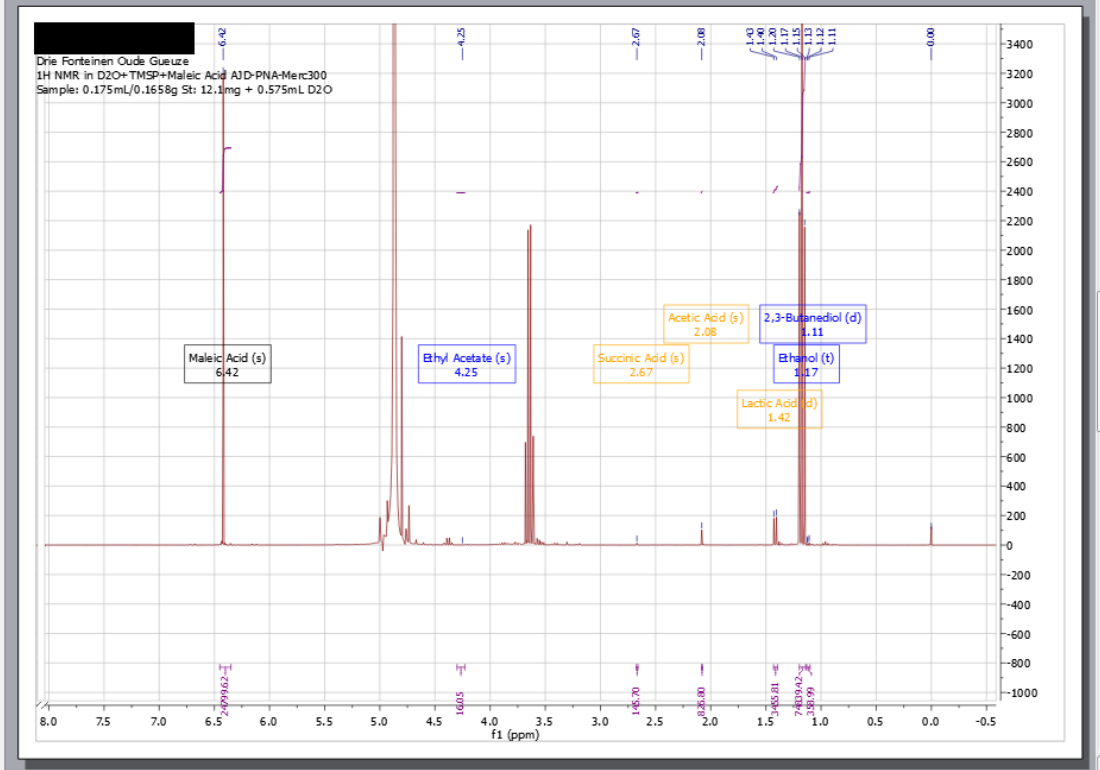
Analyze Settings Report Libraries

Analysis Data | Result

	Compound	Result
1	Ethanol	34222.77 mg/L
2	2,3-Butanediol	160.56 mg/L
3	Citric Acid	0.00 mg/L
4	Lactic Acid	3089.91 mg/L
5	Succinic Acid	128.09 mg/L
6	Ethyl Acetate	21.06 mg/L
7	Acetic Acid	492.81 mg/L

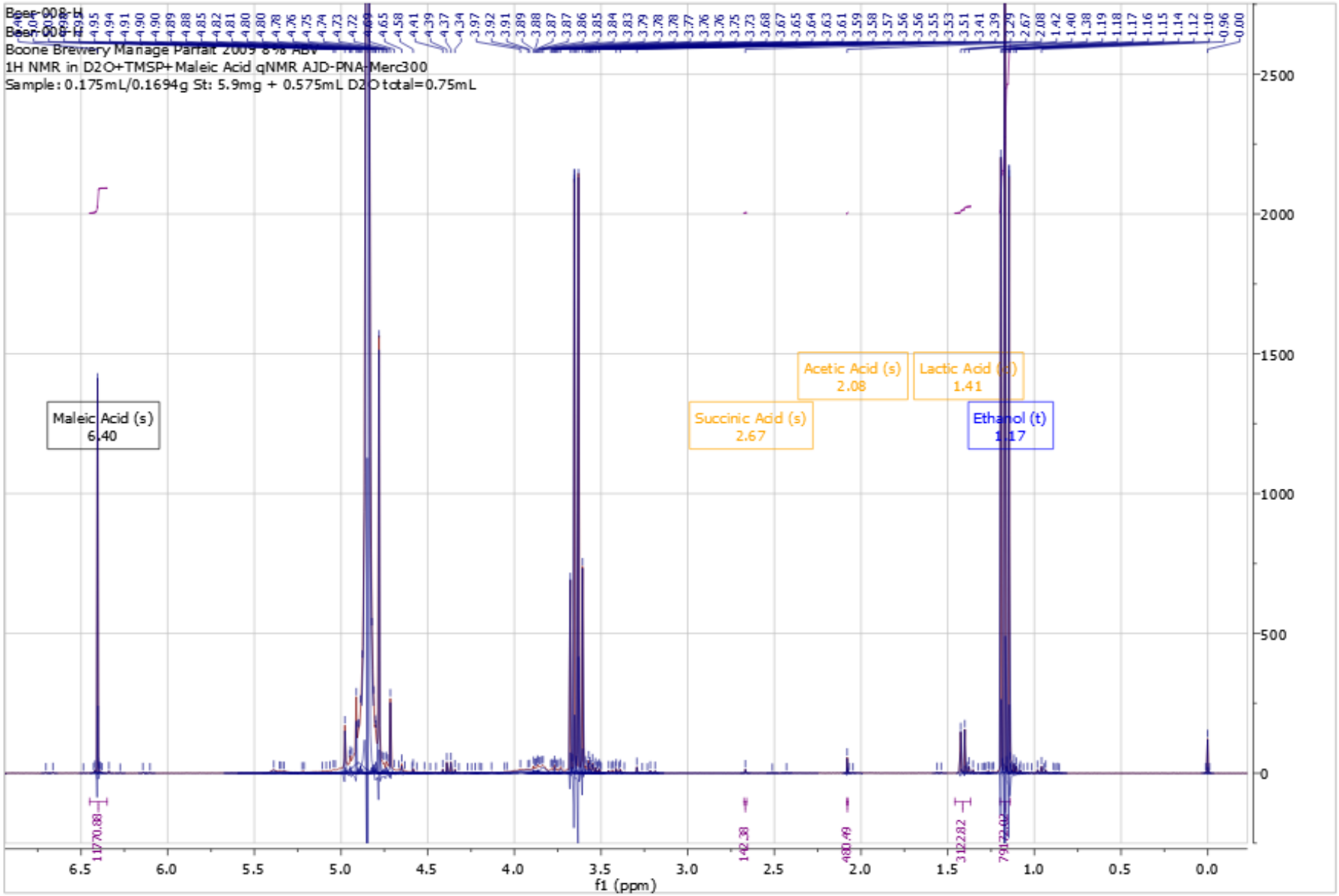
Clear Update

Alerts



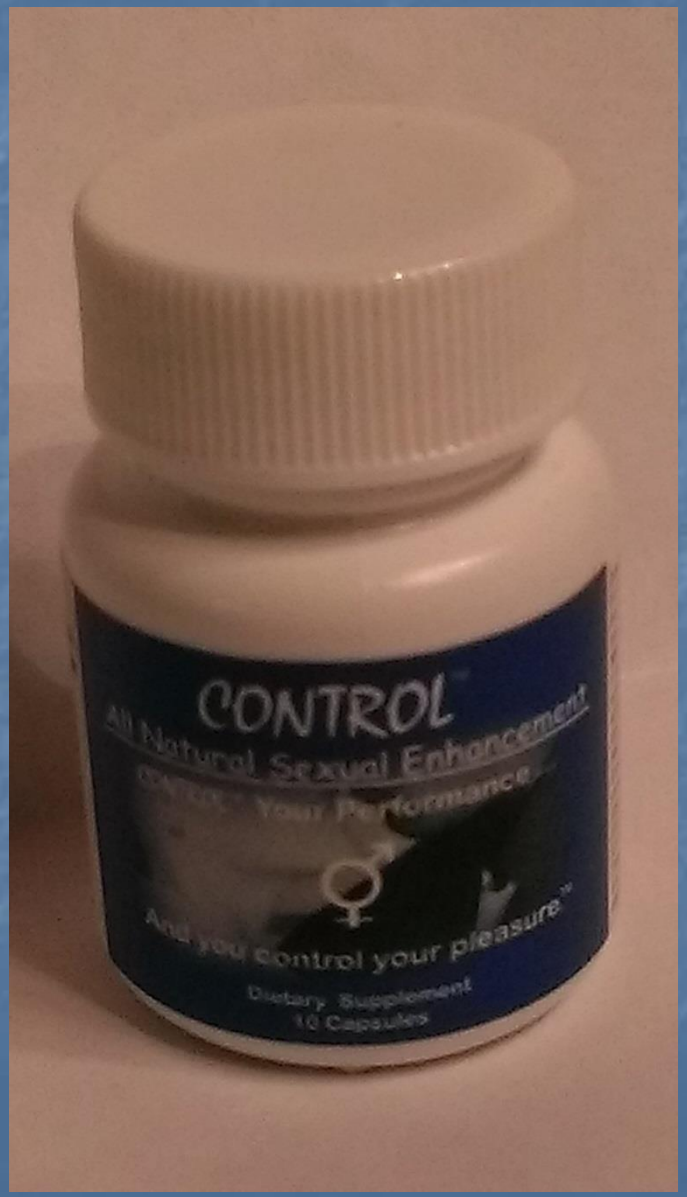
light gray black A black Arial 12

Licenses:


Beer 008 H
Beer 008 H
Boone Brewery Manage Parfait 2009 8% ABV
1H NMR in D2O+TMSP+Maleic Acid qNMR AJD-PNA-Merc300
Sample: 0.175mL/0.1694g St: 5.9mg + 0.575mL D2O total=0.75mL
Maleic Acid (s)
6.40
Succinic Acid (s)
2.67
Acetic Acid (s)
2.08
Lactic Acid (d)
1.41
Ethanol (t)
1.17
2500
2000
1500
1000
500
0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Comparison of results obtained by manual integration (solid border, left) and by automatic Mnova SMA (dotted border, right). Units=mg/L

		Manual Integration				Mnova SMA			
Brewery	**Beer**	**Lactic Acid**	**Acetic Acid**	**Succinic Acid**	**Citric Acid**	**Lactic Acid**	**Acetic Acid**	**Succinic Acid**	**Citric Acid**
Allagash	**Confluence**	850.5	402.4	167.4	248.2	901.3	437.6	184.3	353.2
Budweiser	**Bud Light**	93.9	38.1	35.8	82.7	117.1	17.7	34.2	62.6
Crooked Stave	**Surette**	4699.2	564.9	195.7	265.5	4247.9	549.2	185.6	262.4
Drie Fonteinen	**Oude Gueuze**	5137.6	865.9	234.6	0.0	4935.2	787.1	204.6	0.0
Drie Fonteinen	**Oude Gueuze**	5389.9	917.7	228.9	0.0	5180.0	775.2	194.4	0.0
LoverBeer	**D'Uva Beer**	3071.4	781.5	876.0	358.0	3302.8	759.9	842.4	377.3
Boone	**Mariage Parfait 2009**	4506.5	488.1	216.9	0.0	4591.8	403.2	193.1	0.0



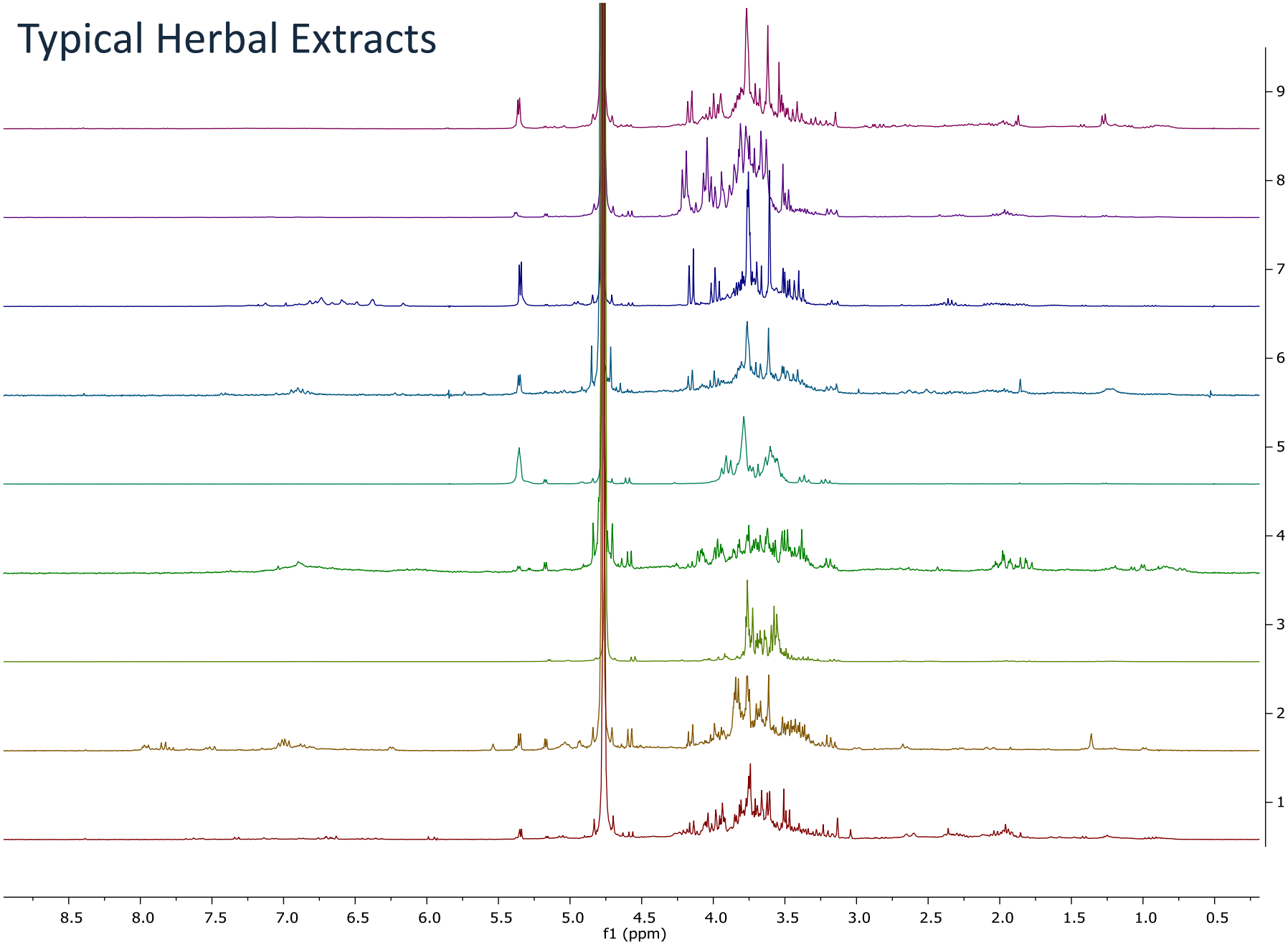
Question:
Why is this competitors product so good?

Ingredients : Oyster, Barberry, Dextrose, Snow Lotus, Bombyx Mori L, Ginger Root, Salfron Crocus.

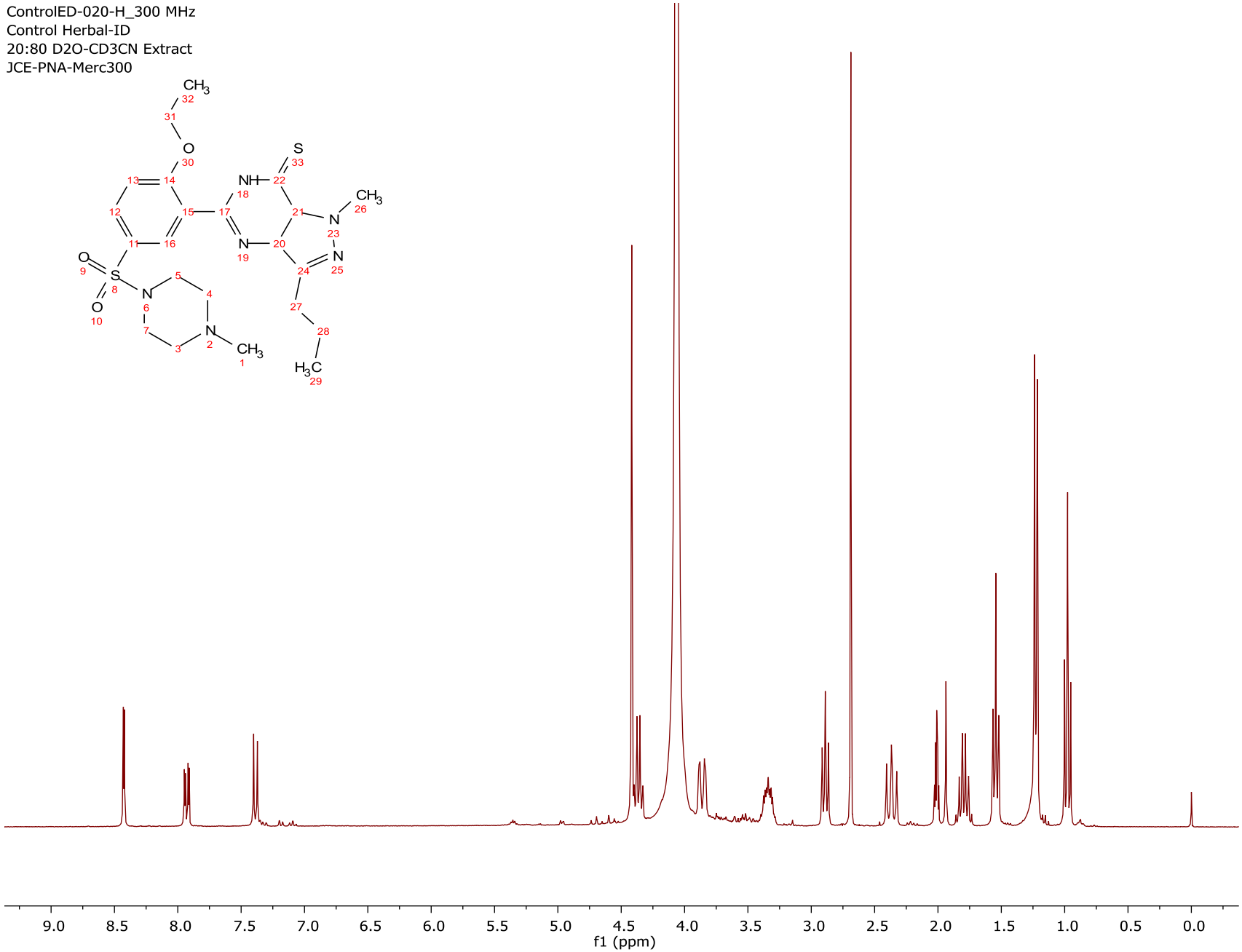
Sample preparation:
Extract with 20:80 D_2O:CD_3CN
Centrifuge or Settle
Supernatant analyzed.

Answer:
Also contained ~10 wt% Sulfoaildenafil
A Viagra Analog

Typical Herbal Extracts

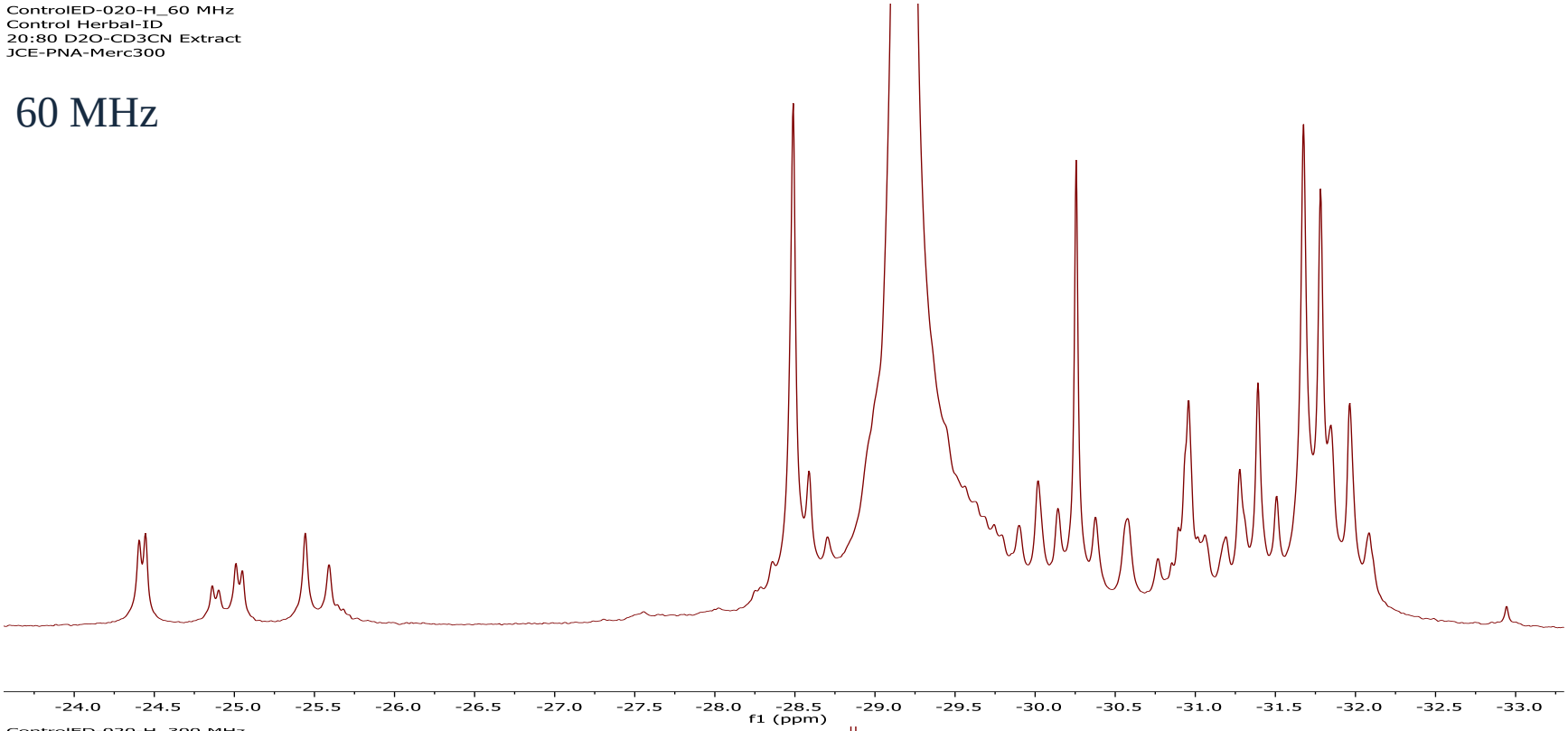


ControlED-020-H_300 MHz
Control Herbal-ID
20:80 D2O-CD3CN Extract
JCE-PNA-Merc300



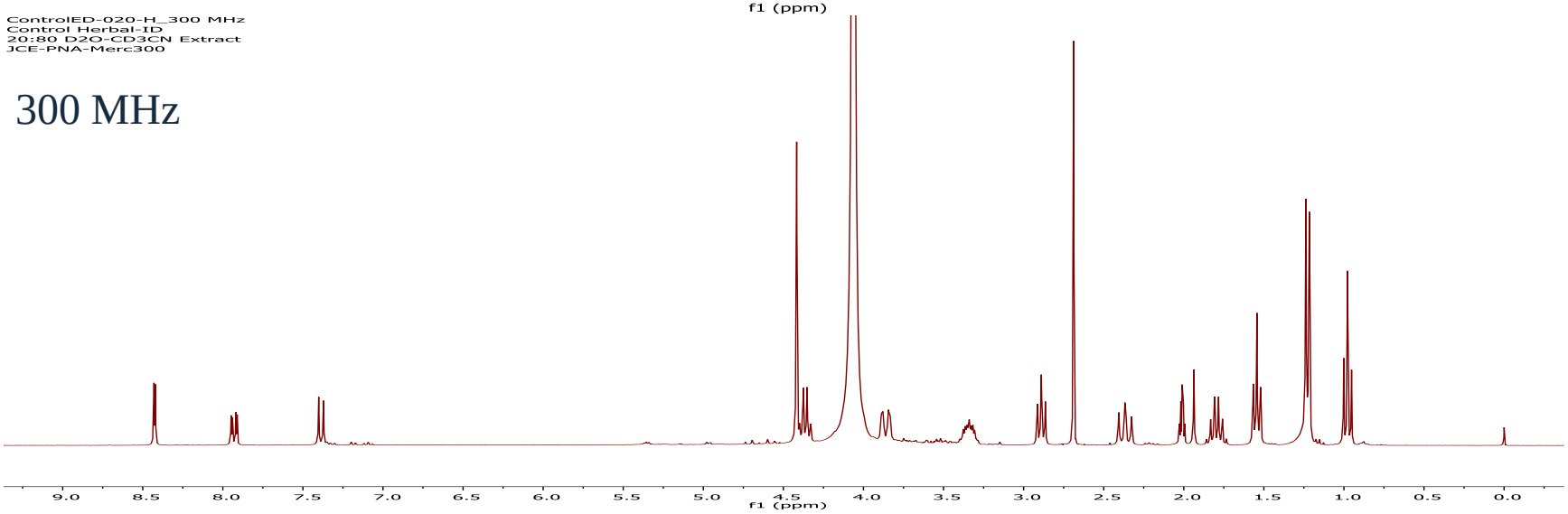
ControlED-020-H_60 MHz
Control Herbal-ID
20:80 D2O-CD3CN Extract
JCE-PNA-Merc300

60 MHz



ControlED-020-H_300 MHz
Control Herbal-ID
20:80 D2O-CD3CN Extract
JCE-PNA-Merc300

300 MHz



Simple Mixture Analysis

Analyze | Settings | Report | Libraries

Analysis Data | Result

Mixture

Library: D:/MNova/USP/ED Herbals

Name: ED Herbals

Description: PDE5i in Herbal Supplements

Sample Details

Sample ID: Herbal ED-300 | Weight(mg): 95.2

Reference Concentration: | Reference Weight(mg): 25.9

Metadata

SOP: 1H qNMR - 300 MHz - Maleic

Investigator: John Edwards

Customer: XXX

Customer Sample Number: XXX

Comments: XXX

Experiment

Experiment

Name: ED Herbals

Description: PDE5i in Herbal Supplements

Units: wt%

Compound	Type	Mol. Weight	Color	Spectrum
Sulfoaidenafil	Compound	504.67	Blue	1
Maleic Acid	Ref	116.07	Red	1
Sulfoaidenafil-60	Compound	504.67	Blue	1

Compound Editor

Name: Sulfoaidenafil-60

Color: Blue | Spectrum Nº: 1 | Molecular Weight: 504.67

Type: Compound | Acceptable Limit From: | To:

Formula

100*(RW*((I1+I2+I3)/(NN1+NN2+NN3))*MW)/(IR1/(NNR1)*MWR*SW)

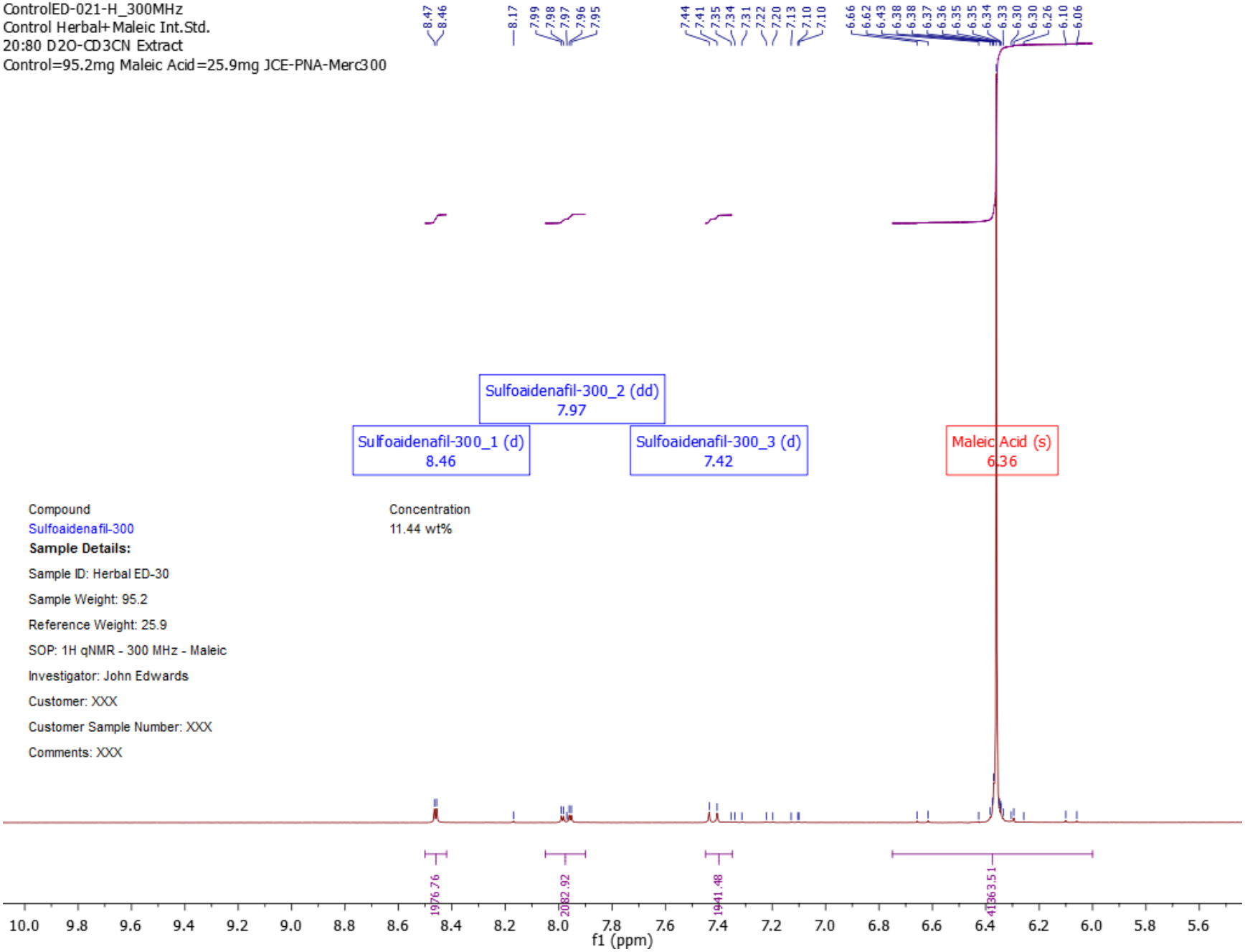
Formula Editor

Ranges

	From	To	NN
1	8.66	8.42	1
2	8.15	7.82	1

OK | Cancel

ControlED-021-H_300MHz
Control Herbal+Maleic Int.Std.
20:80 D2O-CD3CN Extract
Control=95.2mg Maleic Acid=25.9mg JCE-PNA-Merc300



Compound
Sulfoaidenafil-300

Concentration
11.44 wt%

Sample Details:

Sample ID: Herbal ED-30

Sample Weight: 95.2

Reference Weight: 25.9

SOP: 1H qNMR - 300 MHz - Maleic

Investigator: John Edwards

Customer: XXX

Customer Sample Number: XXX

Comments: XXX

ControlED-021-H_60MHz
Control Herbal+Maleic Int.Std.
20:80 D2O-CD3CN Extract
Control=95.2mg Maleic Acid=25.9mg JCE-PNA-Merc300

8.56 8.52 8.09 7.94 7.90 7.50 7.35 6.36

Sulfoaidenafil-60_2 (m)
7.98

Sulfoaidenafil-60_1 (d)
8.54

Sulfoaidenafil-60_3 (d)
7.43

Maleic Acid (s)
6.36

Compound
Sulfoaidenafil-60

Concentration
12.59 wt%

Sample Details:

Sample ID: Herbal ED-60

Sample Weight: 95.2

Reference Weight: 25.9

SOP: 1H qNMR - 60 MHz - Maleic

Investigator: John Edwards

Customer: XXX

Customer Sample Number: XXX

Comments: XXX

3.46 3.36 3.78 48.78

9.6 9.4 9.2 9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6

f1 (ppm)

Peak Picking Options

Method: GSD

Settings

Refinement Level

Ref. 1 (2 fitting cycles)

Optimized for Peaks:

Narrow

Average

Broad

Custom 0.25

Auto Classify

Impurities/Compounds...

Defaults

OK

Cancel

Peak Picking Options

Method: Standard

Peak Picking Options

Noise Factor: 10.00

Peaks Type: Only Positive

Sensitivity: 100.00

Maximum Number of Peaks: 50

Use Parabolic Interpolation

Auto Classify

Impurities/Compounds...

Interactive

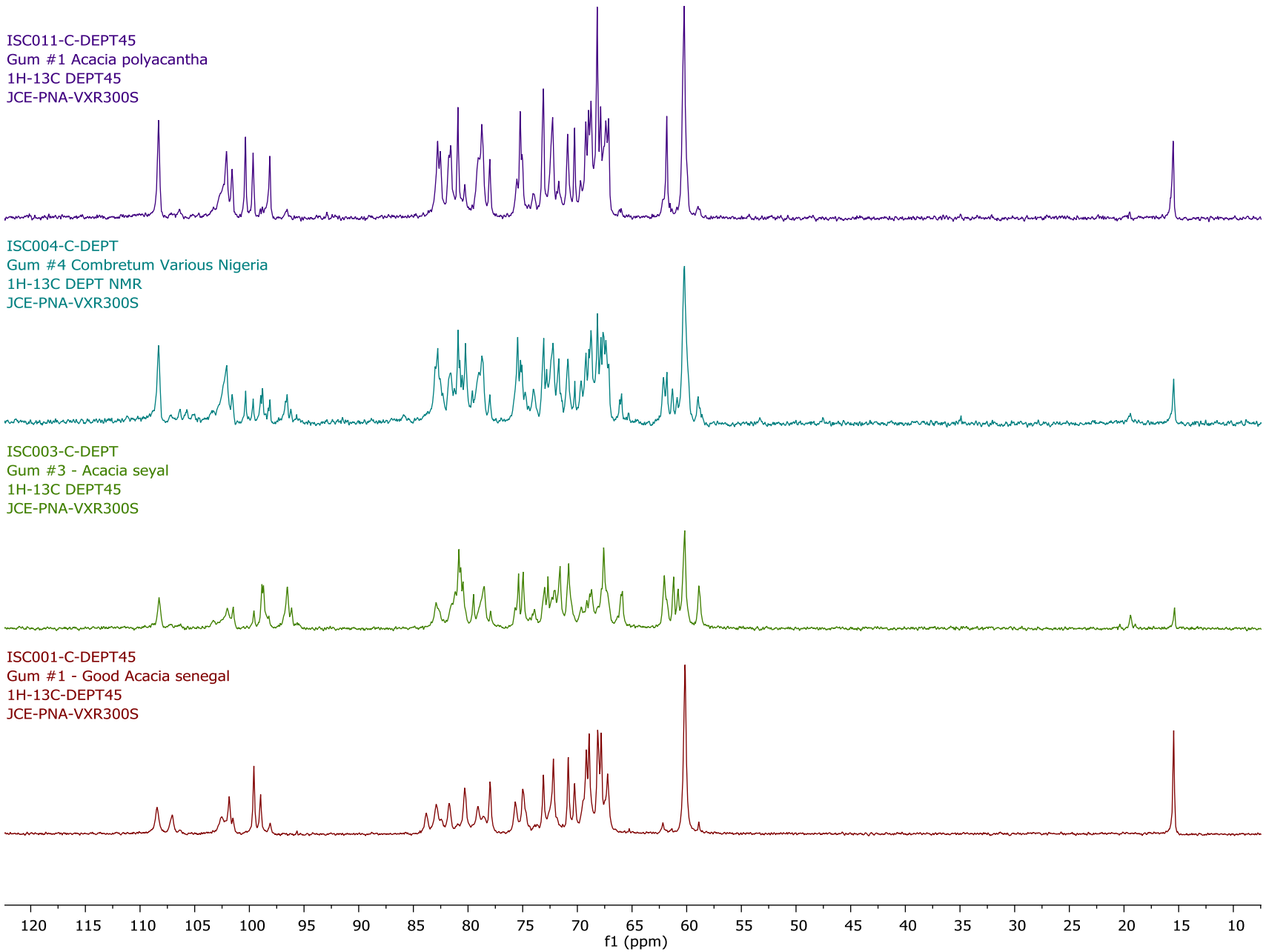
Defaults

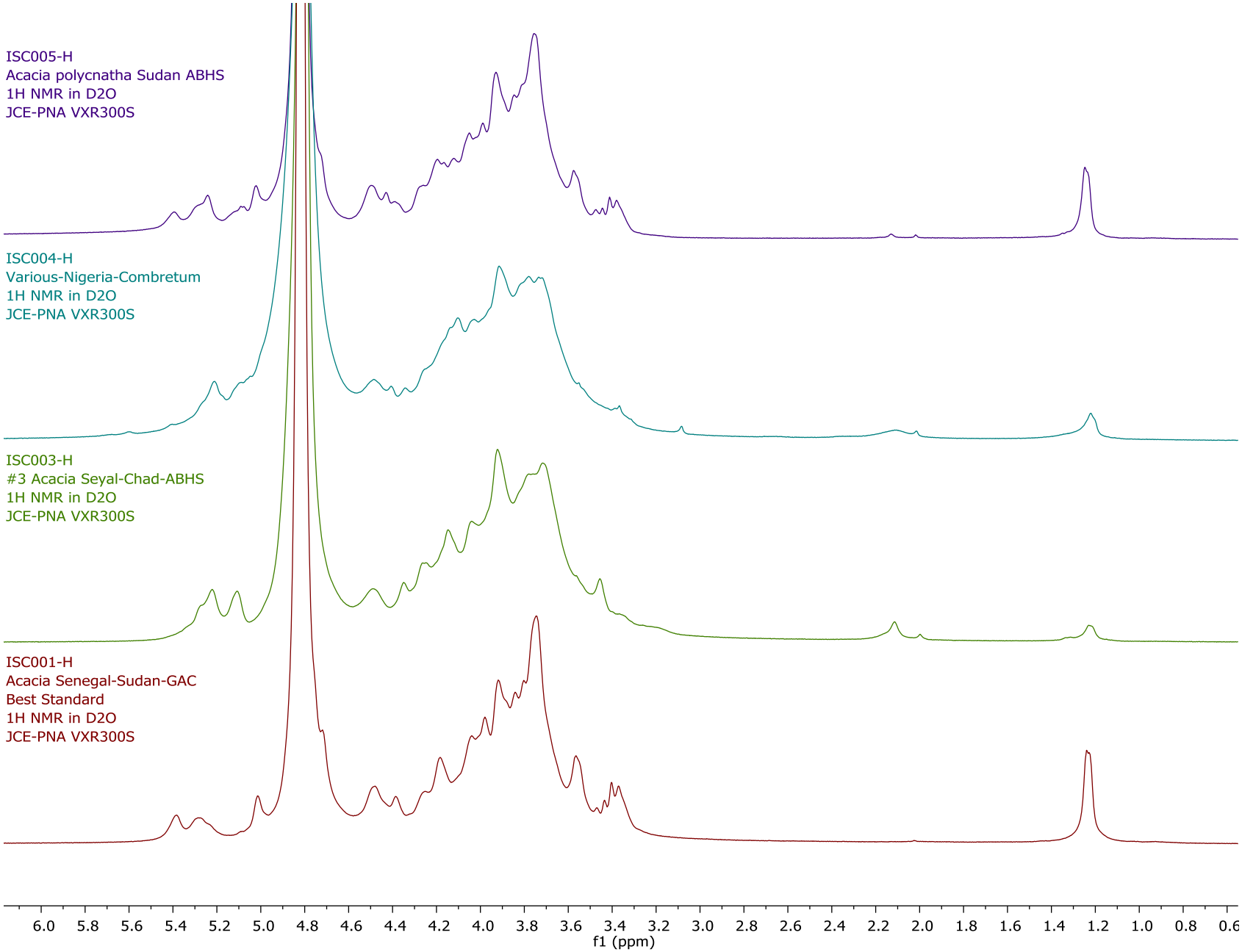
Advanced <<

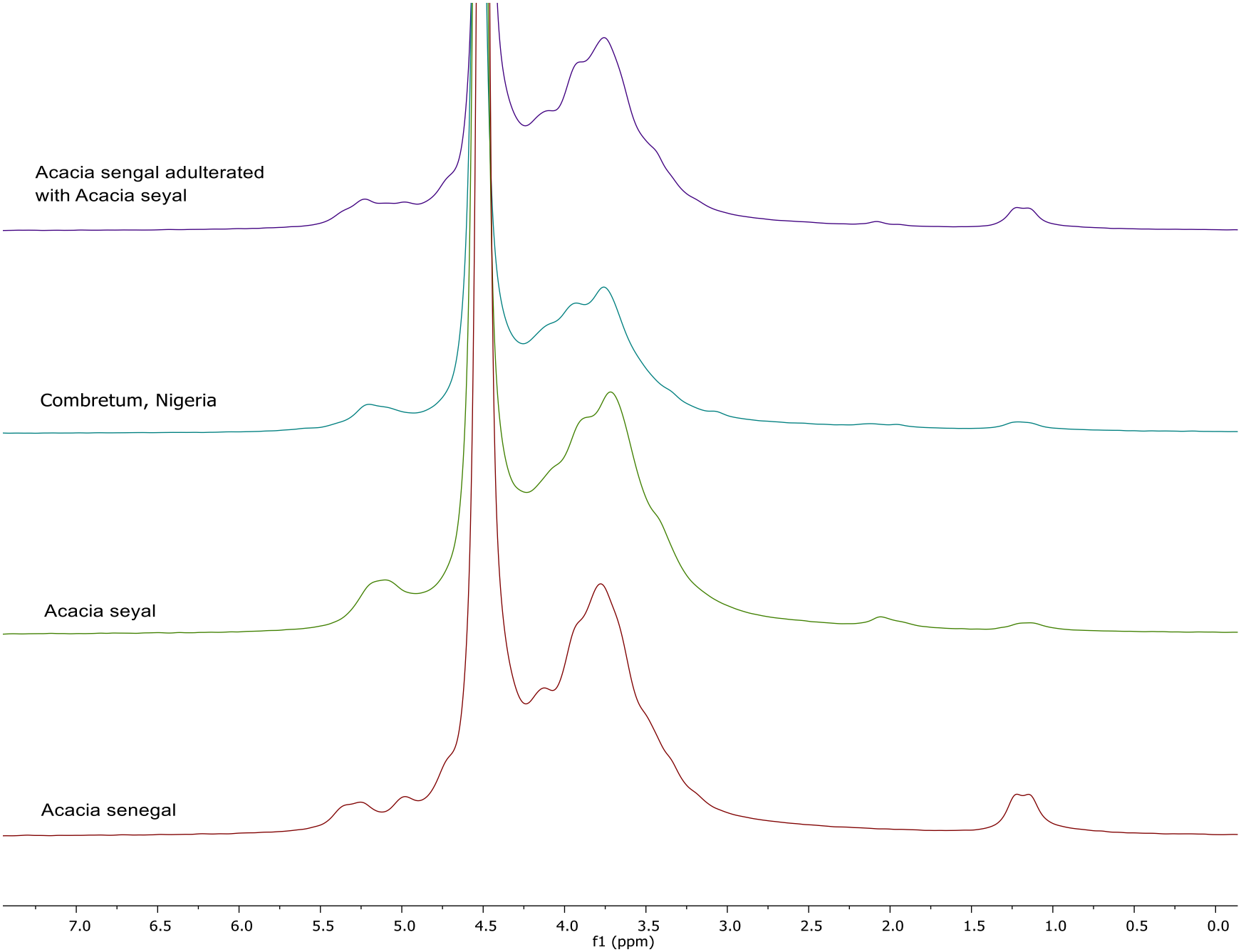
OK

Cancel

Adulteration of Acacia senegal – aka Gum Arabic
Emulsifier in Many Food, Beverage, Pharmaceutical and Cosmetic Products


ISC011-C-DEPT45
Gum #1 Acacia polyacantha
1H-13C DEPT45
JCE-PNA-VXR300S
ISC004-C-DEPT
Gum #4 Combretum Various Nigeria
1H-13C DEPT NMR
JCE-PNA-VXR300S
ISC003-C-DEPT
Gum #3 - Acacia seyal
1H-13C DEPT45
JCE-PNA-VXR300S
ISC001-C-DEPT45
Gum #1 - Good Acacia senegal
1H-13C-DEPT45
JCE-PNA-VXR300S
120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10
f1 (ppm)


ISC005-H
Acacia polycnatha Sudan ABHS
1H NMR in D2O
JCE-PNA VXR300S
ISC004-H
Various-Nigeria-Combretum
1H NMR in D2O
JCE-PNA VXR300S
ISC003-H
#3 Acacia Seyal-Chad-ABHS
1H NMR in D2O
JCE-PNA VXR300S
ISC001-H
Acacia Senegal-Sudan-GAC
Best Standard
1H NMR in D2O
JCE-PNA VXR300S
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 1.6 1.4 1.2 1.0 0.8 0.6
f1 (ppm)


Acacia sengal adulterated
with Acacia seyal
Combretum, Nigeria
Acacia seyal
Acacia senegal
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Acknowledgments

Aloe Project Work – Adam Dicaprio and Daniel Edwards at PNA

Beer Project Work – Adam Dicaprio

ED Herbal Project – Kristie Adams and Anton Bzhelyanky - USP

Chris Berliner – ISC (Provided Gums)