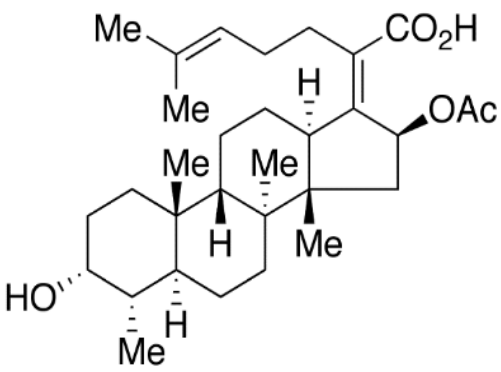
Chembuild Pharma Private Limited
04, VPO Adamwal, Hoshiarpur -146001, Punjab
E-mail : chembuildmktg@gmail.com
Mobile : +91-9779770067

Certificate of Analysis

Product Name: Fusidic Acid Impurity M		**CAS # : 1013937-16-0**
Synonyms	11-Deoxy Fusidic Acid; (Z)-2-((3R,4S,5S,8S,9S,10S,13R,14S,16S)-16-acetoxy-3-hydroxy-4,8,10,14-tetramethyldodecahydro-1H-cyclopenta[a]phenanthren-17(2H,10H,14H)-ylidene)-6-methylhept-5-enoic acid; (3α,4α,8α,9β,13α,14β,16β,17Z)-16-(Acetyloxy)-3-hydroxy-29-nordammara-17(20),24-dien-21-oic Acid	
Catalogue #	CHB 06 01367	**Structure** 
Lot #	CHB-FUS-13	
Chemical Formula	$C_{31}H_{48}O_5$	
Molecular Weight	500.71	
Shipping & Storage Condition	2^0C to 8^0C shipping in dark condition. Store in airtight container protected from light and moisture at -5^0-0^0C	

Analytical Test Result

S. No.	Test	Result
1.	Description	Pale Yellow Semi Solid
2.	Identification i) By IR ii) By ^{1}H-NMR iii) By MASS	 Conforms to structure Conforms to structure Conforms to structure
3.	Solubility	Acetonitrile
4.	Chromatographic Purity (% by HPLC)	95.82%
5.	TGA Value	0.92%
6.	% Potency	Chromatographic Purity% - TGA Value % 95.82% - 0.92% = 94.9%

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Caution	Do not attempt to dry the material. Use as such. Handle under inert atmosphere.
Date of Release	26-July-2021
Validity	24 months from the date of release

Attachments	HPLC Chromatogram, MS Spectra, NMR, IR, TGA

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Checked by: Authorized signatory:

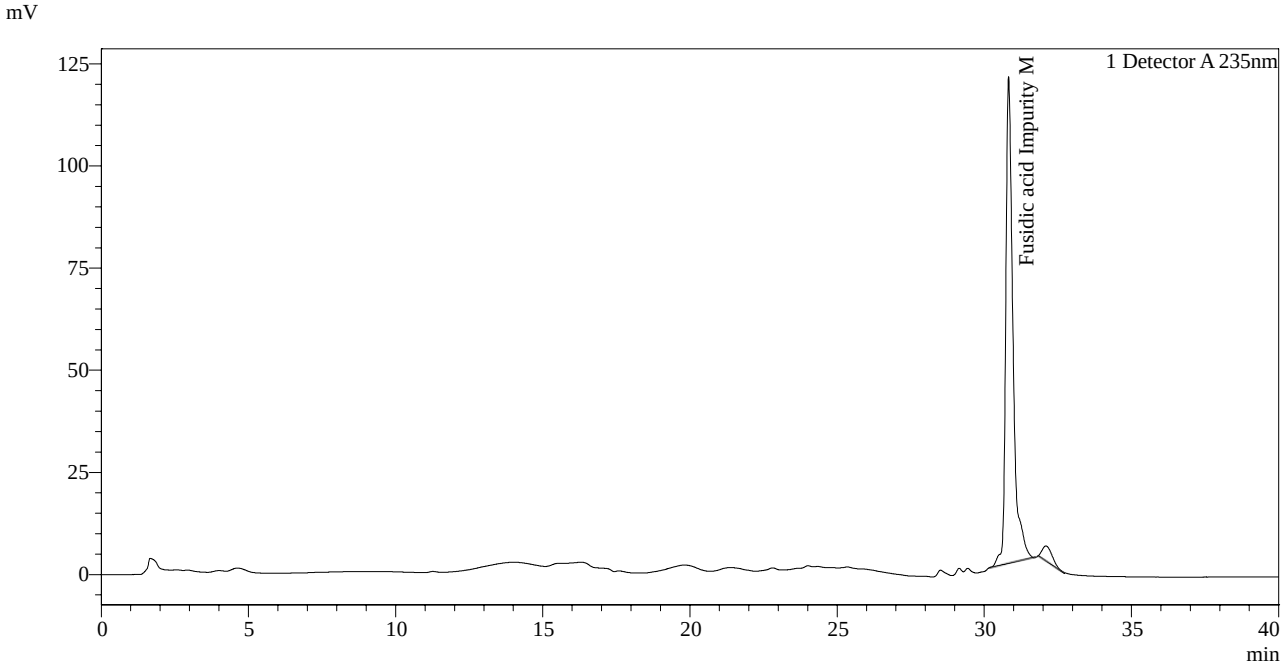
Status:Manual Integration

Chembuild Pharma Pvt. Ltd.
Analytical Test Report

Sample Information LC_008_FUS_21072021_023.lcd

Acquired by	: Common user
Sample Name	: Fusidic acid Impurity M
Sample ID	: CHB-FUS-13
Data File	: LC_008_FUS_21072021_023.lcd
Method File	: FUSIDIC_ACID_EP_20072021.lcm
Batch File	: Fusidic acid_21072021.lcb
Date Acquired	: 7/21/2021 5:24:51 PM
Date Processed	: 7/26/2021 10:02:56 AM

Chromatogram
LC_008_FUS_21072021_023.lcd

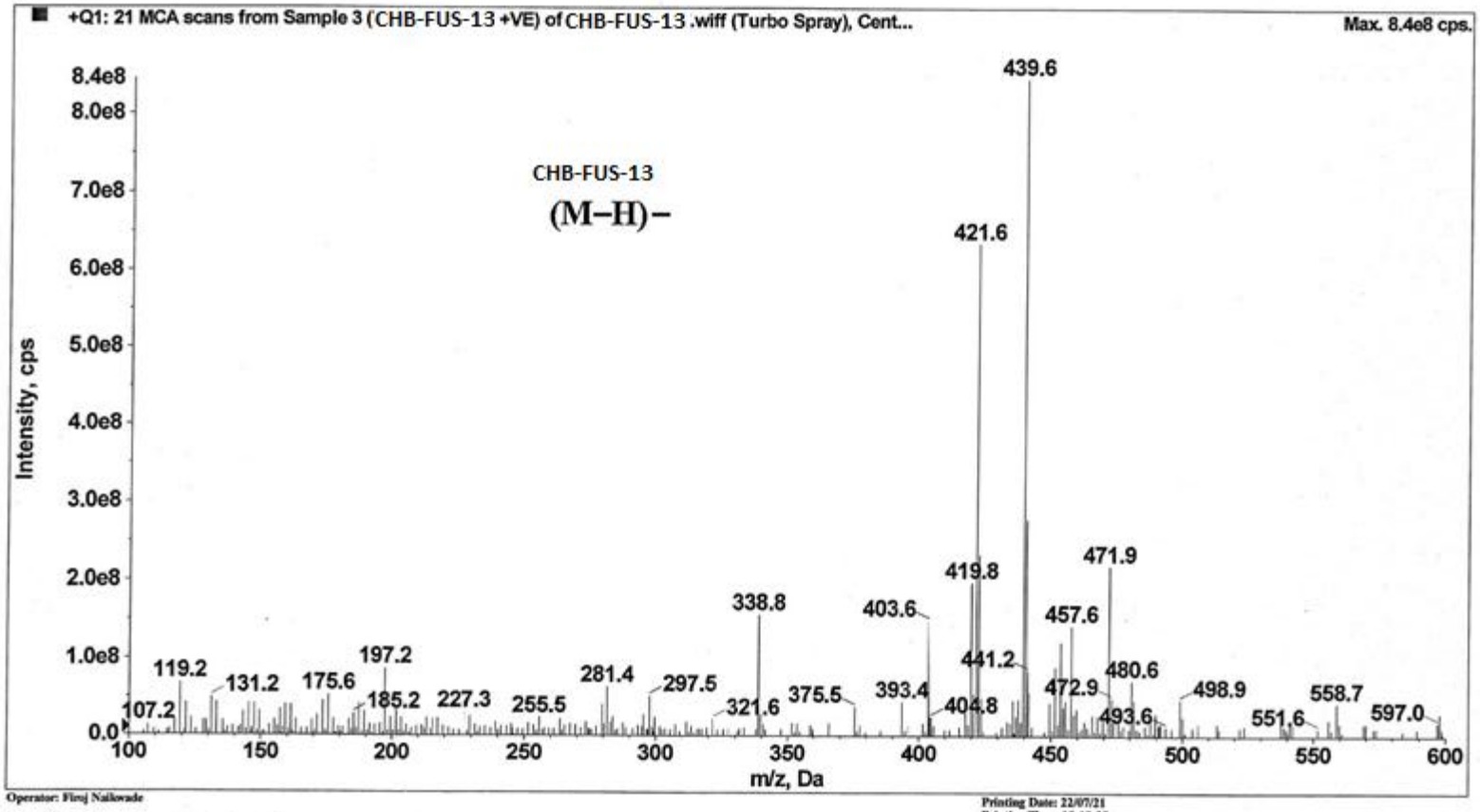


Peak Table LC_008_FUS_21072021_023.lcd

Detector A 235nm

Name	Ret. Time	Area	Area%
Fusidic acid Impurity M	30.828	2023305	95.826
	32.101	88121	4.174
		2111426	100.000

Instrument ID :- PCLPL-INS-1704
Batch Name: ManualTune.bat
Results Path: N/A



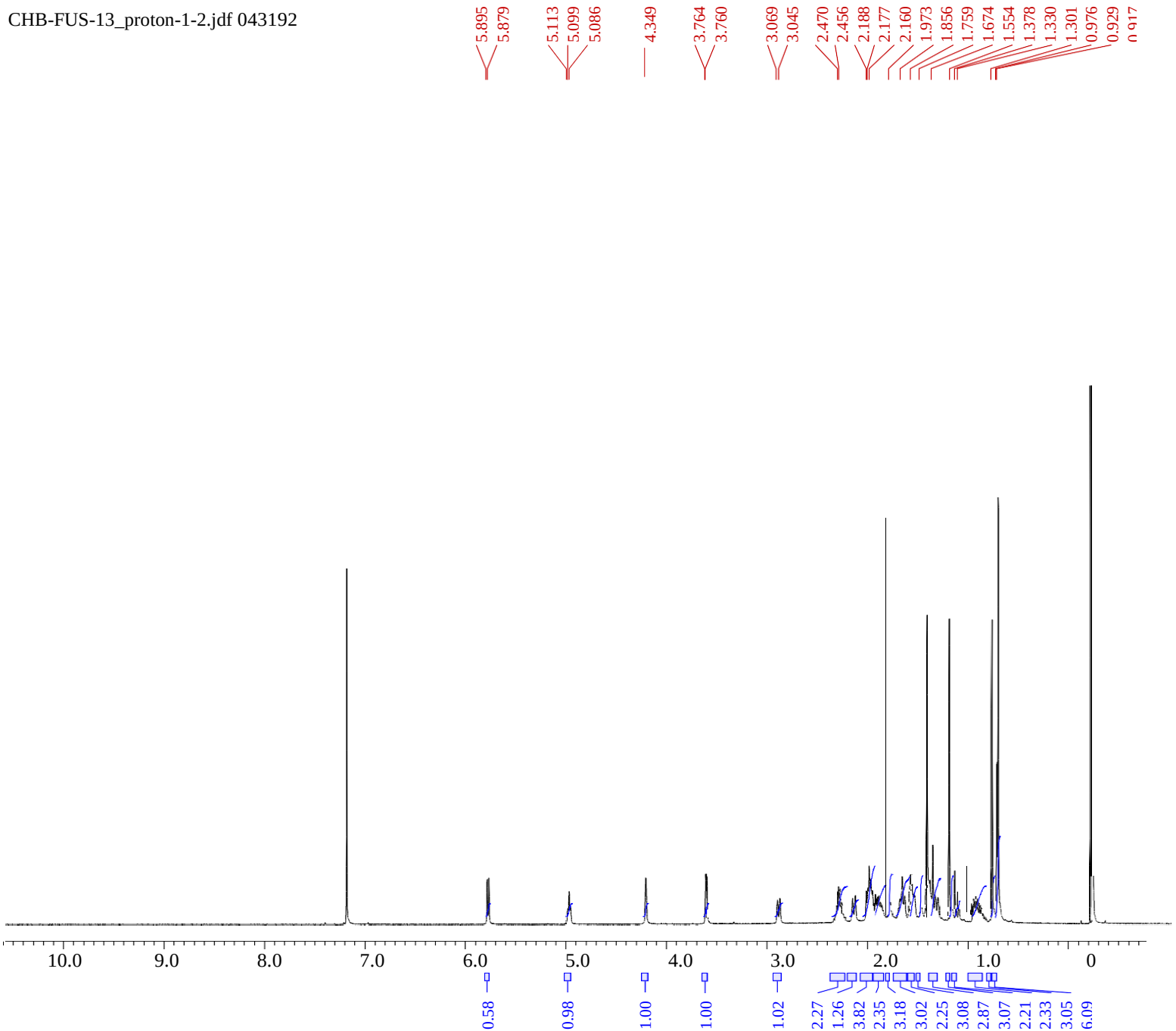
Operator: Firoj Naikwade

Note: Result table, regression and all the relevant chromatograms are verified.

Printing Date: 22/07/21
Printing Time: 18:18:28
Page 1 of 1

Prepared By: 22/07/21

CHB-FUS-13_proton-1-2.jdf 043192



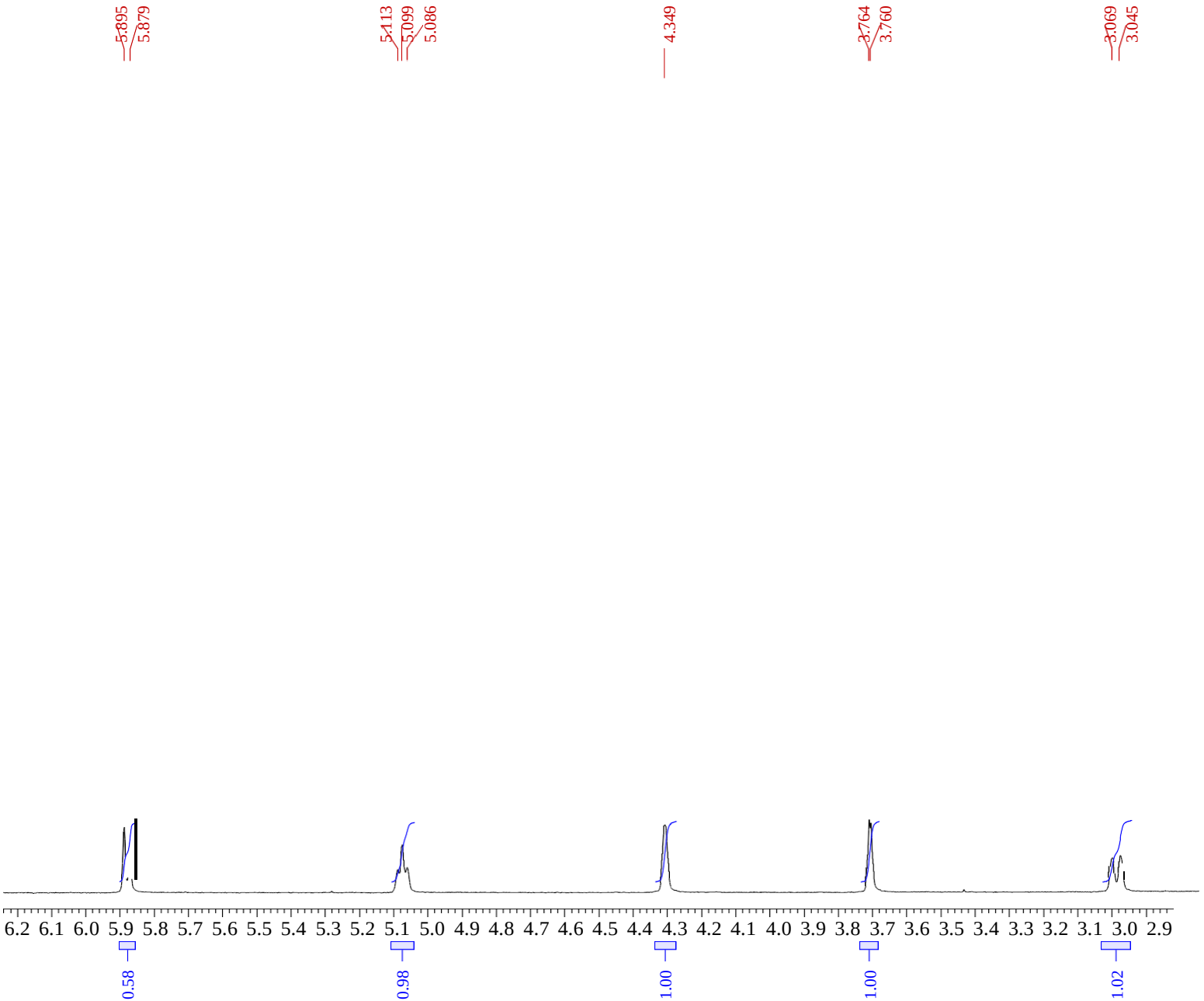
X : ppm : Proton

```
---- PROCESSING PARAMETERS ----
sexp( 0.2[Hz], 0.0[s] )
trapezoid( 0[%], 0[%], 80[%], 100[%] )
zerofill( 1 )
fft( 1, TRUE, TRUE )
machinephase
ppm
auto_reference( 5[%], TRUE )

Derived from: CHB-FUS-13_proton-1-1.
```

```
Filename          = CHB-FUS-13_proton
Instrument        - NMR-500MHz(JEOL)
Instrument id     - NMR-02
Author            = brm1440
Reviewed by       - Anil
Solvent           = CHLOROFORM-D
Spectrometer      = JNM-ECZ500R/S1
Experiment        = proton.jxp
Creation_Time     = 20-JUL-2021 01:53:44
Acquisition Parameter
X_Domain          = Proton
X_Offset          = 7.0[ppm]
X_Sweep           = 11.28158845[kHz]
Scans             = 16
Relaxation_Delay  = 2[s]
```

CHB-FUS-13_proton-1-2.jdf 043192




```
---- PROCESSING PARAMETERS ----
sexp( 0.2[Hz], 0.0[s] )
trapezoid( 0[%], 0[%], 80[%], 100[%] )
zerofill( 1 )
fft( 1, TRUE, TRUE )
machinephase
ppm
auto_reference( 5[%], TRUE )

Derived from: CHB-FUS-13_proton-1-1.
```

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Filename          = CHB-FUS-13_proton
Instrument        - NMR-500MHz(JEOL)
Instrument id     - NMR-02
Author            = brm1440
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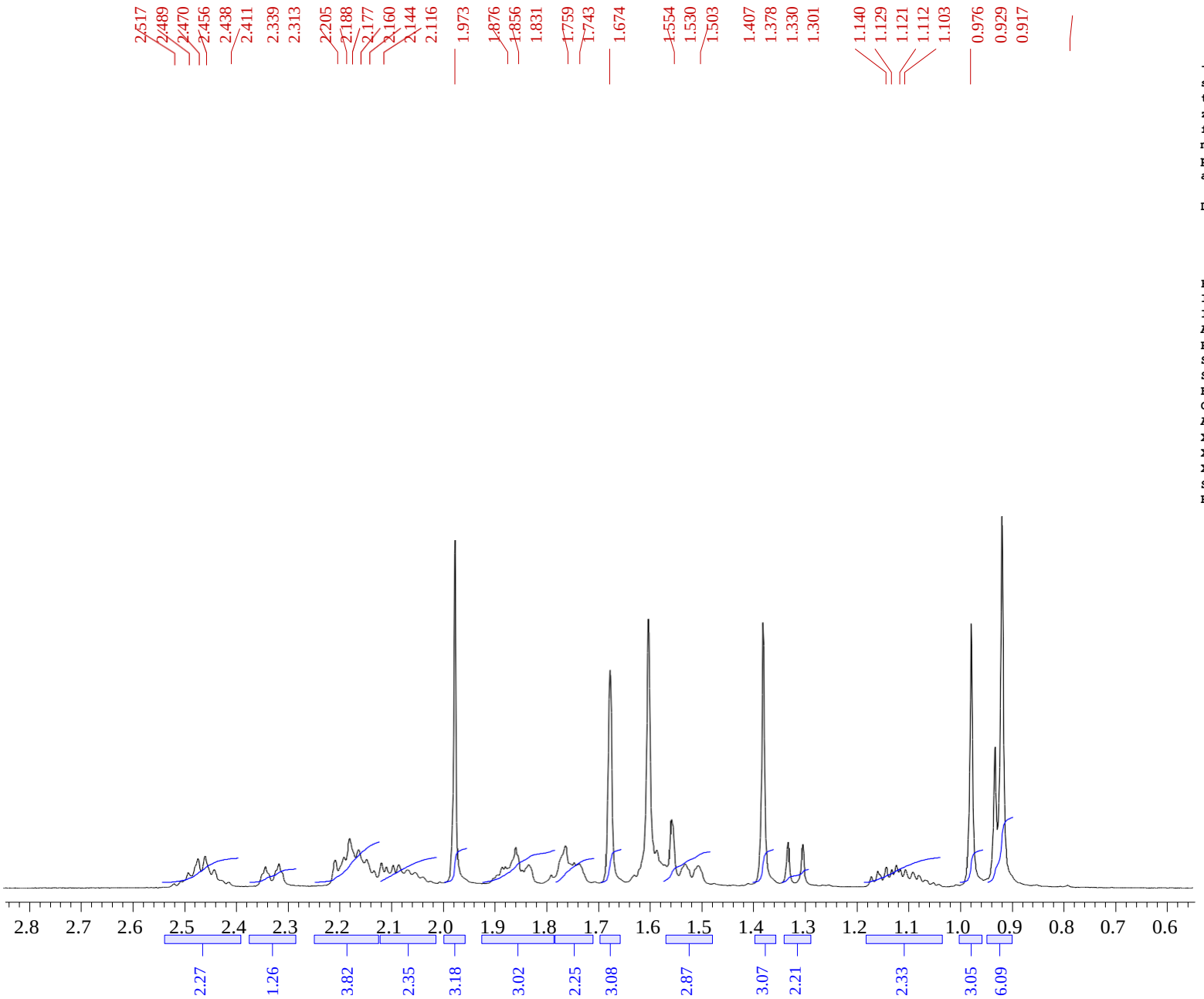
Scans             = 16
Relaxation_Delay = 2[s]
```

CHB-FUS-13_proton-1-2.jdf 043192



X : ppm : Proton

```
---- PROCESSING PARAMETERS ----
sexp( 0.2[Hz], 0.0[s] )
trapezoid( 0[%], 0[%], 80[%], 100[%] )
zerofill( 1 )
fft( 1, TRUE, TRUE )
machinephase
ppm
auto_reference( 5[%], TRUE )

Derived from: CHB-FUS-13_proton-1-1.
```

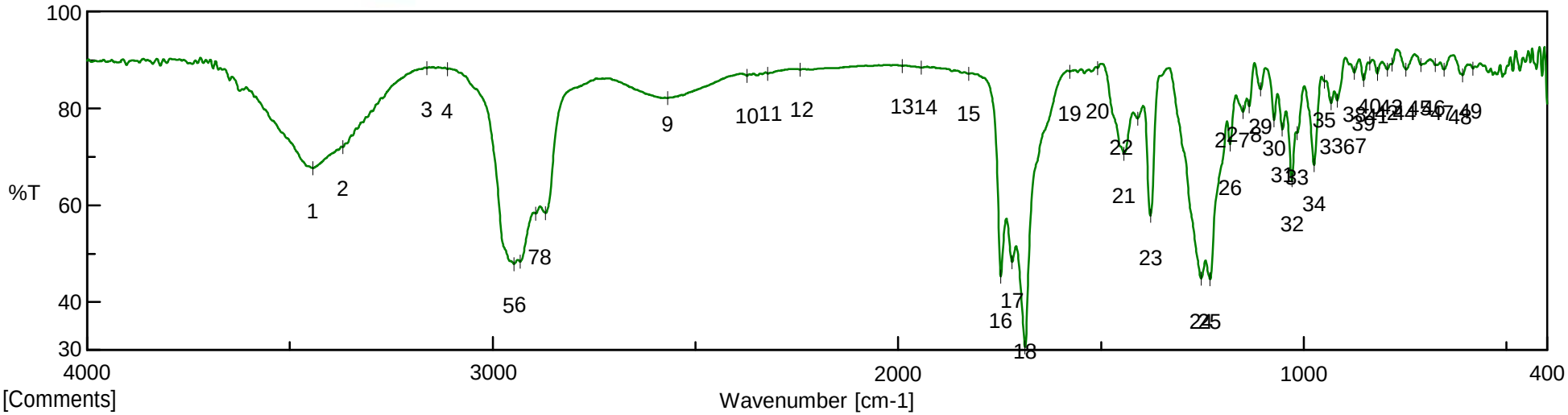
```
Filename          =  CHB-FUS-13_proton
Instrument        - NMR-500MHz(JEOL)
Instrument id     - NMR-02
Author            = brm1440
Reviewed by       - Anil
Solvent           = CHLOROFORM-D
Spectrometer      = JNM-ECZ500R/S1
Experiment        = proton.jxp
Creation_Time     = 20-JUL-2021 01:53:44
Acquisition Parameter
X_Domain          = Proton
X_Offset          = 7.0[ppm]
X_Sweep           = 11.28158845[kHz]
Scans             = 16
Relaxation_Delay = 2[s]
```

J-Coupling Analysis Report

Path = \\dc\$nmr-500$\Raw Data\Jul-2021\CHB-FUS-13_proton-1-2.jdfPosition

Integral	Pattern		J
5.89[ppm]	1	d	J1 = 8.3[Hz]
5.10[ppm]	1	t	J1 = 6.9[Hz]
4.35[ppm]	1	s	
3.76[ppm]	1	d	J1 = 2.1[Hz]
3.06[ppm]	1	d	J1 = 11.7[Hz]
2.46[ppm]	2	m	
2.33[ppm]	1	d	J1 = 13.1[Hz]
2.17[ppm]	4	m	
2.07[ppm]	2	m	
1.97[ppm]	3	s	
1.83[ppm]	3	m	
1.75[ppm]	2	m	
1.67[ppm]	3	s	
1.53[ppm]	3	t	J1 = 12.7[Hz]
1.38[ppm]	3	s	
1.32[ppm]	1	d	J1 = 14.5[Hz]
1.11[ppm]	2	m	
0.98[ppm]	3	s	
0.92[ppm]	6	d	J1 = 6.2[Hz]

SAPALA ORGANICS PVT LTD



[Comments]

Sample name	CHB-FUS-13
Comment	KBr Pellet AR#043193
User	Laxminarayana
Division	QC
Company	Sapala Organics Pvt Ltd

[Measurement Information]

Model Name	FT/IR-4100typeA
Serial Number	B088661016

Measurement Date 20/Jul/2021 12:50

Light Source	Standard
Detector	TGS
Accumulation	16
Resolution	4 cm-1
Zero Filling	On
Apodization	Cosine
Gain	Auto (16)
Aperture	Auto (7.1 mm)
Scanning Speed	Auto (2 mm/sec)
Filter	Auto (30000 Hz)

Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3443.28	67.5967	2	3369.03	71.8959	3	3162.69	88.2513	4	3111.58	88.061
5	2947.66	47.7667	6	2932.23	48.245	7	2893.66	58.1731	8	2869.56	58.3017
9	2568.72	82.0532	10	2372.98	86.6808	11	2321.87	87.1319	12	2241.84	87.9498
13	1990.18	88.7054	14	1942.93	88.3268	15	1826.26	87.085	16	1747.19	45.1276
17	1719.23	48.1899	18	1687.41	30.5817	19	1576.52	87.5534	20	1508.06	88.3121
21	1443.46	70.5417	22	1409.71	77.8387	23	1377.89	57.7011	24	1253.5	44.7525
25	1231.33	44.5917	26	1182.15	72.4336	27	1149.37	79.2147	28	1134.9	80.3977
29	1106.94	83.848	30	1073.19	77.462	31	1052.94	75.6162	32	1029.8	65.1142
33	1016.3	74.8676	34	974.84	68.2265	35	948.806	85.499	36	932.414	81.0349
37	917.95	81.5221	38	875.524	87.3368	39	852.382	85.7954	40	837.919	89.2515
41	818.634	87.1201	42	794.528	87.967	43	782.958	89.0589	44	748.245	87.961
45	711.604	88.9246	46	675.928	88.8778	47	654.715	87.9681	48	608.431	86.8089
49	583.361	88.1741									

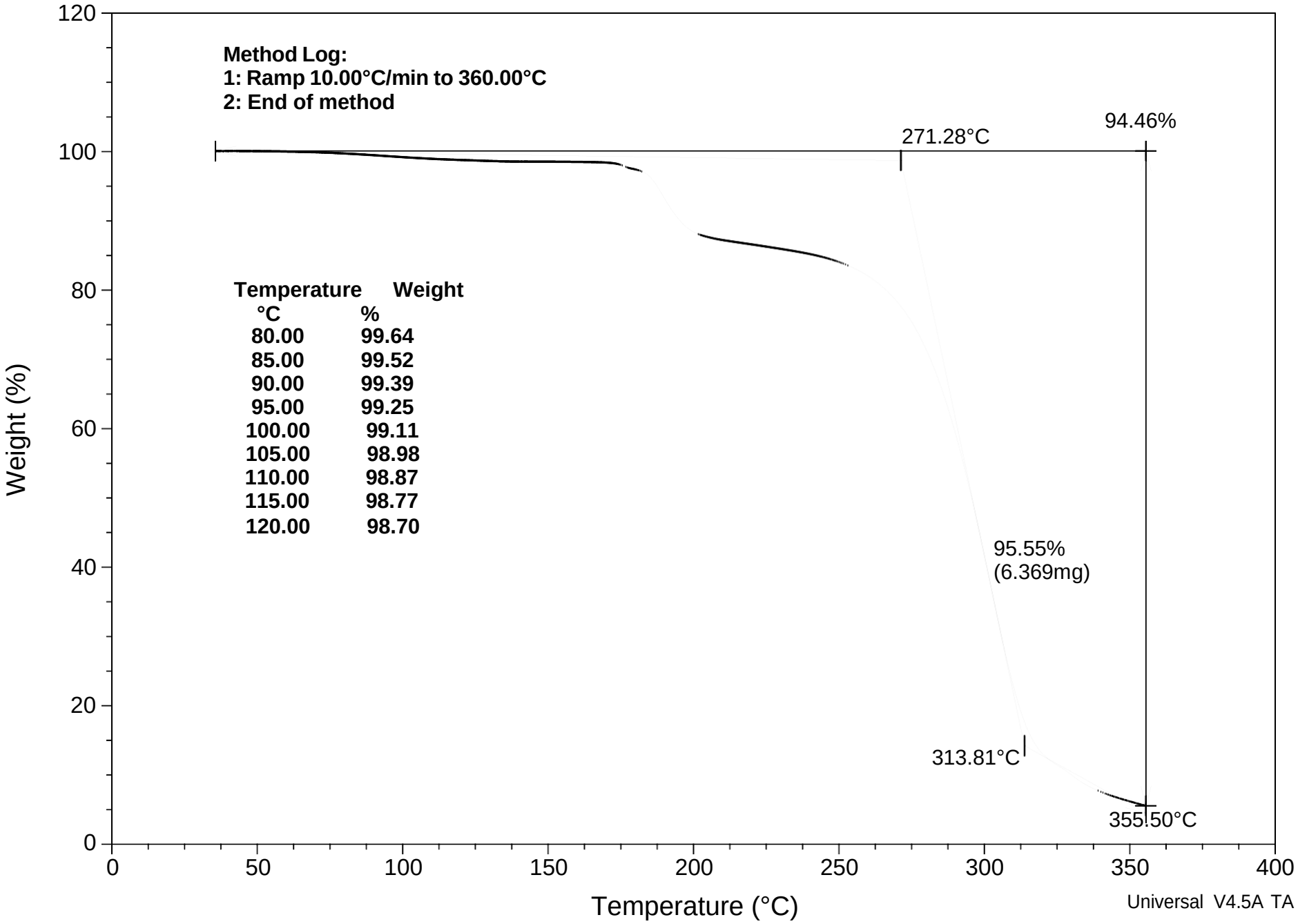
Analysed by:

Reviewed by:

Sample: CHB-FUS-13
Size: 6.6660 mg
Method: Ramp

TGA

File: TAEXPDB 16267 58509
Operator: RAJU1338
Run Date: 20-Jul-2021 11:40
Instrument: TGA Q500 V20.13 Build 39



Temperature °C	Weight %
80.00	99.64
85.00	99.52
90.00	99.39
95.00	99.25
100.00	99.11
105.00	98.98
110.00	98.87
115.00	98.77
120.00	98.70

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STRUCTURE ELUCIDATION REPORT OF

Fusidic Acid Impurity M

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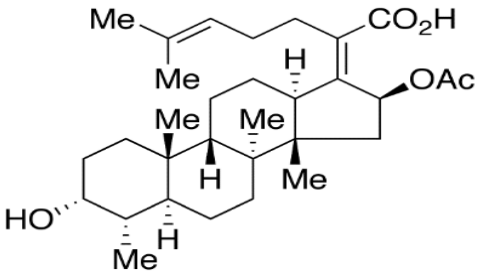
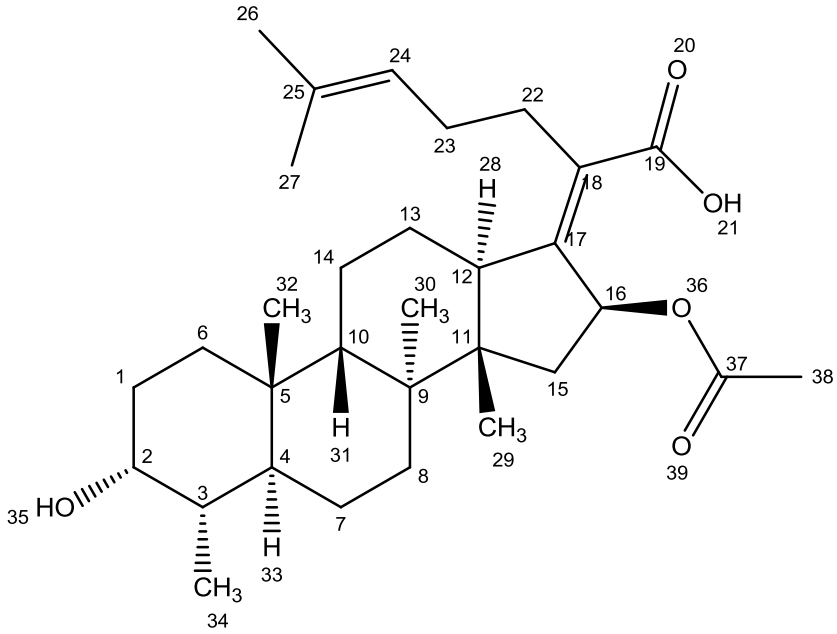
STRUCTURE ELUCIDATION REPORT

PRODUCT INFORMATION		
Name of Impurity	**:**	Fusidic Acid Impurity M
Name of drug Substance	**:**	Fusidic Acid
Chemical Name of Impurity	:	11-Deoxy Fusidic Acid; (Z)-2-((3R,4S,5S,8S,9S,10S,13R,14S,16S)-16-acetoxy-3-hydroxy-4,8,10,14-tetramethyldodecahydro-1H-cyclopenta[a]phenanthren-17(2H,10H,14H)-ylidene)-6-methylhept-5-enoic acid; (3α,4α,8α,9β,13α,14β,16β,17Z)-16-(Acetyloxy)-3-hydroxy-29-nordammara-17(20),24-dien-21-oic Acid
Structure of impurity	**:**	
Molecular formula	:	$C_{31}H_{48}O_5$
Molecular weight	:	500.71
Batch No.	:	CHB-FUS-13
Solubility	:	Acetonitrile.

STRUCTURE ELUCIDATION REPORT

NMR		
^{1}H NMR	**:**	^{1}H NMR spectrum of Fusidic Acid Impurity M recorded in CDCl3 and at 500 MHz instruments. Refer table for assignments.



S. No.	Chemical Shift Value δ (ppm)	Multiplicity	No. of Protons	Assignments
1.	0.917-0.929	d	6	26,27
2.	0.929-0.976	d	3	34
3.	1.103-1.140	m	2	31,33
4.	1.301-1.378	t	2	14
5.	1.407	s	3	29
6.	1.530	s	3	30
7.	1.674	s	3	32
8.	1.743-1.759	d	2	13
9.	1.831-1.876	m	4	7,8
10.	1.973	s	3	38
11.	2.116-2.144	d	2	15

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STRUCTURE ELUCIDATION REPORT

12.	2.160-2.205	m	4	22,23
13.	2.313-2.339	t	2	3,28
14.	2.411-2.517	m	4	1,6
15.	3.045-3.069	d	1	2
16.	4.349	s	1	16
17.	5.086-5.113	t	1	24

MASS	**:**	Mass spectrum of Fusidic Acid Impurity M gave M/Z peak at $(M-H)^-$ = 498.9m/z in the Negative mode i.e. conform with mass of Fusidic Acid Impurity M 500.35
Chromatographic purity	**:**	95.82%

TGA Calculation	**:**	$X1=80.00^0C$ $X2=120.00^0C$ Y1=99.64% Y2=98.72% WT Loss from 80^0C to 120^0C Y1-Y2 = 99.64% - 98.72% = 0.92%

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STRUCTURE ELUCIDATION REPORT

FTIR		
S.No	**Functional group**	**Frequency (Cm^{-1})**
1	-O-H Streching	3443.28
2.	-C-H Streching (Aliphatic Alkane)	2947.66
3.	-C-H Streching (Cyclic alkane)	2869.56
4.	-C=O Streching (Acid)	1747.19
5.	-C=O Streching (Ester)	1687.41