STUDY REPORT ASB29821

- Aloe Vera -

- Quality Control -

Sponsor: ASTRAGALUS
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TABLE OF CONTENTS

FIGURES

TABLES

1 OBJECTIVES

For control of identity and quality ^{1}H-NMR spectra have been recorded.

2 TEST ITEMS

The test item data are given in Chapter 5 (Results).

3 MATERIALS

3.1 Reference and Calibration Items

Tab. 1 Chemicals actually used as reference and calibration items

No	Substance name	Distributor	Order no.
15	3-(Trimethylsilyl)-propionic acid-d_4 Na-salt (TMSP; for NMR Calibration)	E. Merck Darmstadt (D)	8652
9	Nicotinic acid amide (NSA)	Fluka Chemie AG, Buchs (CH)	72340

3.2 Chemicals

Tab. 2 Chemicals used in the study

Substance name	Distributor	Order no.
Deuteriumoxide, Degree of deuteration 99.9%	Deutero GmbH, Kastellaun (D)	-

3.3 Instruments

NMR spectrometer Avance 300 (Bruker, Karlsruhe, D), magnetic flux density 7.05 Tesla
PA BBI probe head; automated sample changer Bruker B-ACS 120
Computer P IV 1.5 GHz under MS Windows 2000 Pro and Bruker XWIN-NMR 3.5 for acquisition
Bruker Win-NMR 6.0 for processing
Standard operation procedure SAA-GMR006-02

Freeze-drying device Type 318 (Christ, Aichach-Oberbernbach, D)
Standard operation procedure SAA-GMR019-02

Electronic semi-micro balance Sartorius R 180 D-*D1 (Göttingen, D)
Standard operation procedure SAA-GMR005-04

4 METHODS

^{1}H-NMR spectra were recorded to characterise the test items. Approx. 50 mg of each freeze-dried test item and approx. 5 mg internal standard NSA have been dissolved in 1 ml D_2O. The actually used NMR parameters appear on the spectrum plot.

5 RESULTS AND DISCUSSION

Fresh Aloe Vera consists of three main components: aloverose, glucose and malic acid; all are detectable by ^{1}H-NMR spectroscopy. These compounds are markers for good material.

High amounts of lactic acid indicate a bacterial degradation (Lactobacillus). Succinic and fumaric acid are produced by Aloe Vera's own enzyme system. Acetic acid and formic acid are caused by hydrolysis of aloverose and thermal degradation of glucose during storage.

Presence of iso-citric acid (WLM) is an indication for utilization of whole leaf. In addition to iso-citric acid whole leaf always contains citric acid in a 2/1 ratio. Higher amounts of citric acid result from added acidifier.

The signals between δ = 9.6 and 8.0 ppm are caused by internal standard.

Aloverose is a polysaccharide, but not all polysaccharides in Aloe Vera are Aloverose. **Aloverose** is partly acetylated polymannose (chemical structure see Figure 1). NMR distinguishes between Aloverose and other polysaccharides selectively. It defines its total amount independent of the chain length and the molecular weight. Acemannan® defines a specific molecular weight fraction.
NMR spectroscopy uses the acetyl proton signals as a fingerprint for identification and quantification (see Figure 2). The method is validated.
The amount of Aloverose changes because Aloe Vera is a natural material. From experiences we know its amount in fresh Aloe Vera gel varies between 5% and 20% w/w of dry matter.

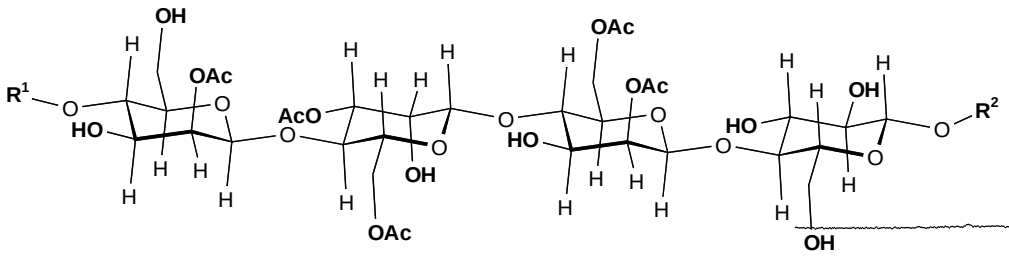

Figure 1 Aloverose

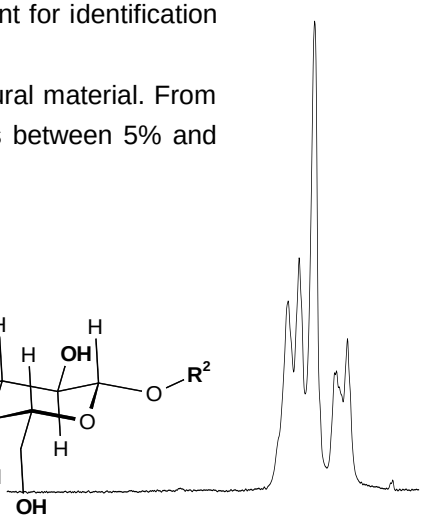

Figure 2

Fig. 1 Definition and determination of Aloverose

Tab. 3 Composition of test item **ASB29821-1**

Sample name:	Aloe Pure of Australia, Natural Juice			
Batch no:	19/07/12		Lab no:	---
Description:	cloudy, yellowish liquid		Results from:	10/03/2011
	Content [%]*	Content [mg/l]*	Origin of component	
Aloverose (polysaccharide)	2.2	96.3	fresh Aloe Vera	
Glucose	not detected		fresh Aloe Vera	
Malic acid	not detected		fresh Aloe Vera	
Lactic acid	1.9	84.6	degradation (bacterial)	
Citric acid	63.6	2,831.0	WLM or added acidifier	
WLM	not detected		whole leaf marker (WLM)	
Maltodextrin	not detected		formulation aid for drying	
Acetic acid	detected		degradation (hydrolysis)	
Succinic acid	not detected		degradation (enzymatic)	
Fumaric acid	not detected		degradation (enzymatic)	
Formic acid	not detected		degradation	
Sodium benzoate	not detected		added preservative	
Potassium sorbate	not detected		added preservative	
Other**	detected		unknown	
Dry matter	0.4			
Aloin***		not determined		
Calcium****		not determined	Density [g/cm^3]:	not determined
Magnesium****		not determined	pH value:	not determined

* The content data [%] refer to dry matter. The content data [mg/l] refer to dry weight 0.7 [%] for powders.
** Organic and inorganic compounds not to be quantified by NMR analysis.
*** Limit of quantification 0.1 ppm.
**** The content data for Calcium/Magnesium are given in [mg/kg TS] for powder resp. [mg/l] for liquids. The determination comes after DIN EN ISO 11885 (liquid) resp. 17294-2 (powder) by Eurofins Umwelt West GmbH (Wesseling, D).

The sample is of Aloe Vera origin without preservative. It shows small degradation by lacto bacteria. The sample contains Vitamin C (not quantified).

Tab. 4 Composition of test item **ASB29821-2**

Sample name:	Aloe Vera, Oleofarm			
Batch no:	11.2012 955		Lab no:	---
Description:	cloudy, yellowish liquid		Results from:	10/03/2011
	Content [%]*	Content [mg/l]*	Origin of component	
Aloverose (polysaccharide)	3.3	207.1	fresh Aloe Vera	
Glucose	5.8	358.6	fresh Aloe Vera	
Malic acid	5.4	332.8	fresh Aloe Vera	
Lactic acid	2.9	180.2	degradation (bacterial)	
Citric acid	46.7	2,895.2	WLM or added acidifier	
WLM	7.4	457.1	whole leaf marker (WLM)	
Maltodextrin	not detected		formulation aid for drying	
Acetic acid	detected		degradation (hydrolysis)	
Succinic acid	detected		degradation (enzymatic)	
Fumaric acid	detected		degradation (enzymatic)	
Formic acid	not detected		degradation	
Sodium benzoate	detected		added preservative	
Potassium sorbate	detected		added preservative	
Other**	detected		unknown	
Dry matter	0.6			
Aloin***		not determined		
Calcium****		not determined	Density [g/cm^3]:	not determined
Magnesium****		not determined	pH value:	not determined

* The content data [%] refer to dry matter. The content data [mg/l] refer to dry weight 0.7 [%] for powders.
** Organic and inorganic compounds not to be quantified by NMR analysis.
*** Limit of quantification 0.1 ppm.
**** The content data for Calcium/Magnesium are given in [mg/kg TS] for powder resp. [mg/l] for liquids. The determination comes after DIN EN ISO 11885 (liquid) resp. 17294-2 (powder) by Eurofins Umwelt West GmbH (Wesseling, D).

The sample is of Aloe Vera origin with preservative. It shows small degradation by lacto bacteria and hydrolysis. The sample contains significant amounts of WLM. It therefore is at least partly produced of whole leaf juice.

Tab. 5 Composition of test item **ASB29821-3**

Sample name:	Aloe Vera A-Z Medica			
Batch no:	686171		Lab no:	---
Description:	cloudy, yellowish liquid		Results from:	10/03/2011
	Content [%]*	Content [mg/l]*	Origin of component	
Aloverose (polysaccharide)	2.0	208.1	fresh Aloe Vera	
Glucose	2.5	267.2	fresh Aloe Vera	
Malic acid	7.6	795.5	fresh Aloe Vera	
Lactic acid	trace		degradation (bacterial)	
Citric acid	67.3	7,074.7	WLM or added acidifier	
WLM	4.6	478.7	whole leaf marker (WLM)	
Maltodextrin	not detected		formulation aid for drying	
Acetic acid	detected		degradation (hydrolysis)	
Succinic acid	detected		degradation (enzymatic)	
Fumaric acid	not detected		degradation (enzymatic)	
Formic acid	not detected		degradation	
Sodium benzoate	detected		added preservative	
Potassium sorbate	detected		added preservative	
Other**	detected		unknown	
Dry matter	1.1			
Aloin***		not determined		
Calcium****		not determined	Density [g/cm^3]:	not determined
Magnesium****		not determined	pH value:	not determined

* The content data [%] refer to dry matter. The content data [mg/l] refer to dry weight 0.7 [%] for powders.
** Organic and inorganic compounds not to be quantified by NMR analysis.
*** Limit of quantification 0.1 ppm.
**** The content data for Calcium/Magnesium are given in [mg/kg TS] for powder resp. [mg/l] for liquids. The determination comes after DIN EN ISO 11885 (liquid) resp. 17294-2 (powder) by Eurofins Umwelt West GmbH (Wesseling, D).

The sample is of Aloe Vera origin with preservative. It shows no degradation. The sample contains significant amounts of WLM. It therefore is at least partly produced of whole leaf juice.

Tab. 6 Composition of test item **ASB29821-4**

Sample name:	Aloe Vera, Alter Medica			
Batch no:	A1010/131		Lab no:	---
Description:	cloudy, yellowish liquid		Results from:	10/03/2011
	Content [%]*	Content [mg/l]*	Origin of component	
Aloverose (polysaccharide)	not detected		fresh Aloe Vera	
Glucose	28.4	1,925.3	fresh Aloe Vera	
Malic acid	not detected		fresh Aloe Vera	
Lactic acid	not detected		degradation (bacterial)	
Citric acid	2.0	135.6	WLM or added acidifier	
WLM	not detected		whole leaf marker (WLM)	
Maltodextrin	51.0	3,464.6	formulation aid for drying	
Acetic acid	not detected		degradation (hydrolysis)	
Succinic acid	not detected		degradation (enzymatic)	
Fumaric acid	not detected		degradation (enzymatic)	
Formic acid	not detected		degradation	
Sodium benzoate	detected		added preservative	
Potassium sorbate	detected		added preservative	
Other**	detected		unknown	
Dry matter	0.7			
Aloin***		not determined		
Calcium****		not determined	Density [g/cm^3]:	not determined
Magnesium****		not determined	pH value:	not determined

* The content data [%] refer to dry matter. The content data [mg/l] refer to dry weight 0.7 [%] for powders.
** Organic and inorganic compounds not to be quantified by NMR analysis.
*** Limit of quantification 0.1 ppm.
**** The content data for Calcium/Magnesium are given in [mg/kg TS] for powder resp. [mg/l] for liquids. The determination comes after DIN EN ISO 11885 (liquid) resp. 17294-2 (powder) by Eurofins Umwelt West GmbH (Wesseling, D).

The sample is not of Aloe Vera origin, as principal components: Aloverose and Malic acid could not be detected. It shows no degradation.

Tab. 7 Composition of test item **ASB29821-5**

Sample name:	Aloe Vera Gel Forever Living			
Batch no:	150610		Lab no:	---
Description:	cloudy, yellowish liquid		Results from:	10/03/2011
	Content [%]*	Content [mg/l]*	Origin of component	
Aloverose (polysaccharide)	1.2	566.4	fresh Aloe Vera	
Glucose	2.1	1,023.5	fresh Aloe Vera	
Malic acid	3.0	1,461.8	fresh Aloe Vera	
Lactic acid	not detected		degradation (bacterial)	
Citric acid	2.9	1,412.3	WLM or added acidifier	
WLM	not detected		whole leaf marker (WLM)	
Maltodextrin	not detected		formulation aid for drying	
Acetic acid	not detected		degradation (hydrolysis)	
Succinic acid	not detected		degradation (enzymatic)	
Fumaric acid	not detected		degradation (enzymatic)	
Formic acid	not detected		degradation	
Sodium benzoate	detected		added preservative	
Potassium sorbate	detected		added preservative	
Other**	detected		unknown	
Dry matter	4.8			
Aloin***		not determined		
Calcium****		not determined	Density [g/cm^3]:	not determined
Magnesium****		not determined	pH value:	not determined

* The content data [%] refer to dry matter. The content data [mg/l] refer to dry weight 0.7 [%] for powders.
** Organic and inorganic compounds not to be quantified by NMR analysis.
*** Limit of quantification 0.1 ppm.
**** The content data for Calcium/Magnesium are given in [mg/kg TS] for powder resp. [mg/l] for liquids. The determination comes after DIN EN ISO 11885 (liquid) resp. 17294-2 (powder) by Eurofins Umwelt West GmbH (Wesseling, D).

The sample is a concentrate of Aloe Vera origin with preservative. It shows no degradation. The sample contains sorbitol (not quantified).

Tab. 8 Composition of test item **ASB29821-6**

Sample name:	Aloe Vera Laboratoria Natury			
Batch no:	051210		Lab no:	---
Description:	cloudy, yellowish liquid		Results from:	10/03/2011
	Content [%]*	Content [mg/l]*	Origin of component	
Aloverose (polysaccharide)	8.7	517.9	fresh Aloe Vera	
Glucose	10.2	604.5	fresh Aloe Vera	
Malic acid	13.0	773.7	fresh Aloe Vera	
Lactic acid	11.5	687.0	degradation (bacterial)	
Citric acid	10.5	622.9	WLM or added acidifier	
WLM	not detected		whole leaf marker (WLM)	
Maltodextrin	not detected		formulation aid for drying	
Acetic acid	detected		degradation (hydrolysis)	
Succinic acid	detected		degradation (enzymatic)	
Fumaric acid	detected		degradation (enzymatic)	
Formic acid	not detected		degradation	
Sodium benzoate	detected		added preservative	
Potassium sorbate	detected		added preservative	
Other**	detected		unknown	
Dry matter	0.6			
Aloin***		not determined		
Calcium****		not determined	Density [g/cm^3]:	not determined
Magnesium****		not determined	pH value:	not determined

* The content data [%] refer to dry matter. The content data [mg/l] refer to dry weight 0.7 [%] for powders.
** Organic and inorganic compounds not to be quantified by NMR analysis.
*** Limit of quantification 0.1 ppm.
**** The content data for Calcium/Magnesium are given in [mg/kg TS] for powder resp. [mg/l] for liquids. The determination comes after DIN EN ISO 11885 (liquid) resp. 17294-2 (powder) by Eurofins Umwelt West GmbH (Wesseling, D).

The sample is of Aloe Vera origin with preservative. It shows strong degradation by lacto bacteria.

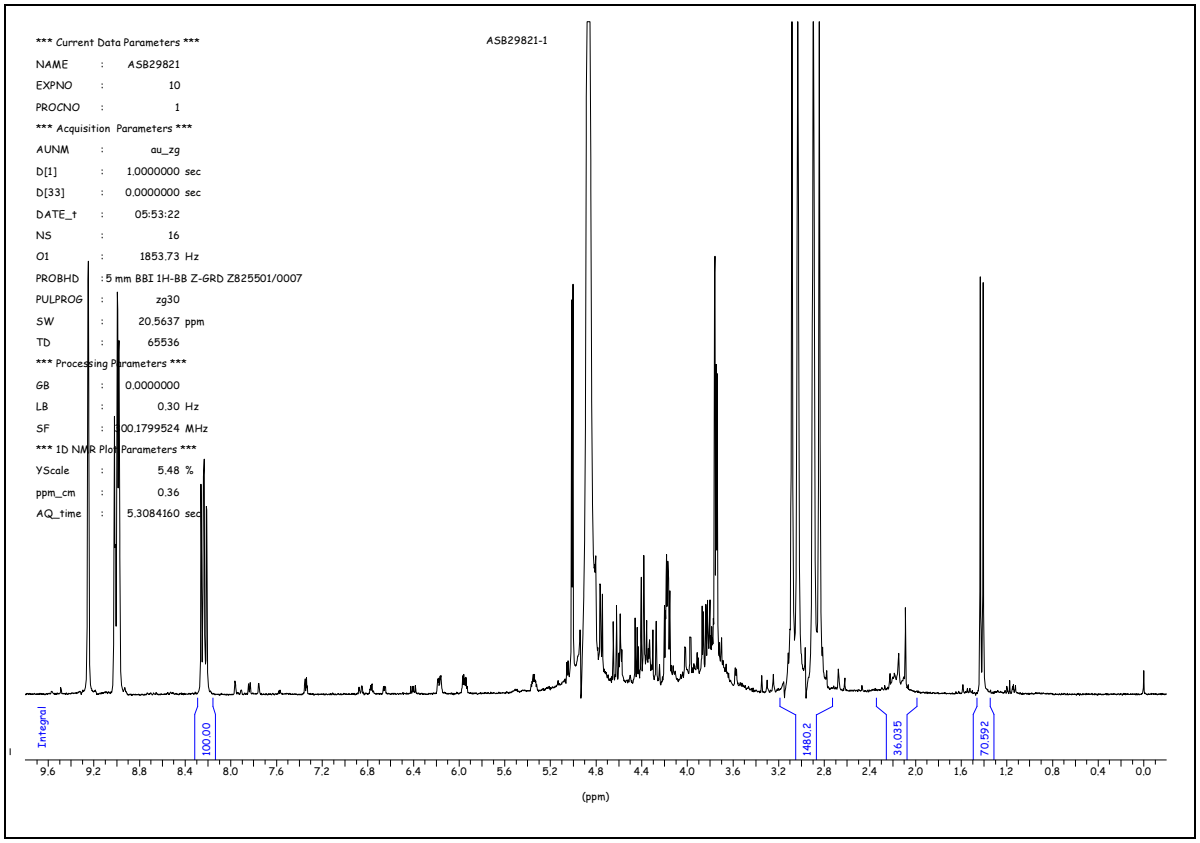

Fig. 2 ^{1}H-NMR spectrum of test item ABS29821-1

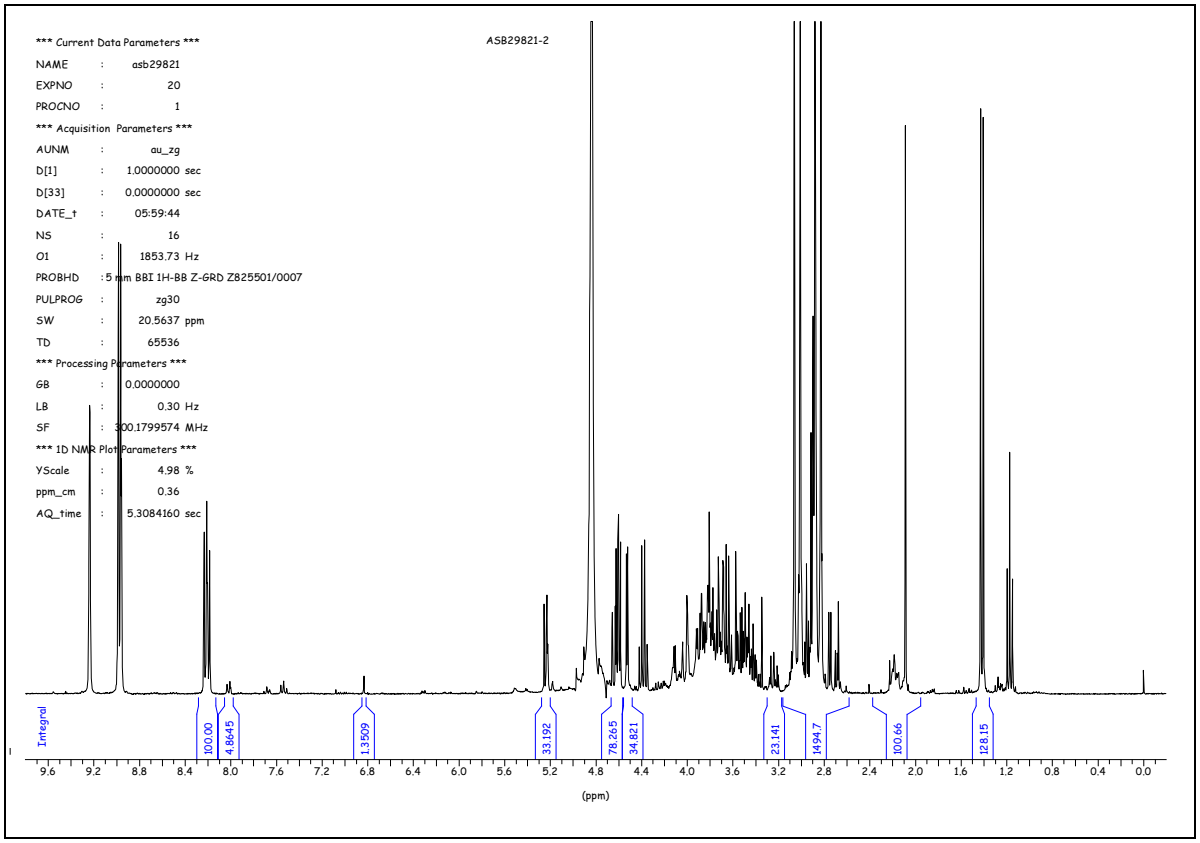

Fig. 3 ^{1}H-NMR spectrum of test item ABS29821-2

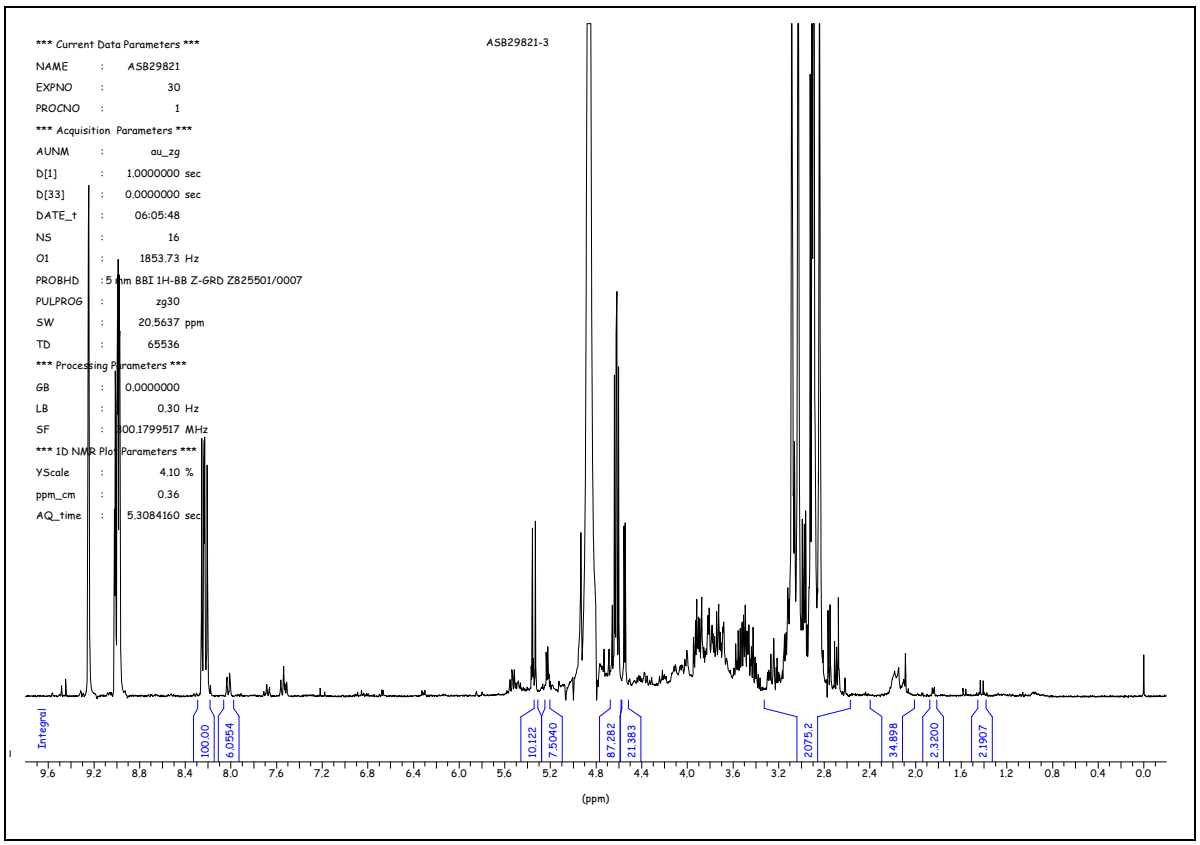

Fig. 4 ^{1}H-NMR spectrum of test item ABS29821-3

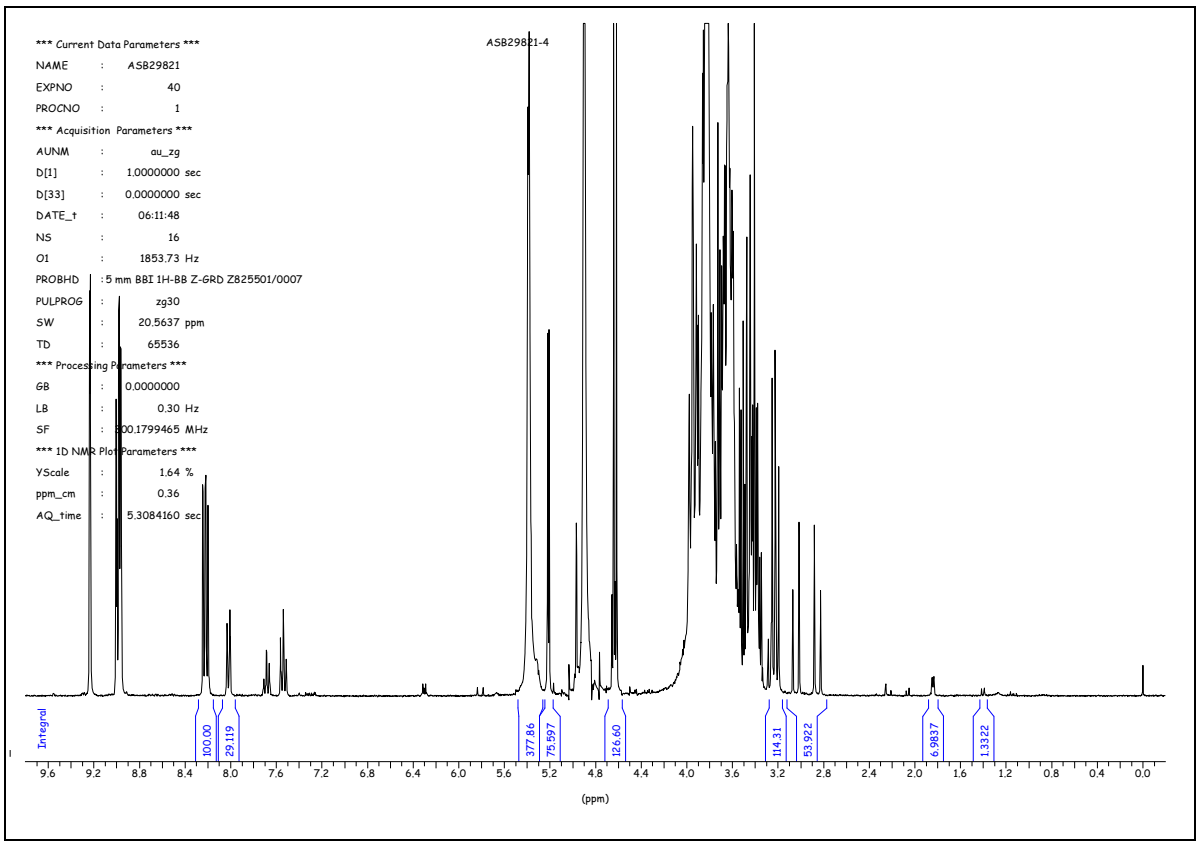

Fig. 5 ^{1}H-NMR spectrum of test item ABS29821-4

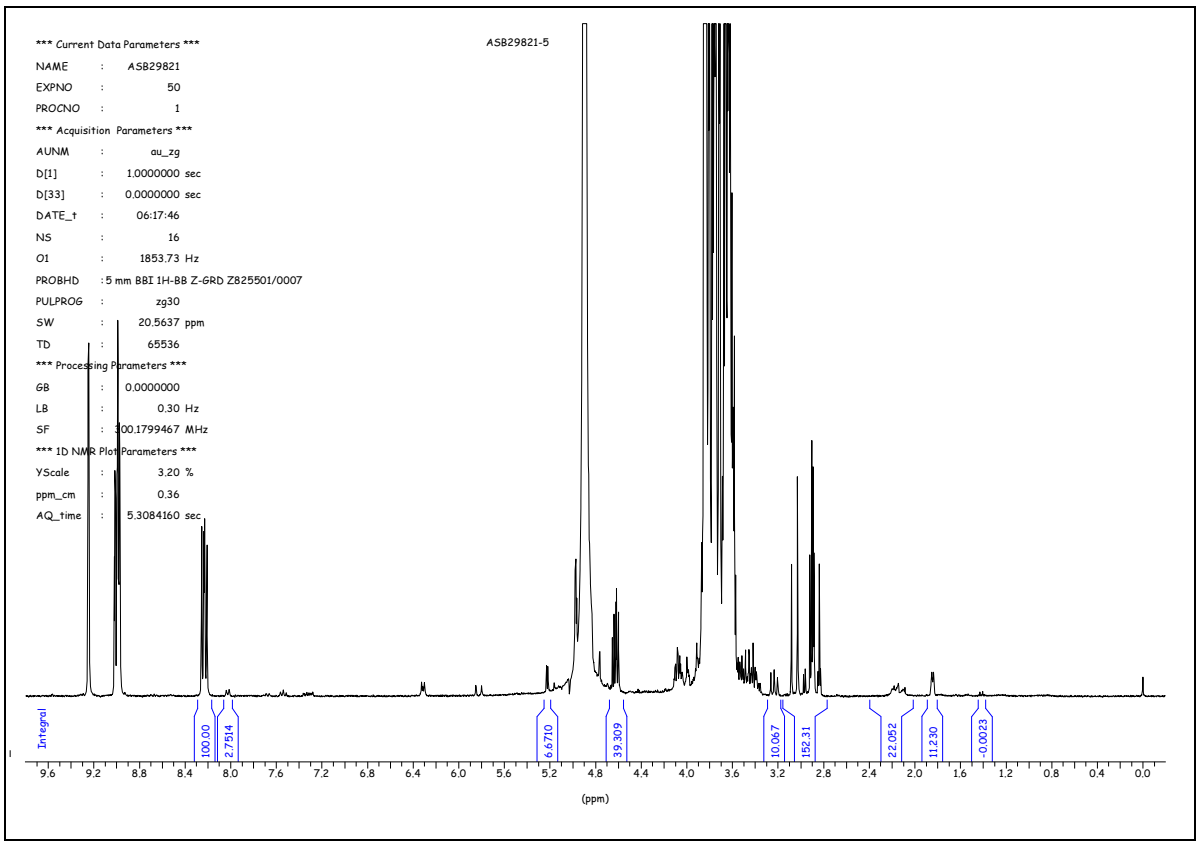

Fig. 6 ^{1}H-NMR spectrum of test item ABS29821-5

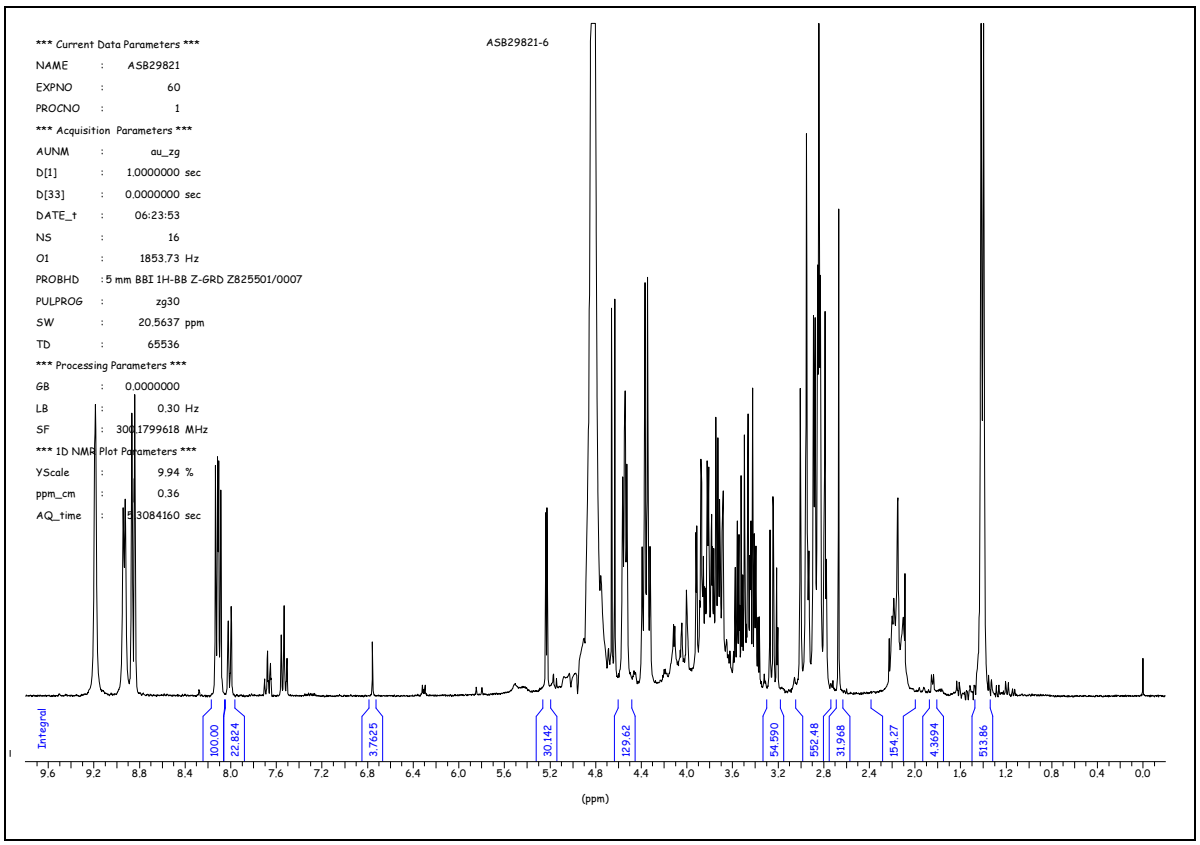

Fig. 7 ^{1}H-NMR spectrum of test item ABS29821-6

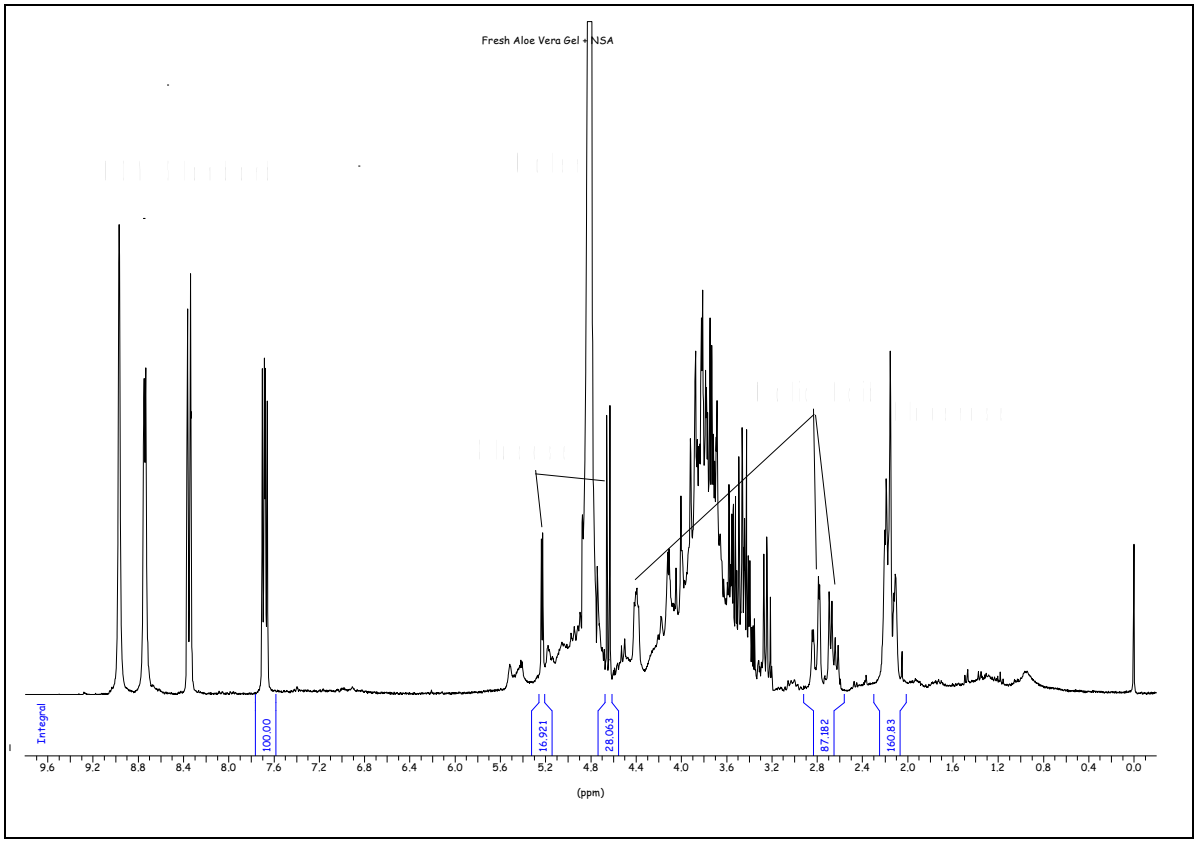

Fig. 8 For comparison: ^{1}H-NMR spectrum of fresh Aloe Vera gel + NSA Standard

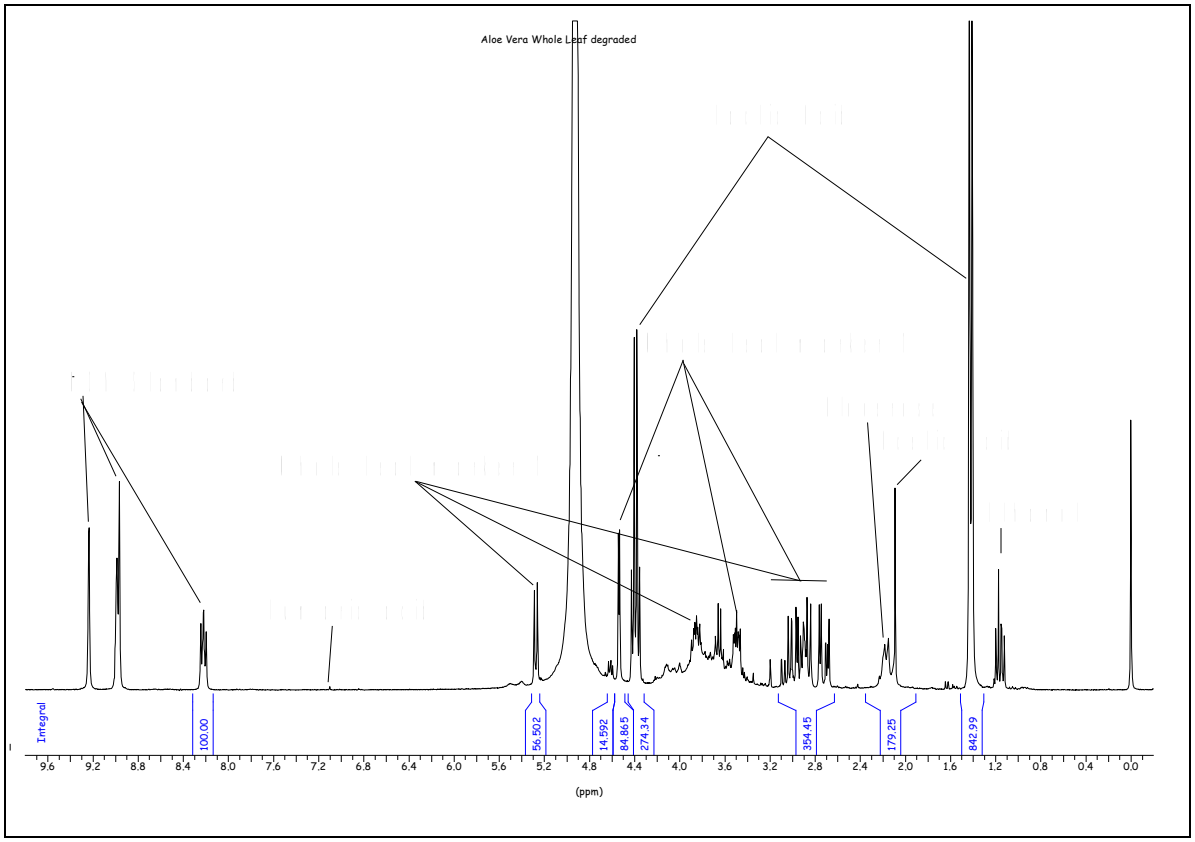

Fig. 9 For comparison: ^{1}H-NMR spectrum of Aloe Vera Whole Leaf degraded

6 PERSONNEL

Study director:	Dr. Bernd Diehl,	Chemist
Co-worker:	Andreas Beyer,	Biological technical assistant

All are staff members of the test facility.

7 CONFIRMATION OF THE STUDY REPORT

Date: 14 March 2011 **Study director:** _____ Dr. Bernd Diehl	**Company stamp:**

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