

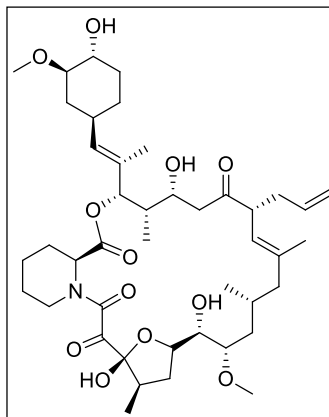
Analysis Date: 14-01-2025

Re-test Date: 15-01-2028

## DESMETHYL TACROLIMUS

### Identification

<b>Chemical Name</b>	: (1R,9S,12S,13R,14S,17R,18E,21S,23S,24R,25S,27R)-1,14-dihydroxy-12-[(E)-1-[(1R,3R,4R)-4-hydroxy-3-methoxy cyclo hexyl]prop-1-en-2-yl]-23,25-dimethoxy-13,21,27-trimethyl -17-prop-2-enyl-11,28-dioxa-4-azatricyclo[22.3.1.04,9]octacos-18-ene-2,3,10,16-tetrone
<b>CAT No</b>	: ALL-T03931
<b>CAS No</b>	: 132172-14-6
<b>Molecular Formula</b>	: C43H67NO12
<b>Molecular Weight</b>	: 789.99



### Analytical Information

<b>Batch Code</b>	: ALL-T03931	<b>HPLC Purity</b>	: 96.39 %
<b>Solubility</b>	: MeOH: ACN	<b>Potency</b>	: 95.70 %
<b>Appearance of Product</b>	: Off White Solid	<b>Mass</b>	: Confirm
<b>Long Term Storage</b>	: 2-8 0C	<b>IR Analysis</b>	: Confirm
<b>Weight Loss By TGA</b>	: 0.183 %	<b>1HNMR</b>	: Confirm
<b>Residue Of Ignition</b>	: 0.529 %	<b>13C NMR</b>	: Confirm

### Additional Information

%Potency = [100 - ( Inorganic Impurities % + Water %) × Chromatographic Purity%]/100 =

$$[100 - (0.183+0.529) \times 96.39]/100 = 95.70 \%$$

**Recommendation** : Released

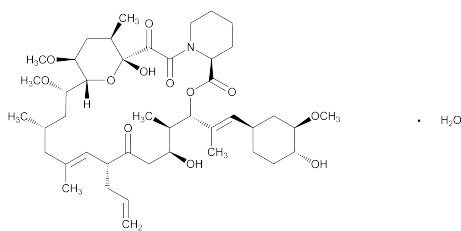
	Department	Name	Signature
Prepared and Reviewed by	Analytical	Mr. Swapnil Kausatkar Jr. Executive	
Approved By	QA&QC	Dr. Ashish Keche Director QA&QC	

**Attachment** : HPLC, Mass, 1H NMR, IR, TGA, Q-NMR, 13C NMR, UV REPORT

**Shipping Condition** : All Product are stable to be shipped at room temperature, unless otherwise specified

#### Corporate Office

## Tacrolimus



$C_{44}H_{69}NO_{12} \cdot H_2O$  822.03

15,19-Epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-, monohydrate, [3*S*\*,3*R*\*,*E*(1*S*\*,2*S*\*,4*S*\*)],4*S*\*,5*R*\*,8*S*\*,9*E*,12*R*\*,14*R*\*,15*S*\*,16*R*\*,18*S*\*,19*S*\*,26*aR*\*];  
(-)-(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone monohydrate CAS RN@: 109581-93-3; UNII: WM0HAQ4WNM.

### DEFINITION

Tacrolimus contains NLT 98.0% and NMT 102.0% of tacrolimus ( $C_{44}H_{69}NO_{12}$ ), calculated on the anhydrous and solvent-free basis.

### IDENTIFICATION

- A. SPECTROSCOPIC IDENTIFICATION TESTS** (197), *Infrared Spectroscopy*: 197M
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

Protect solutions containing tacrolimus from light.

**Solution A:** 6 mM phosphoric acid

**Solution B:** Acetonitrile and *tert*-butyl methyl ether (81:19)

**Solution C:** *Solution A* and *Solution B* (4:1)

**Solution D:** *Solution A* and *Solution B* (1:4)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution C (%)	Solution D (%)
0	72	28
30	72	28
53	15	85
54	72	28
60	72	28

**Diluent:** Acetonitrile and water (7:3)

**System suitability solution:** 3 mg/mL of USP Tacrolimus System Suitability Mixture RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.

**Standard solution:** 3 mg/mL of USP Tacrolimus RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.

**Sample solution:** 3 mg/mL of Tacrolimus in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 15-cm; 3-μm packing L1

**Temperatures**

**Column:** 60°

**Autosampler:** 4°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 3* for relative retention times.]

### Suitability requirements

**Resolution:** NLT 3.0 between ascomycin and tacrolimus, *System suitability solution*

**Relative standard deviation:** NMT 1.0% for the sum of the responses of tacrolimus, tacrolimus open ring, and tacrolimus 19-epimer, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of tacrolimus ( $C_{44}H_{69}NO_{12}$ ) in the portion of Tacrolimus taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = sum of the peak responses of tacrolimus open ring, tacrolimus 19-epimer, and tacrolimus from the *Sample solution*

$r_s$  = sum of the peak responses of tacrolimus open ring, tacrolimus 19-epimer, and tacrolimus from the *Standard solution*

$C_s$  = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Tacrolimus in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis

### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **ORGANIC IMPURITIES, PROCEDURE 1**

Use *Organic Impurities, Procedure 1* when the impurity profile includes tacrolimus methylacrylaldehyde and tacrolimus diene. It is suggested that new columns be conditioned with about 500 mL of alcohol before use to meet the resolution criterion.

**Mobile phase:** Hexane, *n*-butyl chloride, and acetonitrile (7:2:1). Add *n*-butyl chloride to hexane, and mix well before adding acetonitrile. After adding acetonitrile, mix the *Mobile phase* for 2 h to get a clear solution. Any deviations from the ratio of components in the *Mobile phase* and the order of mixing will result in a two-phase solution.

**System suitability solution:** 0.1 mg/mL each of USP Tacrolimus RS and USP Tacrolimus Related Compound A RS in *Mobile phase*

**Sample solution:** 2.0 mg/mL of Tacrolimus in *Mobile phase*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 225 nm

**Column:** Two 4.6-mm × 25-cm columns; 5-μm packing L20

**Column temperature:** 28 ± 2°

**Flow rate:** 1.5 mL/min. Adjust the *Flow rate* so that the retention time of tacrolimus is approximately 15 min.

**Injection volume:** 20 µL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 1.1 between tacrolimus and tacrolimus related compound A

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Tacrolimus taken:

$$\text{Result} = (r_u / F_i) \times \{1 / [r_T + \Sigma(r_u / F_i)]\} \times 100$$

$r_u$  = peak response of each impurity from the *Sample solution*

$F_i$  = relative response factor for each corresponding impurity (see *Table 2*)

$r_T$  = peak response of tacrolimus from the *Sample solution*

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tacrolimus methylacryl aldehyde <sup>a</sup>	0.55	16.7	0.2
Tacrolimus diene <sup>b</sup>	0.79	2.2	0.2
Tacrolimus impurity 1 <sup>c</sup>	0.96	1.0	0.2
Tacrolimus related compound A <sup>d</sup>	0.96	—	—
Tacrolimus	1.0	1.0	—
Tacrolimus 19-epimer <sup>d,e</sup>	1.1	—	—
Tacrolimus open ring <sup>d,f</sup>	1.3	—	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities <sup>g</sup>	—	—	0.3

<sup>a</sup> (E)-3-[[1R,3R,4R]-4-Hydroxy-3-methoxycyclohexyl]-2-methylacrylaldehyde.

<sup>b</sup> (14E,18E)-17-Allyl-1-hydroxy-12-[(E)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0<sup>4,9</sup>] octacos-14,18-diene-2,3,10,16-tetrone.

<sup>c</sup> Specified unidentified impurity.

<sup>d</sup> For informational purposes only; not to be reported.

<sup>e</sup> (3S,4R,5S,8R,9E,12S,14S,15R,16S,18R,19S,26aS)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a,hexadecahydro-5,19-dihydroxy-3-[(E)-2-[[1R,3R,4R]-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone.

<sup>f</sup> (3S,4R,5S,8R,12S,14S,15R,16S,18R,26aS,E)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26a-tetradecahydro-5,15,20,20-tetrahydroxy-3-[(E)-2-[[1R,3R,4R]-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-3H-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,19,21(4H,8H,20H,23H)-tetrone.

<sup>g</sup> Total impurities limit does not include tacrolimus open ring and tacrolimus 19-epimer.

**• ORGANIC IMPURITIES, PROCEDURE 2**

Use *Organic Impurities, Procedure 2* when the impurity profile includes ascomycin, desmethyl tacrolimus, tacrolimus

8-epimer, and tacrolimus 8-propyl analog. Protect solutions containing tacrolimus from light.

**Solution A, Solution B, Solution C, Solution D, Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 30 µg/mL of USP Tacrolimus RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.

**Reporting threshold solution:** 1.5 µg/mL of USP Tacrolimus RS in *Diluent*

**Peak identification solution 1:** 10 µg/mL of USP Tacrolimus 8-epimer RS in acetonitrile

**Peak identification solution 2:** 10 µg/mL of USP Tacrolimus 8-propyl Analog RS in acetonitrile

**System suitability**

[NOTE—Identify the related compounds by the relative retention times provided in *Table 3*.]

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 3.0 between tacrolimus and ascomycin, *System suitability solution*

**Relative standard deviation:** NMT 10.0% for the sum of the responses of tacrolimus and tacrolimus 19-epimer, *Standard solution*

**Analysis**

**Samples:** *Sample solution, Standard solution, Reporting threshold solution, Peak identification solution 1, and Peak identification solution 2*

Calculate the percentage of each impurity in the portion of Tacrolimus taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times 100$$

$r_u$  = peak response of each impurity from the *Sample solution*

$r_s$  = sum of the peak responses of tacrolimus 19-epimer and tacrolimus from the *Standard solution*

$C_s$  = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Tacrolimus in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 3*. Identify tacrolimus 8-epimer and tacrolimus 8-propyl analog using *Peak identification solution 1* and *Peak identification solution 2*. Report impurity peaks with responses NLT that of the peak in the *Reporting threshold solution* (0.05%). Disregard peaks with retention times less than 3 min.

**Table 3**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tacrolimus open ring <sup>a,b</sup>	0.52	—
Ascomycin 19-epimer (if present) <sup>c,d</sup>	0.54	0.1
Tacrolimus 19-epimer <sup>b,e</sup>	0.63	—
Ascomycin <sup>f</sup>	0.87	0.50
Desmethyl tacrolimus (if present) <sup>d,g</sup>	0.94	0.1
Tacrolimus	1.00	—
Tacrolimus 8-epimer <sup>h</sup>	1.28	0.15
Tacrolimus 8-propyl analog <sup>i</sup>	1.33	0.15
Any individual unspecified impurity	—	0.1

**Table 3** (continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities <sup>l</sup>	—	1.0

<sup>a</sup> (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26*aS*,*E*)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26*a*-tetradecahydro-5,15,20,20-tetrahydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,19,21(4*H*,8*H*,20*H*,23*H*)-tetrone.

<sup>b</sup> Tacrolimus open ring and tacrolimus 19-epimer are isomers of tacrolimus, which are present in equilibrium with the active ingredient. They are not to be reported as degradation products.

<sup>c</sup> (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26*aS*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

<sup>d</sup> If possible from the manufacturing process.

<sup>e</sup> (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26*aS*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

<sup>f</sup> (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

<sup>g</sup> (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-trimethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

<sup>h</sup> (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

<sup>i</sup> (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-Hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

<sup>l</sup> Total impurities limit does not include tacrolimus open ring and tacrolimus 19-epimer.

### SPECIFIC TESTS

- **OPTICAL ROTATION**, *Specific Rotation* (781*S*)  
Sample solution: 10 mg/mL in *N,N*-dimethylformamide  
Acceptance criteria: −110° to −115° on the “as-is” basis
- **WATER DETERMINATION**, *Method I* (921): NMT 4.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers. Store at controlled room temperature.
- **LABELING**: If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which test for *Organic Impurities* the article complies.

### Change to read:

#### • USP REFERENCE STANDARDS (11)

USP Tacrolimus RS

15,19-Epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-, monohydrate, [3*S*-[3*R*\*,*E*(1*S*\*,3*S*\*,4*S*\*)],4*S*\*,5*R*\*,8*S*\*,9*E*,12*R*\*,14*R*\*,15*S*\*,16*R*\*,18*S*\*,19*S*\*,26*aR*\*]−.

C<sub>44</sub>H<sub>69</sub>NO<sub>12</sub> · H<sub>2</sub>O 822.03

#### USP Tacrolimus Related Compound A RS

(*E*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-Hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

C<sub>43</sub>H<sub>69</sub>NO<sub>12</sub> ▲792.02▲ (ERR 1-Sep-2021)

#### USP Tacrolimus 8-epimer RS

(3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

C<sub>44</sub>H<sub>69</sub>NO<sub>12</sub> ▲804.03▲ (ERR 1-Sep-2021)

#### USP Tacrolimus 8-propyl Analog RS

(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-Hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

C<sub>44</sub>H<sub>71</sub>NO<sub>12</sub> 806.03

#### USP Tacrolimus System Suitability Mixture RS

This is a mixture of tacrolimus, ascomycin

(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

C<sub>43</sub>H<sub>69</sub>NO<sub>12</sub> 792.01

and tacrolimus 8-propyl analog

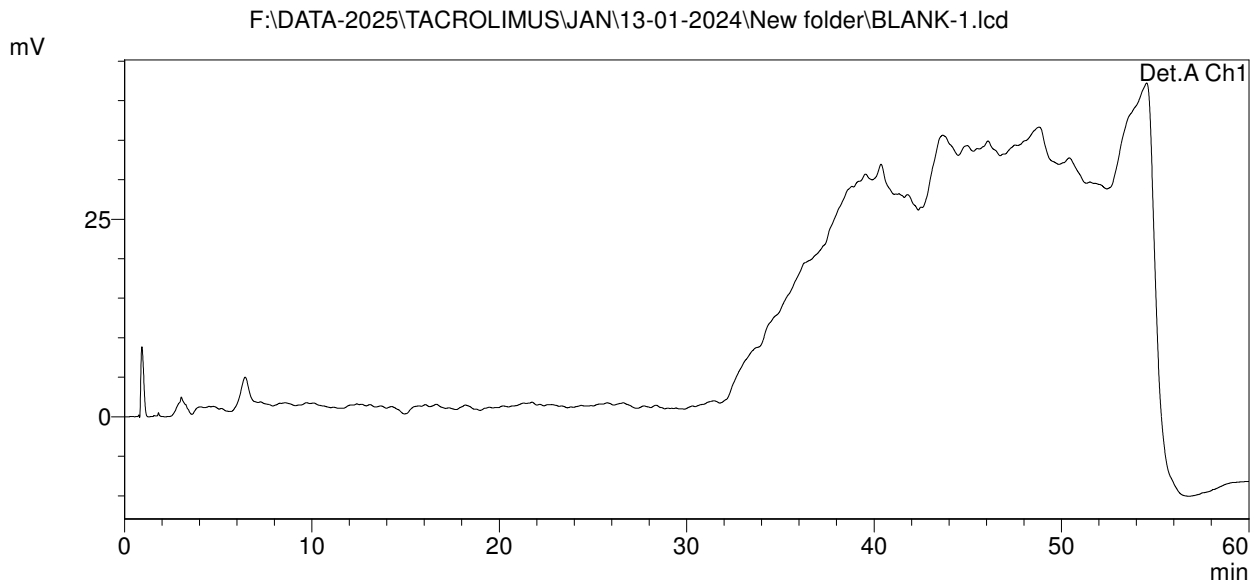
(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-Hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

C<sub>44</sub>H<sub>71</sub>NO<sub>12</sub> 806.03

**==== Shimadzu LCsolution Analysis Report ====**

Acquired by : Admin  
Sample Name : BLANK-1  
Sample ID : BLANK-1  
Tray# : 1  
Vail # : 1  
Injection Volume : 20 uL  
Data File Name : BLANK-1.lcd  
Method File Name : TACROLIMUS 60 MIN.lcm  
Batch File Name : SEQ.lcb  
Report File Name : Default.lcr  
Data Acquired : 1/13/2025 6:10:07 PM  
Data Processed : 1/13/2025 7:10:10 PM

---

**<Chromatogram>**

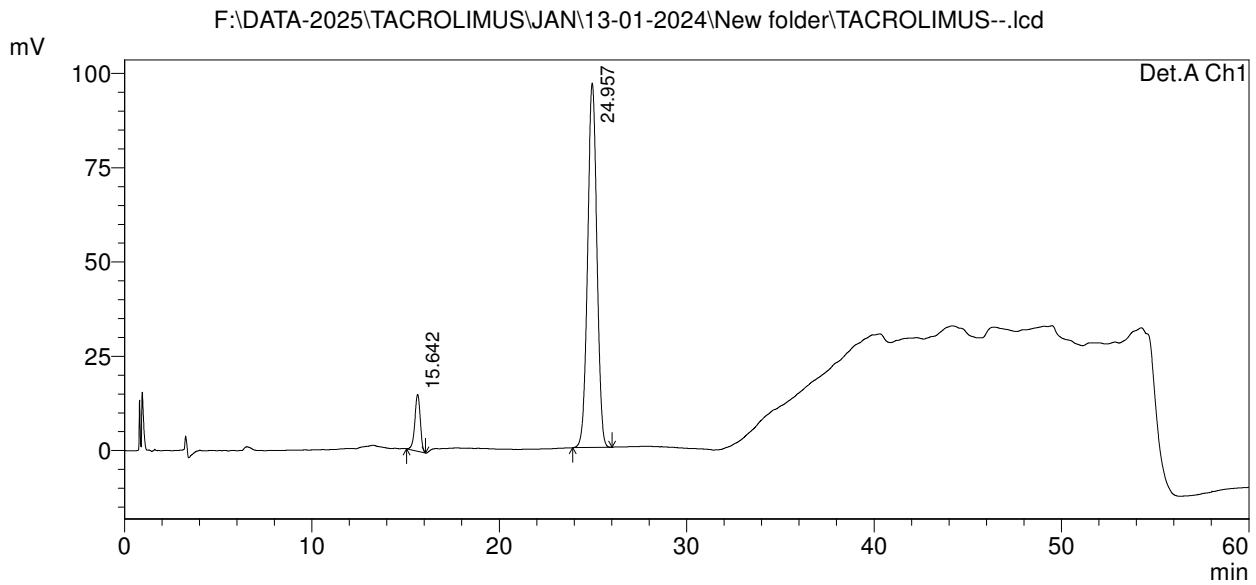
1 Det.A Ch1/220nm

# ==== Shimadzu LCsolution Analysis Report ====

F:\DATA-2025\tACROLIMUS\JAN\13-01-2024\New folder\tACROLIMUS--.lcd

Acquired by : Admin  
 Sample Name : TACROLIMUS  
 Sample ID : TACROLIMUS  
 Tray# : 1  
 Vial # : 2  
 Injection Volume : 20 uL  
 Data File Name : TACROLIMUS--.lcd  
 Method File Name : TACROLIMUS 60 MIN.lcm  
 Batch File Name : SEQ.lcb  
 Report File Name : Default.lcr  
 Data Acquired : 1/14/2025 10:14:17 AM  
 Data Processed : 1/14/2025 11:14:21 AM

## <Chromatogram>



1 Det.A Ch1/220nm

PeakTable

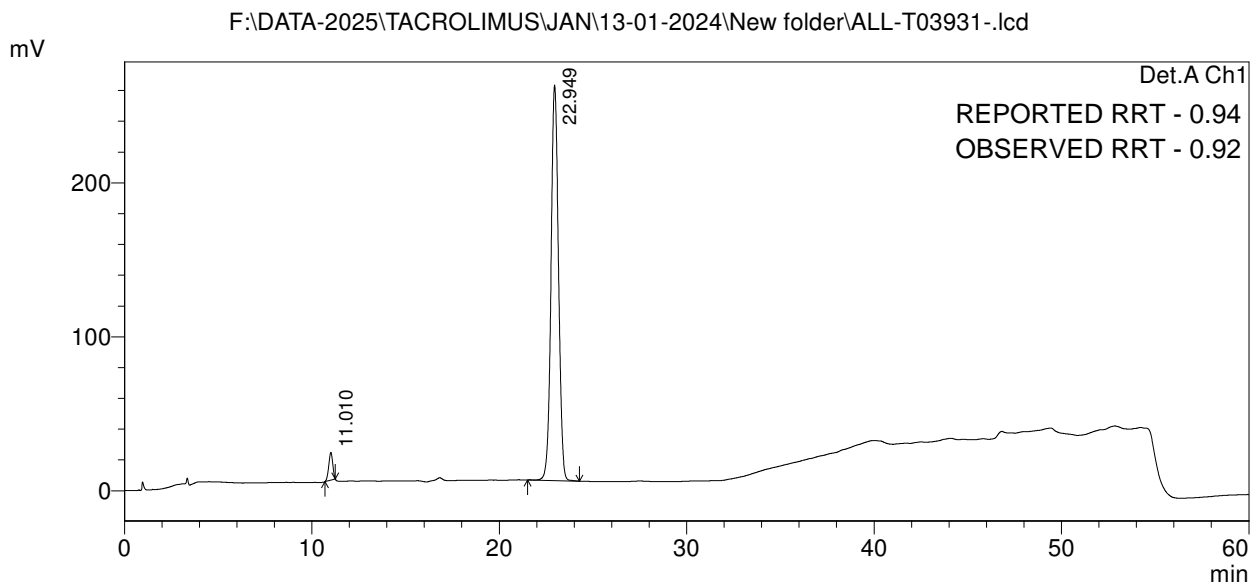
Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	15.642	303388	15074	8.377	13.494
2	24.957	3318189	96637	91.623	86.506
Total		3621576	111712	100.000	100.000

# ==== Shimadzu LCsolution Analysis Report =====

F:\DATA-2025\TACROLIMUS\JAN\13-01-2024\New folder\ALL-T03931-.lcd  
 Acquired by : Admin  
 Sample Name : ALL-T03931  
 Sample ID : ALL-T03931  
 Tray# : 1  
 Vial # : 11  
 Injection Volume : 20 uL  
 Data File Name : ALL-T03931-.lcd  
 Method File Name : TACROLIMUS 60 MIN.lcm  
 Batch File Name : SEQ.lcb  
 Report File Name : Default.lcr  
 Data Acquired : 1/14/2025 3:00:27 PM  
 Data Processed : 1/14/2025 4:00:30 PM

## <Chromatogram>



1 Det.A Ch1/220nm

PeakTable

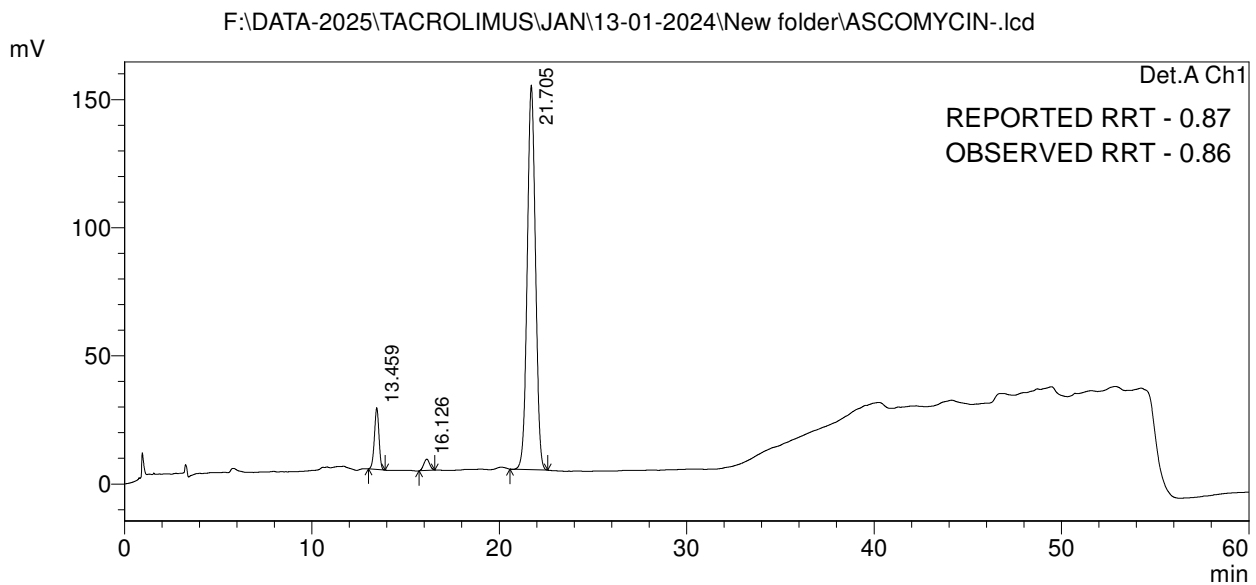
Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	11.010	266430	18091	3.610	6.578
2	22.949	7113125	256947	96.390	93.422
Total		7379555	275038	100.000	100.000

# ==== Shimadzu LCsolution Analysis Report ====

F:\DATA-2025\TACROLIMUS\JAN\13-01-2024\New folder\ASCOMYCIN-.lcd  
 Acquired by : Admin  
 Sample Name : ASCOMYCIN  
 Sample ID : ASCOMYCIN  
 Tray# : 1  
 Vial # : 3  
 Injection Volume : 20 uL  
 Data File Name : ASCOMYCIN-.lcd  
 Method File Name : TACROLIMUS 60 MIN.lcm  
 Batch File Name : SEQ.lcb  
 Report File Name : Default.lcr  
 Data Acquired : 1/14/2025 11:24:17 AM  
 Data Processed : 1/14/2025 12:24:19 PM

## <Chromatogram>



1 Det.A Ch1/220nm

PeakTable

Detector A Ch1 220nm

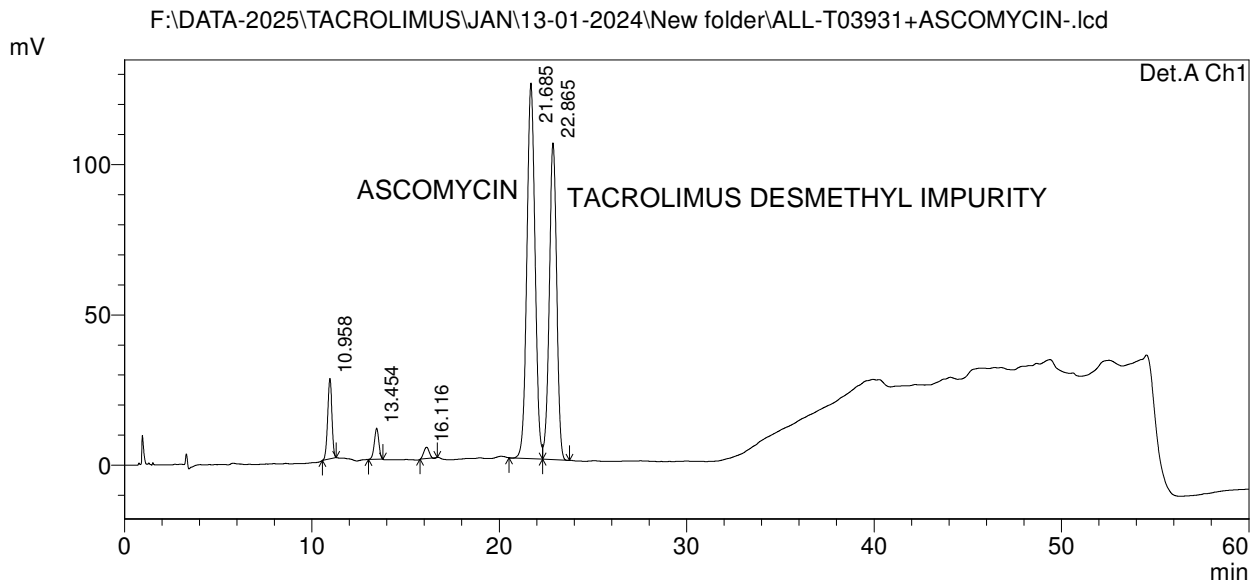
Peak#	Ret. Time	Area	Height	Area %	Height %
1	13.459	408199	24156	8.034	13.521
2	16.126	95983	4413	1.889	2.470
3	21.705	4576619	150082	90.077	84.008
Total		5080801	178652	100.000	100.000

# ==== Shimadzu LCsolution Analysis Report ====

F:\DATA-2025\TACROLIMUS\JAN\13-01-2024\New folder\ALL-T03931+ASCOMYCIN-.lcd

Acquired by : Admin  
 Sample Name : ALL-T03931+ASCOMYCIN  
 Sample ID : ALL-T03931+ASCOMYCIN  
 Tray# : 1  
 Vial # : 5  
 Injection Volume : 20 uL  
 Data File Name : ALL-T03931+ASCOMYCIN-.lcd  
 Method File Name : TACROLIMUS 60 MIN.lcm  
 Batch File Name : SEQ.lcb  
 Report File Name : Default.lcr  
 Data Acquired : 1/14/2025 12:49:59 PM  
 Data Processed : 1/14/2025 1:50:02 PM

## <Chromatogram>



1 Det.A Ch1/220nm

PeakTable

Detector A Ch1 220nm

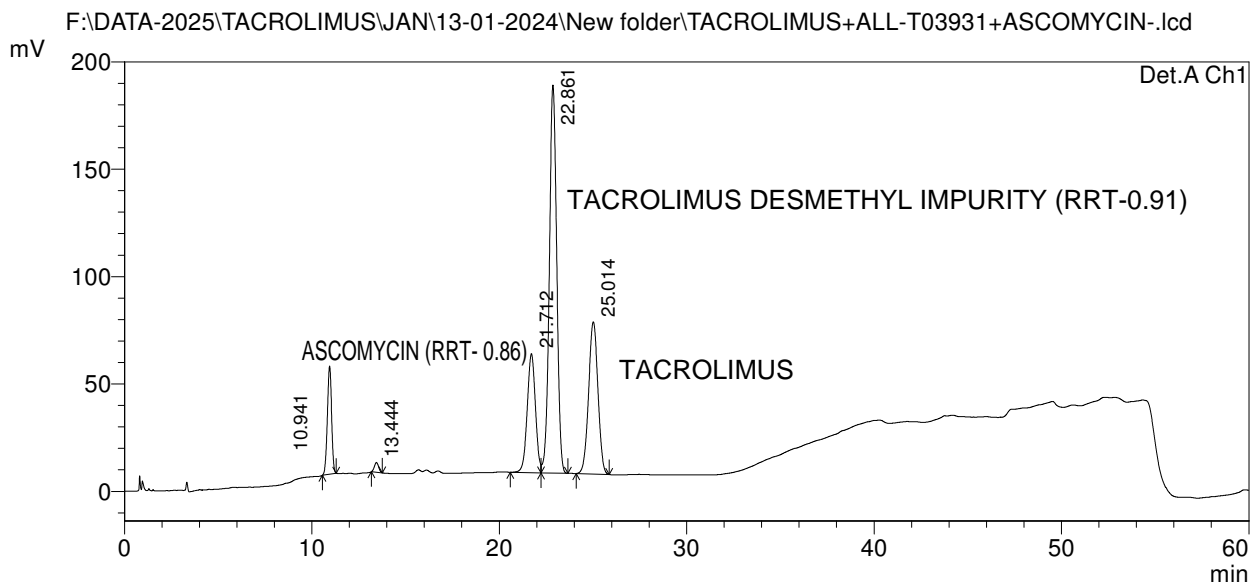
Peak#	Ret. Time	Area	Height	Area %	Height %
1	10.958	418267	26754	5.697	9.863
2	13.454	174644	10391	2.379	3.831
3	16.116	72777	3756	0.991	1.385
4	21.685	3787407	124988	51.584	46.078
5	22.865	2889106	105366	39.349	38.844
Total		7342200	271255	100.000	100.000

# ==== Shimadzu LCsolution Analysis Report =====

F:\DATA-2025\TACROLIMUS\JAN\13-01-2024\New folder\TACROLIMUS+ALL-T03931+ASCOMYCIN-.lcd

Acquired by : Admin  
 Sample Name : TACROLIMUS+ALL-T03931+ASCOMYCIN  
 Sample ID : TACROLIMUS+ALL-T03931+ASCOMYCIN  
 Tray# : 1  
 Vial # : 6  
 Injection Volume : 20 uL  
 Data File Name : TACROLIMUS+ALL-T03931+ASCOMYCIN-.lcd  
 Method File Name : TACROLIMUS 60 MIN.lcm  
 Batch File Name : SEQ.lcb  
 Report File Name : Default.lcr  
 Data Acquired : 1/14/2025 1:51:40 PM  
 Data Processed : 1/14/2025 2:51:44 PM

## <Chromatogram>



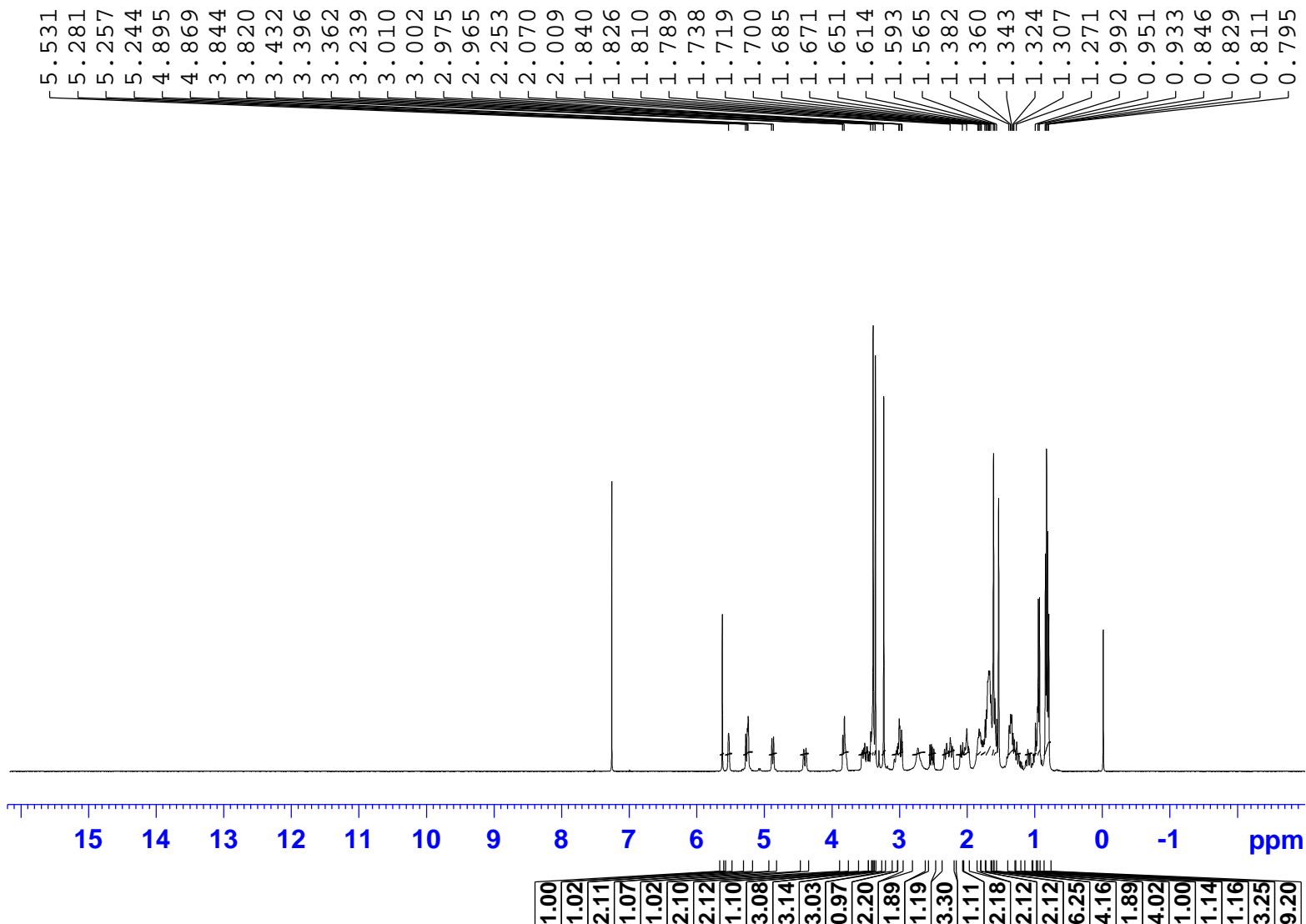
1 Det. A Ch1/220nm

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	10.941	785111	50313	7.892	13.902
2	13.444	72394	4565	0.728	1.261
3	21.712	1684068	55472	16.928	15.328
4	22.861	4950760	180666	49.766	49.920
5	25.014	2455840	70893	24.686	19.589
Total		9948173	361910	100.000	100.000

ALLMPUS LABORATORIES PVT LTD  
ALL-T03931  
1H-NMR/CDC13  
31-AUG-2023

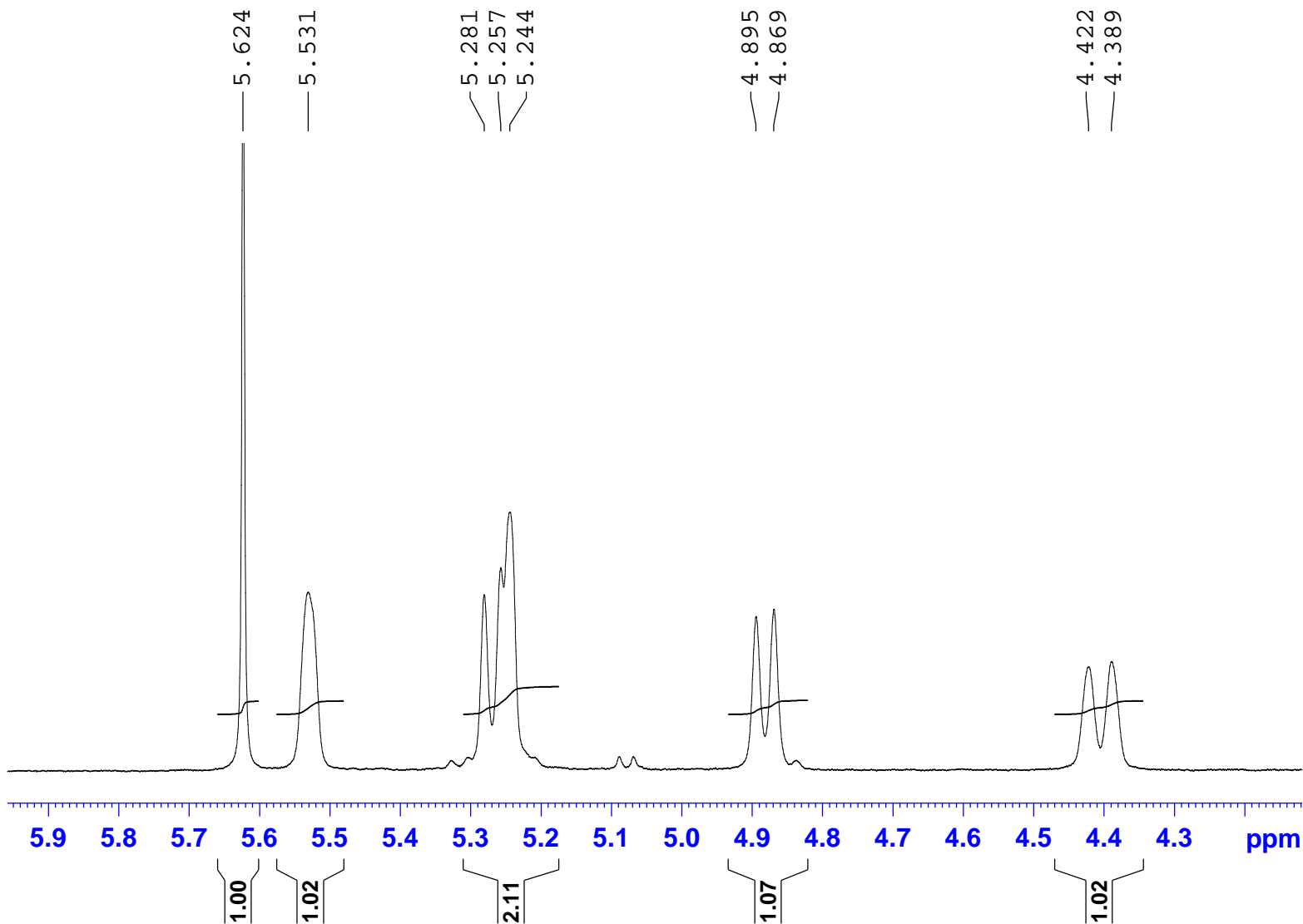


Current Data Parameters  
NAME QC11230831003  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20230831  
Time 14.07 h  
INSTRUM spect  
PROBHD Z108618\_0828 (  
PULPROG zg30  
TD 65536  
SOLVENT CDC13  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 174.45  
DW 62.400 usec  
DE 16.75 usec  
TE 0 K  
D1 1.00000000 sec  
TD0 1  
SFO1 400.0904705 MHz  
NUC1 1H  
P0 5.33 usec  
P1 16.00 usec  
PLW1 12.89900017 W

F2 - Processing parameters  
SI 65536  
SF 400.0880107 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

ALLMPUS LABORATORIES PVT LTD  
ALL-T03931  
1H-NMR/CDC13  
31-AUG-2023

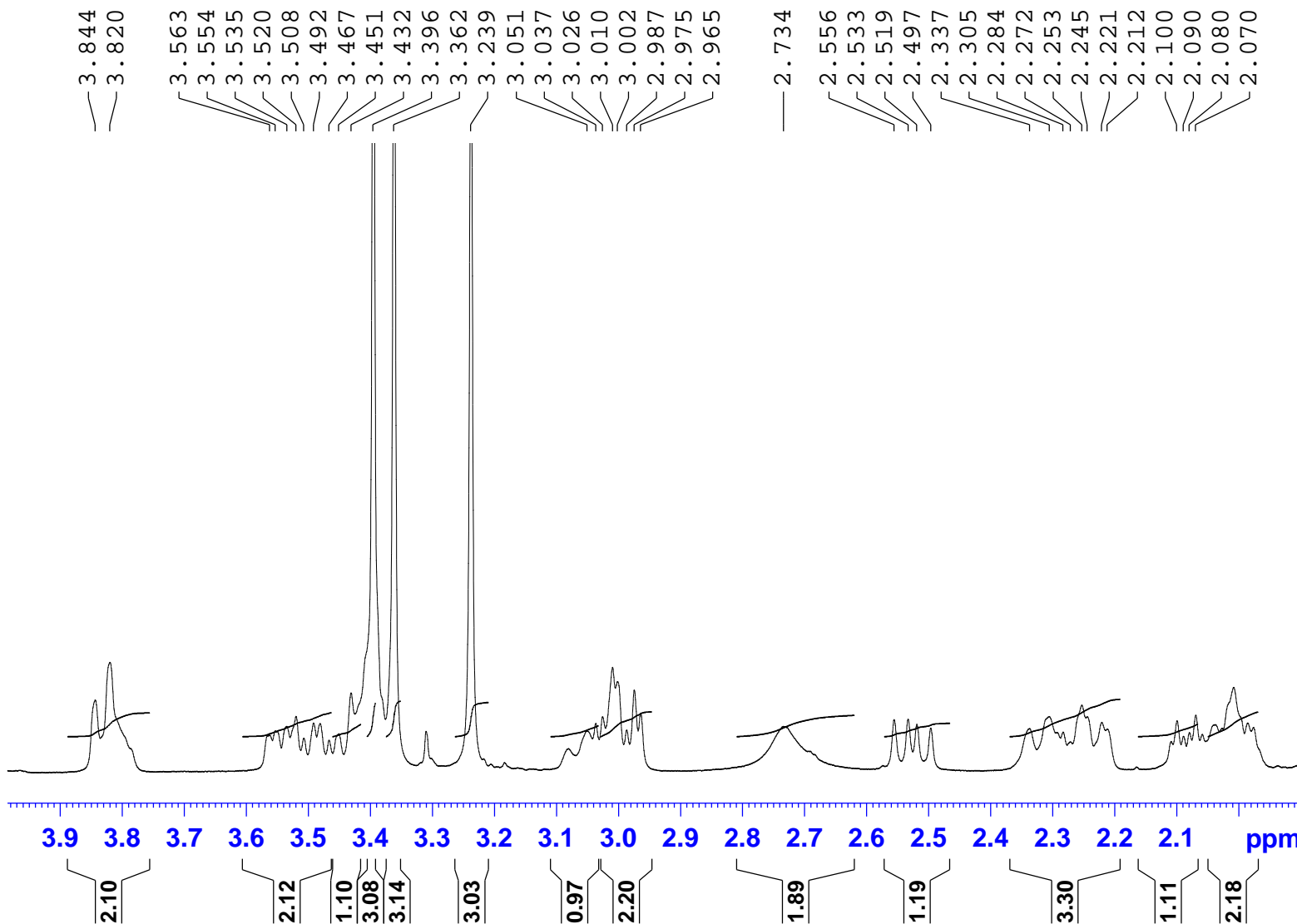


Current Data Parameters  
NAME QC11230831003  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20230831  
Time 14.07 h  
INSTRUM spect  
PROBHD Z108618\_0828 (  
PULPROG zg30  
TD 65536  
SOLVENT CDC13  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 174.45  
DW 62.400 usec  
DE 16.75 usec  
TE 0 K  
D1 1.00000000 sec  
TD0 1  
SFO1 400.0904705 MHz  
NUC1 1H  
P0 5.33 usec  
P1 16.00 usec  
PLW1 12.89900017 W

F2 - Processing parameters  
SI 65536  
SF 400.0880107 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

ALLMPUS LABORATORIES PVT LTD  
ALL-T03931  
1H-NMR/CDC13  
31-AUG-2023



Current Data Parameters  
NAME QC11230831003  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20230831  
Time 14.07 h  
INSTRUM spect  
PROBHD Z108618\_0828 (  
PULPROG zg30  
TD 65536  
SOLVENT CDC13  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 174.45  
DW 62.400 usec  
DE 16.75 usec  
TE 0 K  
D1 1.00000000 sec  
TD0 1  
SFO1 400.0904705 MHz  
NUC1 1H  
P0 5.33 usec  
P1 16.00 usec  
PLW1 12.89900017 W

F2 - Processing parameters  
SI 65536  
SF 400.0880107 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

ALLMPUS LABORATORIES PVT LTD  
 ALL-T03931  
 1H-NMR/CDC13  
 31-AUG-2023



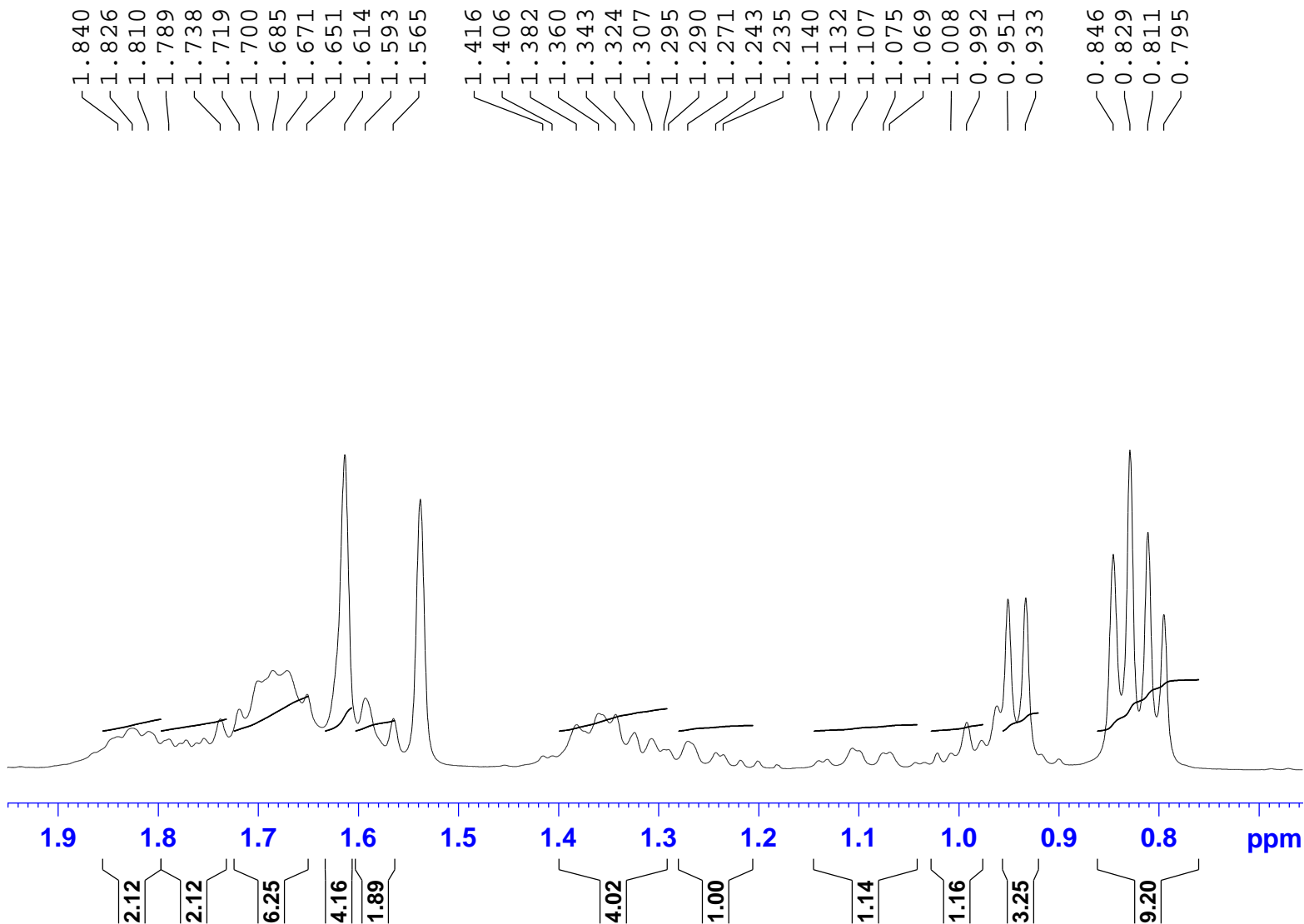
Current Data Parameters  
 NAME QC11230831003  
 EXPNO 1  
 PROCNO 1

F2 - Acquisition Parameters

Date\_ 20230831  
 Time 14.07 h  
 INSTRUM spect  
 PROBHD Z108618\_0828 (  
 PULPROG zg30  
 TD 65536  
 SOLVENT CDC13  
 NS 16  
 DS 2  
 SWH 8012.820 Hz  
 FIDRES 0.244532 Hz  
 AQ 4.0894465 sec  
 RG 174.45  
 DW 62.400 usec  
 DE 16.75 usec  
 TE 0 K  
 D1 1.00000000 sec  
 TD0 1  
 SFO1 400.0904705 MHz  
 NUC1 1H  
 P0 5.33 usec  
 P1 16.00 usec  
 PLW1 12.89900017 W

F2 - Processing parameters

SI 65536  
 SF 400.0880107 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00



ALLMPUS LABORATORIES PVT LTD  
 ALL-T03931  
 1H-NMR/CDC13+3,5-DIBROMO NITROBENZENE  
 02-SEP-2023



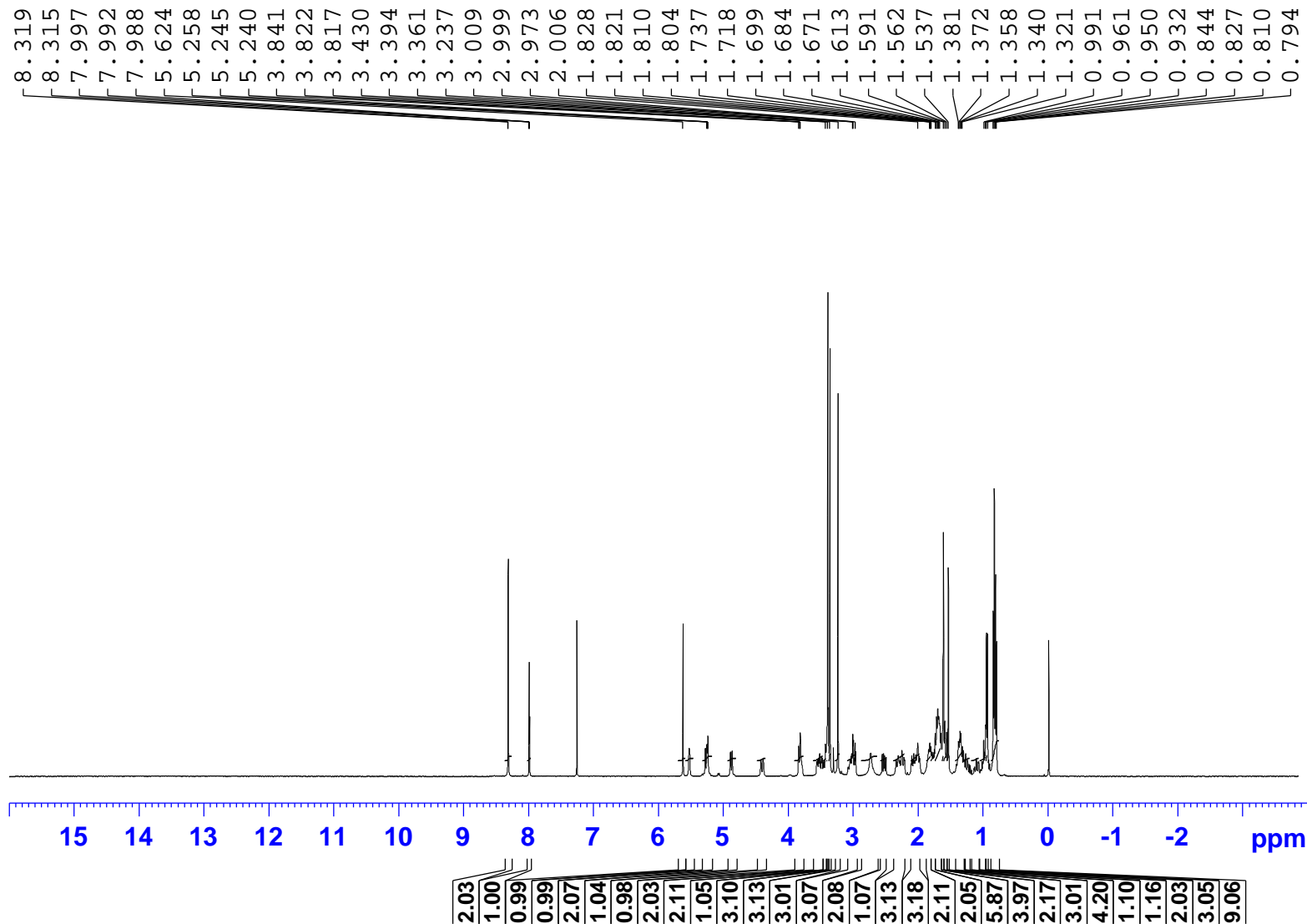
Current Data Parameters  
 NAME QC11230902027  
 EXPNO 1  
 PROCNO 1

F2 - Acquisition Parameters

Date\_ 20230902  
 Time 12.56 h  
 INSTRUM spect  
 PROBHD Z108618\_0828 (  
 PULPROG zg30  
 TD 65536  
 SOLVENT CDC13  
 NS 16  
 DS 2  
 SWH 8012.820 Hz  
 FIDRES 0.244532 Hz  
 AQ 4.0894465 sec  
 RG 140.13  
 DW 62.400 usec  
 DE 16.75 usec  
 TE 0 K  
 D1 1.00000000 sec  
 TD0 1  
 SFO1 400.0904705 MHz  
 NUC1 1H  
 P0 5.33 usec  
 P1 16.00 usec  
 PLW1 12.89900017 W

F2 - Processing parameters

SI 65536  
 SF 400.0880107 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00



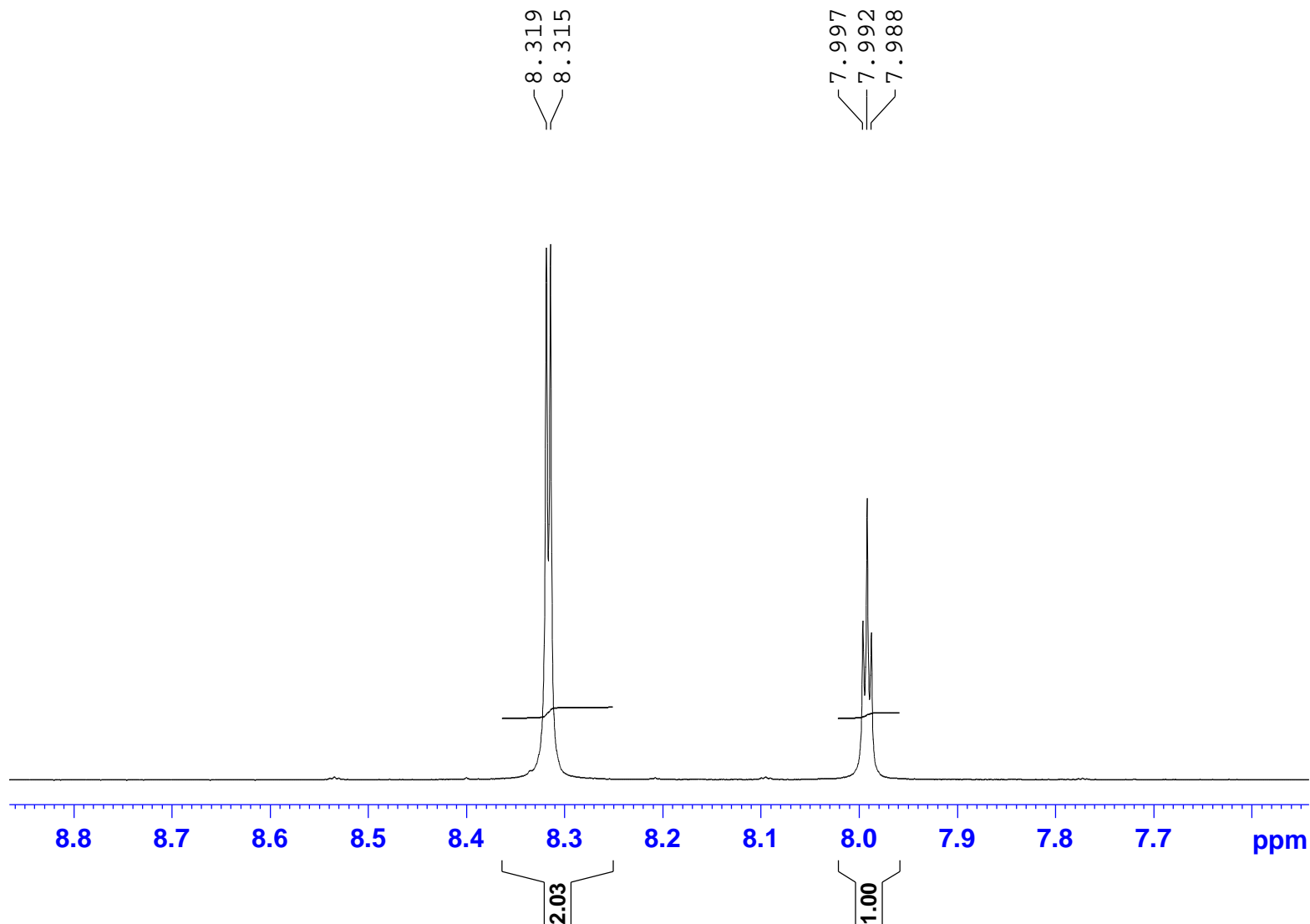
ALLMPUS LABORATORIES PVT LTD  
ALL-T03931  
1H-NMR/CDC13+3,5-DIBROMO NITROBENZENE  
02-SEP-2023



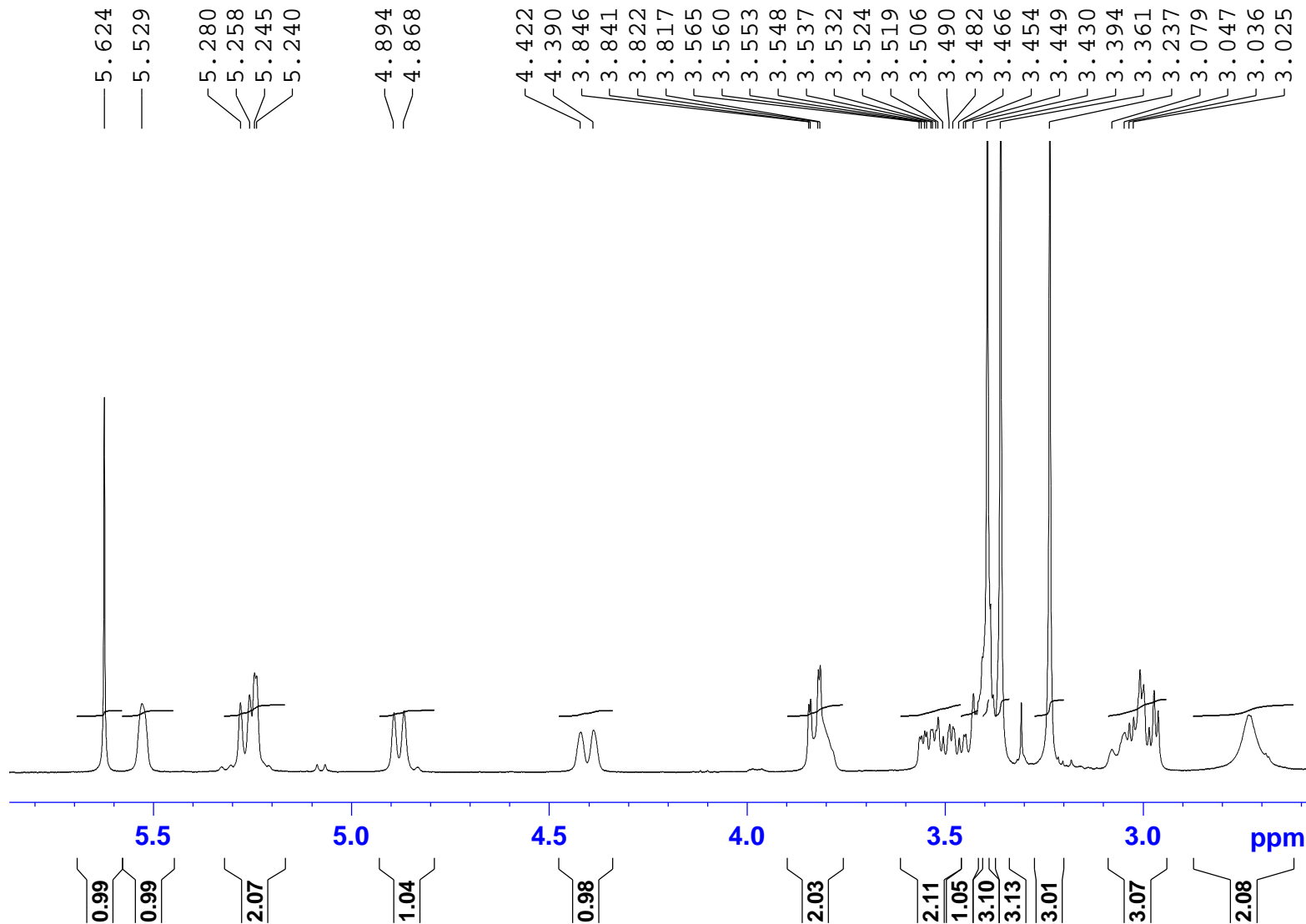
Current Data Parameters  
NAME QC11230902027  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20230902  
Time 12.56 h  
INSTRUM spect  
PROBHD Z108618\_0828 (  
PULPROG zg30  
TD 65536  
SOLVENT CDC13  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 140.13  
DW 62.400 usec  
DE 16.75 usec  
TE 0 K  
D1 1.00000000 sec  
TD0 1  
SF01 400.0904705 MHz  
NUC1 1H  
P0 5.33 usec  
P1 16.00 usec  
PLW1 12.89900017 W

F2 - Processing parameters  
SI 65536  
SF 400.0880107 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



ALLMPUS LABORATORIES PVT LTD  
 ALL-T03931  
 1H-NMR/CDC13+3,5-DIBROMO NITROBENZENE  
 02-SEP-2023



Current Data Parameters  
 NAME QC11230902027  
 EXPNO 1  
 PROCNO 1

F2 - Acquisition Parameters

Date\_ 20230902  
 Time 12.56 h  
 INSTRUM spect  
 PROBHD Z108618\_0828 (  
 PULPROG zg30  
 TD 65536  
 SOLVENT CDC13  
 NS 16  
 DS 2  
 SWH 8012.820 Hz  
 FIDRES 0.244532 Hz  
 AQ 4.0894465 sec  
 RG 140.13  
 DW 62.400 usec  
 DE 16.75 usec  
 TE 0 K  
 D1 1.00000000 sec  
 TD0 1  
 SFO1 400.0904705 MHz  
 NUC1 1H  
 P0 5.33 usec  
 P1 16.00 usec  
 PLW1 12.89900017 W

F2 - Processing parameters

SI 65536  
 SF 400.0880107 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00

ALLMPUS LABORATORIES PVT LTD  
 ALL-T03931  
 1H-NMR/CDC13+3,5-DIBROMO NITROBENZENE  
 02-SEP-2023



2.555  
 2.532  
 2.252  
 2.243  
 2.069  
 2.037  
 2.016  
 2.006  
 1.997  
 1.846  
 1.839  
 1.828  
 1.821  
 1.810  
 1.804  
 1.793  
 1.788  
 1.771  
 1.761  
 1.752  
 1.737  
 1.718  
 1.699  
 1.684  
 1.671  
 1.613  
 1.591  
 1.562  
 1.537  
 1.381  
 1.372  
 1.358  
 1.340  
 1.324  
 1.321  
 1.305  
 1.269  
 0.991  
 0.976  
 0.961  
 0.950  
 0.932  
 0.844  
 0.827  
 0.810  
 0.794

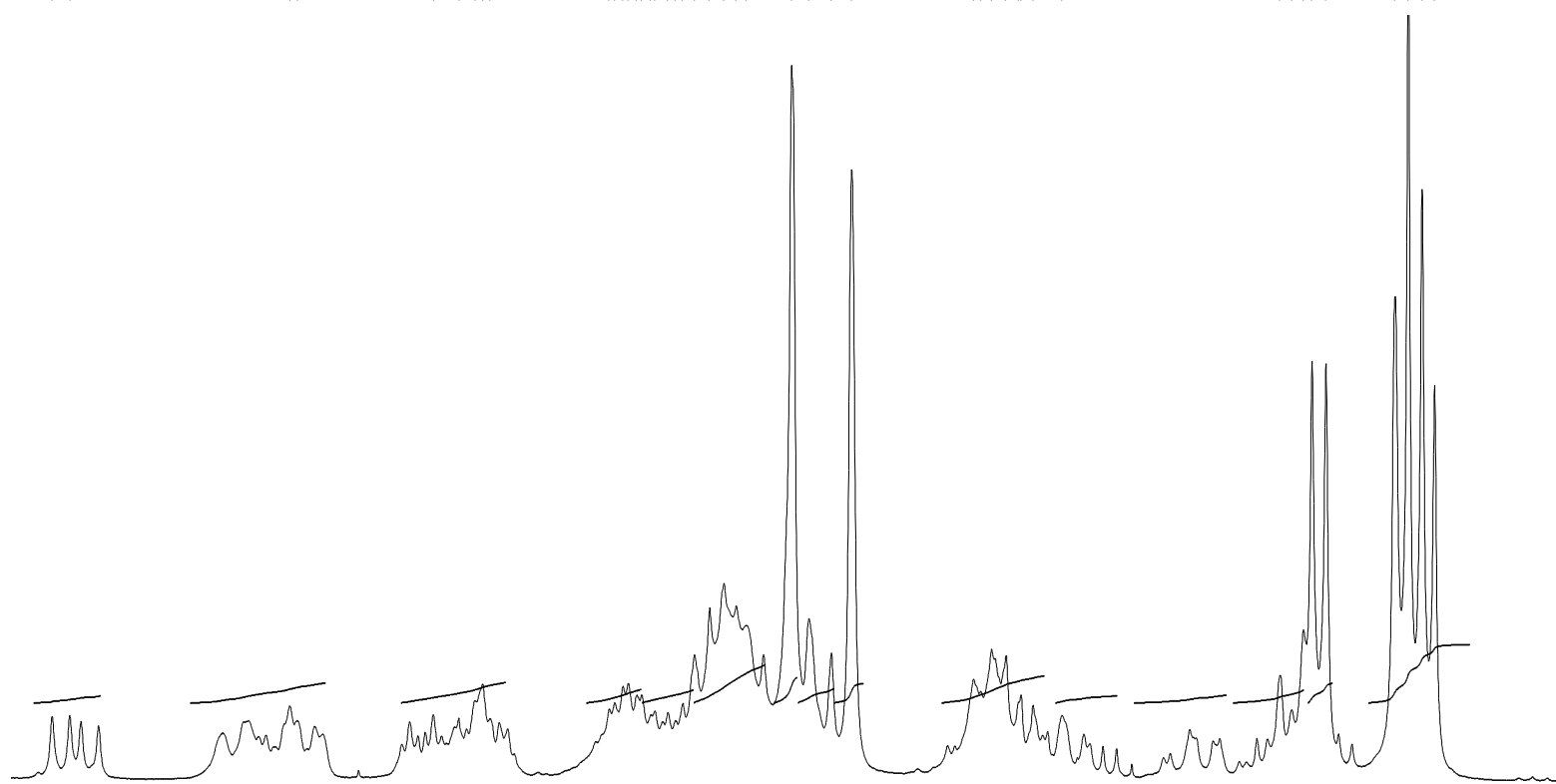
Current Data Parameters  
 NAME QC11230902027  
 EXPNO 1  
 PROCNO 1

F2 - Acquisition Parameters

Date\_ 20230902  
 Time 12.56 h  
 INSTRUM spect  
 PROBHD Z108618\_0828 (  
 PULPROG zg30  
 TD 65536  
 SOLVENT CDC13  
 NS 16  
 DS 2  
 SWH 8012.820 Hz  
 FIDRES 0.244532 Hz  
 AQ 4.0894465 sec  
 RG 140.13  
 DW 62.400 usec  
 DE 16.75 usec  
 TE 0 K  
 D1 1.00000000 sec  
 TD0 1  
 SFO1 400.0904705 MHz  
 NUC1 1H  
 P0 5.33 usec  
 P1 16.00 usec  
 PLW1 12.89900017 W

F2 - Processing parameters

SI 65536  
 SF 400.0880107 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00



2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8 ppm

1.07 3.13 3.18 2.11 2.05 5.87 3.97 2.17 3.01 4.20 1.10 1.16 2.03 3.05 9.06

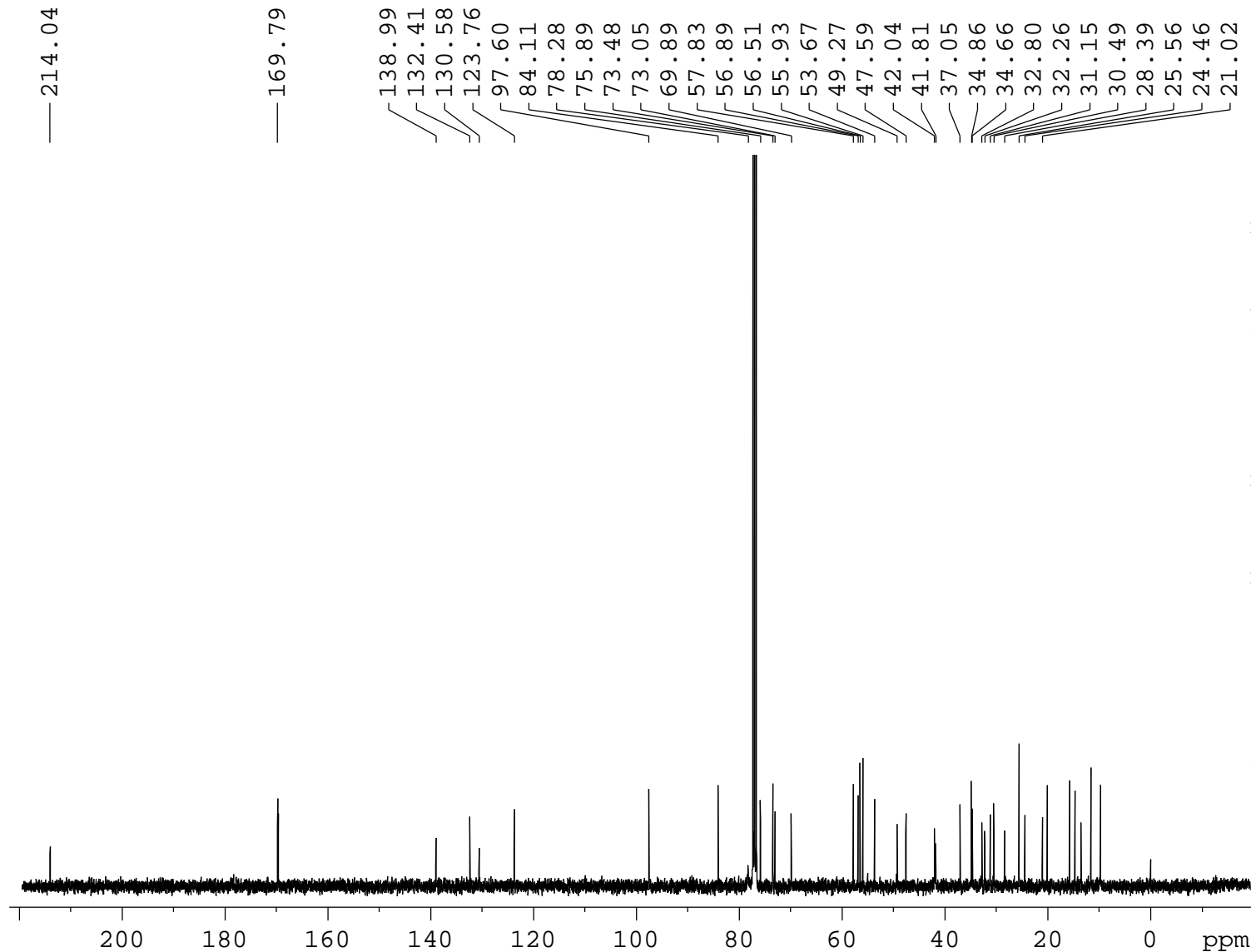
## ALLMPUS LABORATORIES

### Assay by NMR

B.No: ALL-T03931 Internal Standard: 3,5-DIBROMO NITROBENZENE	
Integral of sample resonace at 5.6 ppm (Is)	0.99
Number of protons internal standard (Nr)	1
Molecular weight of sample (Ms)	789
Weight of intenal standard (Wr)	4.76
Purity of internal Std (Pr)	98
Integral of internal standard resonance at 7.9 ppm (Ir)	1
Number of sample protons (Ns)	1
Molecular weight of internal standard (Mr)	280.9
Weight of the sample (mg) (Ws)	13.09

$\text{Assay by NMR} = \frac{(\text{Is} \times \text{Wr} \times \text{Ms} \times \text{Nr} \times \text{Pr})}{(\text{Ir} \times \text{Ws} \times \text{Mr} \times \text{Ns})}$	
Assay by NMR =	99.10

ALLMPUS LABORATORIES PVT LTD  
ALL-T03931  
13C-NMR/CDC13  
31-AUG-2023



Current Data Parameters  
NAME QC11230831003  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20230901  
Time 1.39 h  
INSTRUM spect  
PROBHD z108618\_0828 (  
PULPROG zgpg30  
TD 65536  
SOLVENT CDC13  
NS 1024  
DS 4  
SWH 24038.461 Hz  
FIDRES 0.733596 Hz  
AQ 1.3631488 sec  
RG 195.98  
DW 20.800 usec  
DE 6.50 usec  
TE 0 K  
D1 2.00000000 sec  
D11 0.03000000 sec  
TD0 1  
SFO1 100.6122678 MHz  
NUC1 13C  
P0 3.67 usec  
P1 11.00 usec  
PLW1 40.78400040 W  
SFO2 400.0896004 MHz  
NUC2 1H  
CPDPRG[2] waltz65  
PCPD2 90.00 usec  
PLW2 12.89900017 W  
PLW12 0.40766999 W  
PLW13 0.20506001 W

F2 - Processing parameters  
SI 32768  
SF 100.6022115 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40

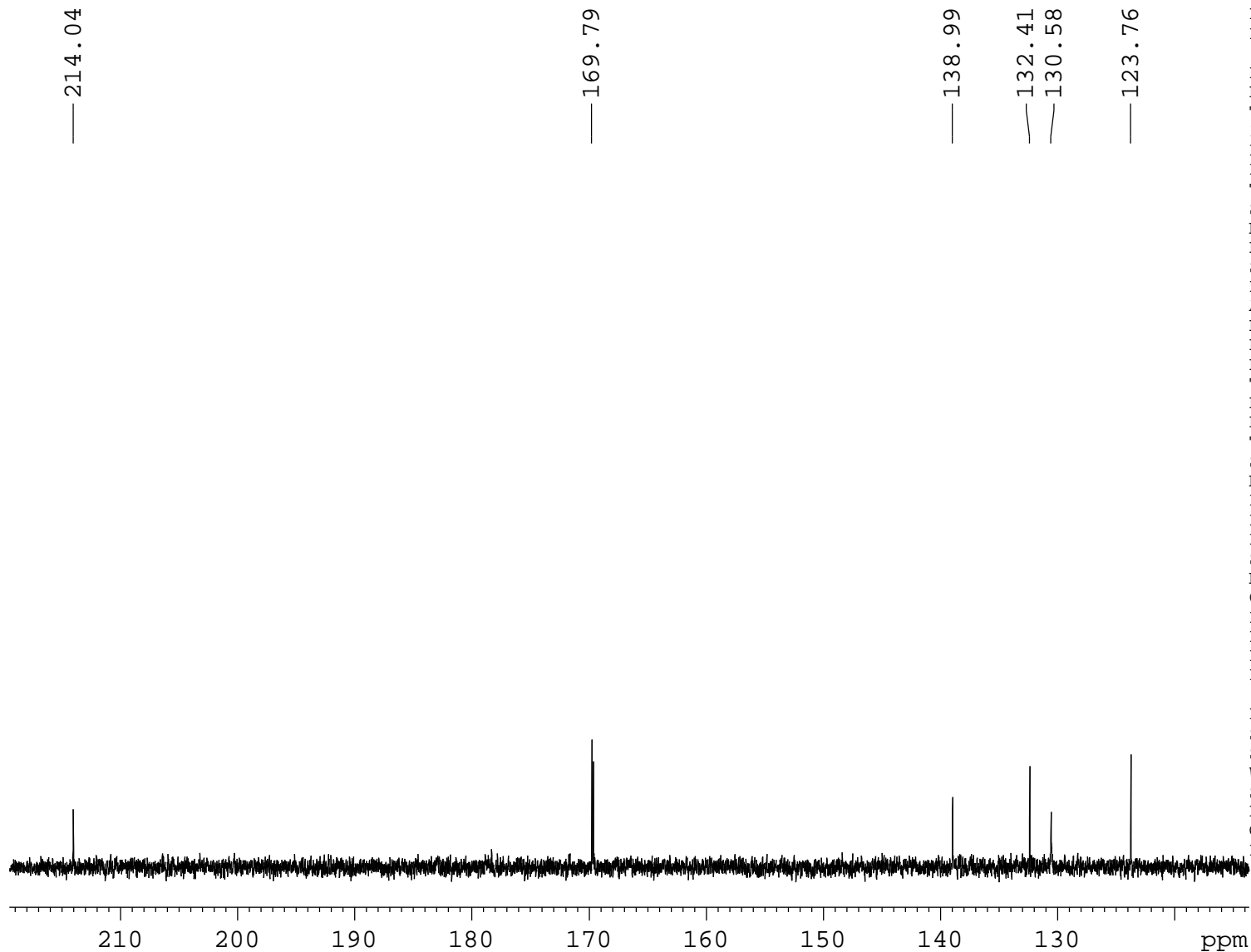
ALLMPUS LABORATORIES PVT LTD  
ALL-T03931  
13C-NMR/CDC13  
31-AUG-2023



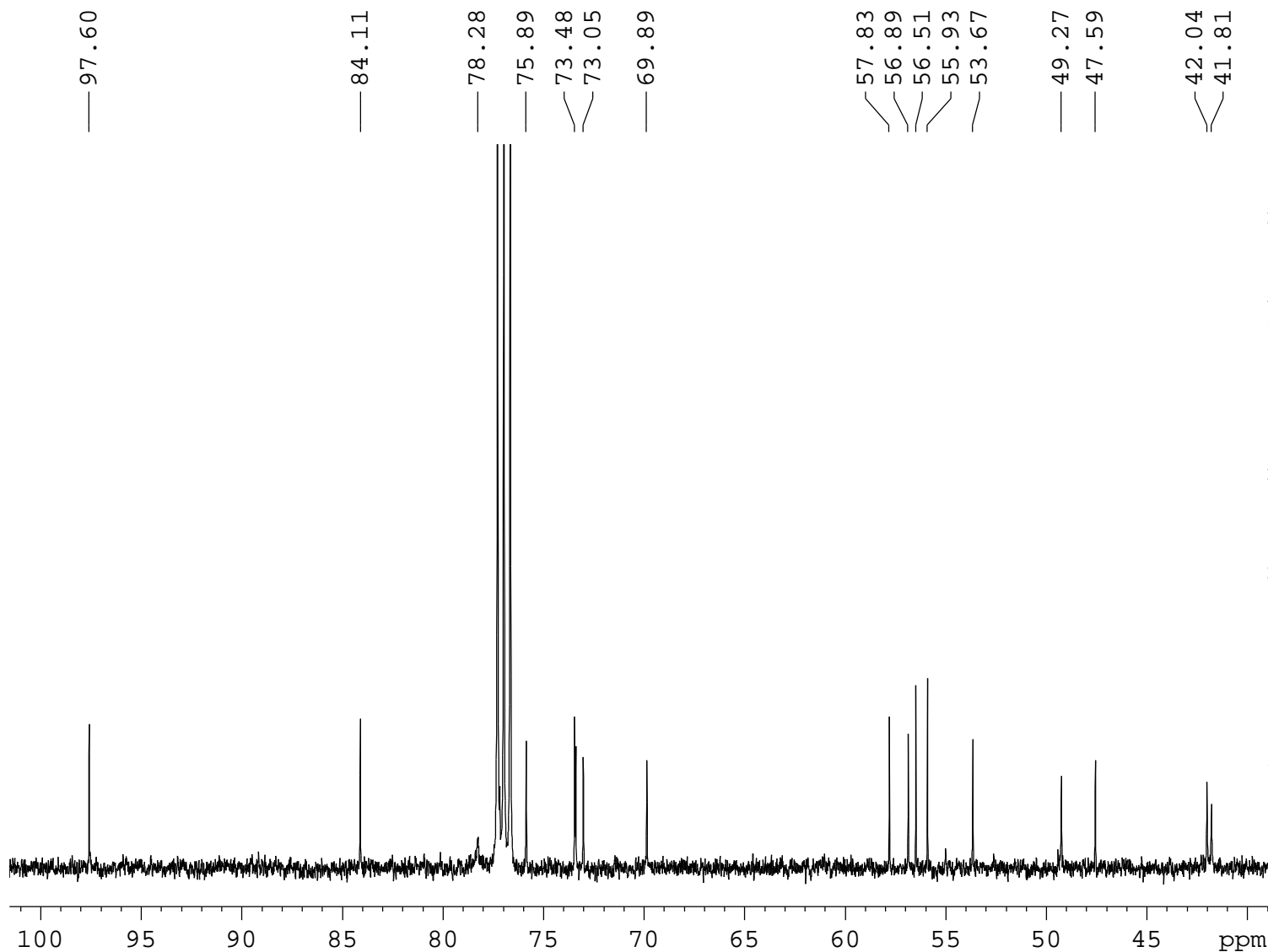
Current Data Parameters  
NAME QC11230831003  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20230901  
Time 1.39 h  
INSTRUM spect  
PROBHD z108618\_0828 (  
PULPROG zgpg30  
TD 65536  
SOLVENT CDC13  
NS 1024  
DS 4  
SWH 24038.461 Hz  
FIDRES 0.733596 Hz  
AQ 1.3631488 sec  
RG 195.98  
DW 20.800 usec  
DE 6.50 usec  
TE 0 K  
D1 2.00000000 sec  
D11 0.03000000 sec  
TD0 1  
SFO1 100.6122678 MHz  
NUC1 13C  
P0 3.67 usec  
P1 11.00 usec  
PLW1 40.78400040 W  
SFO2 400.0896004 MHz  
NUC2 1H  
CPDPRG[2] waltz65  
PCPD2 90.00 usec  
PLW2 12.89900017 W  
PLW12 0.40766999 W  
PLW13 0.20506001 W

F2 - Processing parameters  
SI 32768  
SF 100.6022115 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40



ALLMPUS LABORATORIES PVT LTD  
ALL-T03931  
13C-NMR/CDC13  
31-AUG-2023



Current Data Parameters  
NAME QC11230831003  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20230901  
Time 1.39 h  
INSTRUM spect  
PROBHD z108618\_0828 (  
PULPROG zgpg30  
TD 65536  
SOLVENT CDC13  
NS 1024  
DS 4  
SWH 24038.461 Hz  
FIDRES 0.733596 Hz  
AQ 1.3631488 sec  
RG 195.98  
DW 20.800 usec  
DE 6.50 usec  
TE 0 K  
D1 2.00000000 sec  
D11 0.03000000 sec  
TD0 1  
SFO1 100.6122678 MHz  
NUC1 13C  
P0 3.67 usec  
P1 11.00 usec  
PLW1 40.78400040 W  
SFO2 400.0896004 MHz  
NUC2 1H  
CPDPRG[2] waltz65  
PCPD2 90.00 usec  
PLW2 12.89900017 W  
PLW12 0.40766999 W  
PLW13 0.20506001 W

F2 - Processing parameters  
SI 32768  
SF 100.6022115 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40

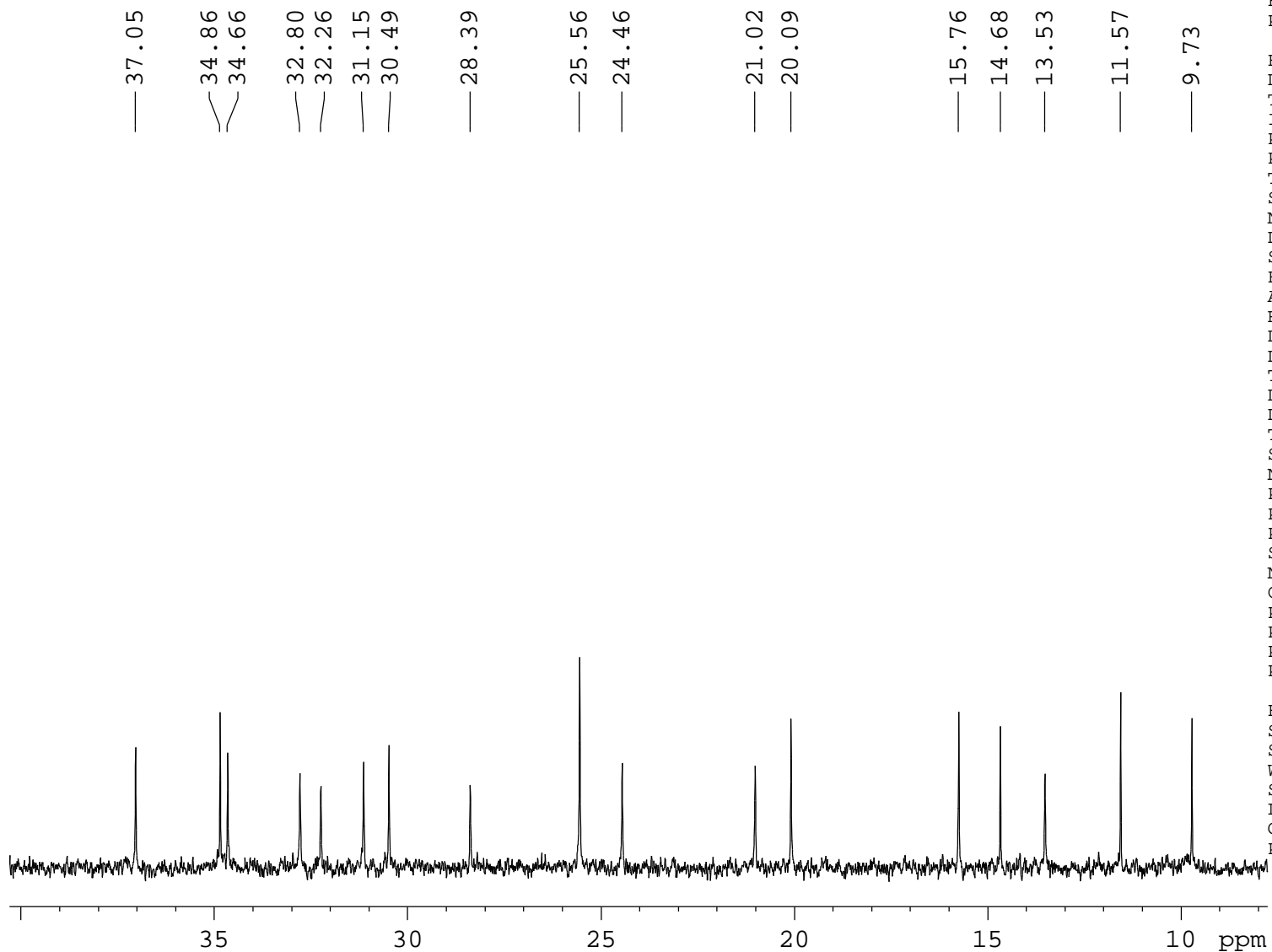
ALLMPUS LABORATORIES PVT LTD  
ALL-T03931  
13C-NMR/CDC13  
31-AUG-2023



Current Data Parameters  
NAME QC11230831003  
EXPNO 2  
PROCNO 1

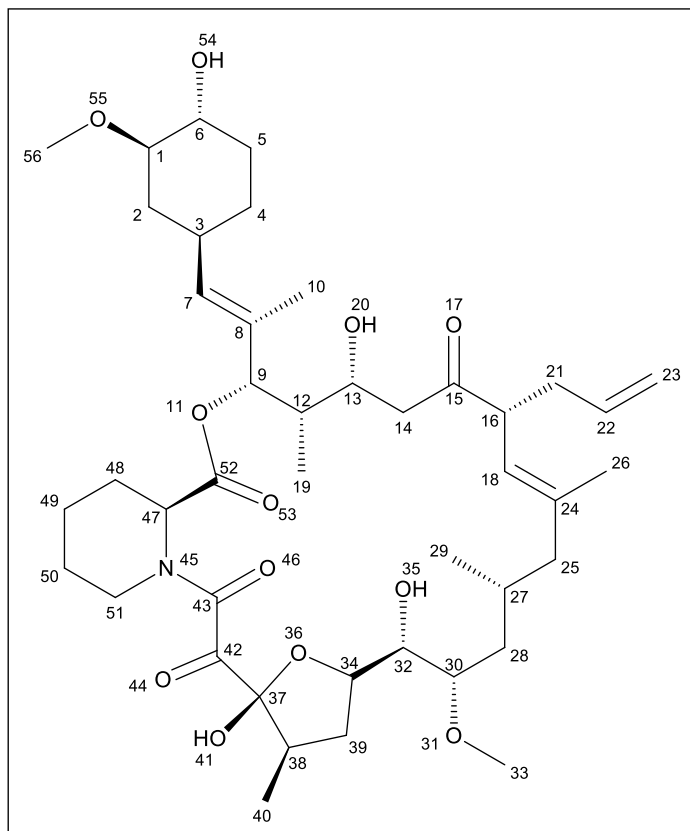
F2 - Acquisition Parameters  
Date\_ 20230901  
Time 1.39 h  
INSTRUM spect  
PROBHD z108618\_0828 (  
PULPROG zgpg30  
TD 65536  
SOLVENT CDC13  
NS 1024  
DS 4  
SWH 24038.461 Hz  
FIDRES 0.733596 Hz  
AQ 1.3631488 sec  
RG 195.98  
DW 20.800 usec  
DE 6.50 usec  
TE 0 K  
D1 2.00000000 sec  
D11 0.03000000 sec  
TD0 1  
SFO1 100.6122678 MHz  
NUC1 13C  
P0 3.67 usec  
P1 11.00 usec  
PLW1 40.78400040 W  
SFO2 400.0896004 MHz  
NUC2 1H  
CPDPRG[2] waltz65  
PCPD2 90.00 usec  
PLW2 12.89900017 W  
PLW12 0.40766999 W  
PLW13 0.20506001 W

F2 - Processing parameters  
SI 32768  
SF 100.6022115 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40



## SER- DESMETHYL TACROLIMUS / TACROLIMUS 13 DMT

### Structure



**Chemical Name-** (1R,9S,12S,13R,14S,17R,18E,21S,23S,24R,25S,27R)-1,14-dihydroxy-12-[(E)-1-[(1R,3R,4R)-4-hydroxy-3-methoxy cyclo hexyl]prop-1-en-2-yl]-23,25-dimethoxy-13,21,27-trimethyl -17-prop-2-enyl-11,28-dioxa-4-azatricyclo[22.3.1.04,9]octacos-18-ene-2,3,10,16-tetrone

**BATCH No-** ALL-T03931

### **NMR Study**

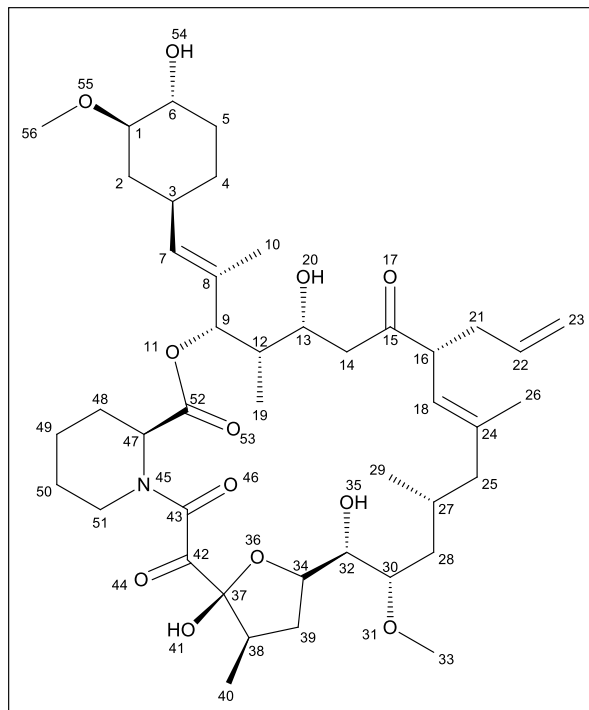
#### **1. <sup>1</sup>H NMR interpretation**

The NMR experiment was recorder in CDCl<sub>3</sub> using 400 MHz Bruker instrument. Chemical shifts were reported on the δ scale in ppm, relative to (TMS as reference =0.000 ppm).<sup>1</sup>H NMR assignment of **DESMETHYL TACROLIMUS OR TACROLIMUS 13 DMT** as below.

<b>Sr.No.</b>	<b>1H Label/ Assignment's</b>	<b><sup>1</sup>H (δ=0.000 ppm)</b>	<b>No. of Protons</b>	<b>Multiplicity m, J (Hz)</b>
1	19,29,40	0.795-0.846	9H	m
2	49,50,2,4,28	0.933-1.416	10H	m
3	10,3,12,26,38,48,39,5,27	1.565-1.840	16H	m
4	20,54,21,25	2.070-2.337	6H	m
5	14	2.497-2.556	1H	m
6	35,41	2.734	2H	m
7	1,14,6	2.965-3.051	3H	m
8	56	3.239	3H	s
9	33	3.362	3H	s
10	51,32,30,16,13	3.396-3.563	6H	m
11	34,47	3.820-3.844	2H	m
12	23	4.389-4.422	1H	d
13	23	4.869-4.895	1H	d
14	18,9	5.244-5.281	2H	m
15	22,7	5.531-5.624	2H	m

## 2. C13-NMR

The NMR experiment was recorder in CDCl<sub>3</sub> using 400 MHz Bruker instrument.  
<sup>13</sup>CNMR assignment as below.



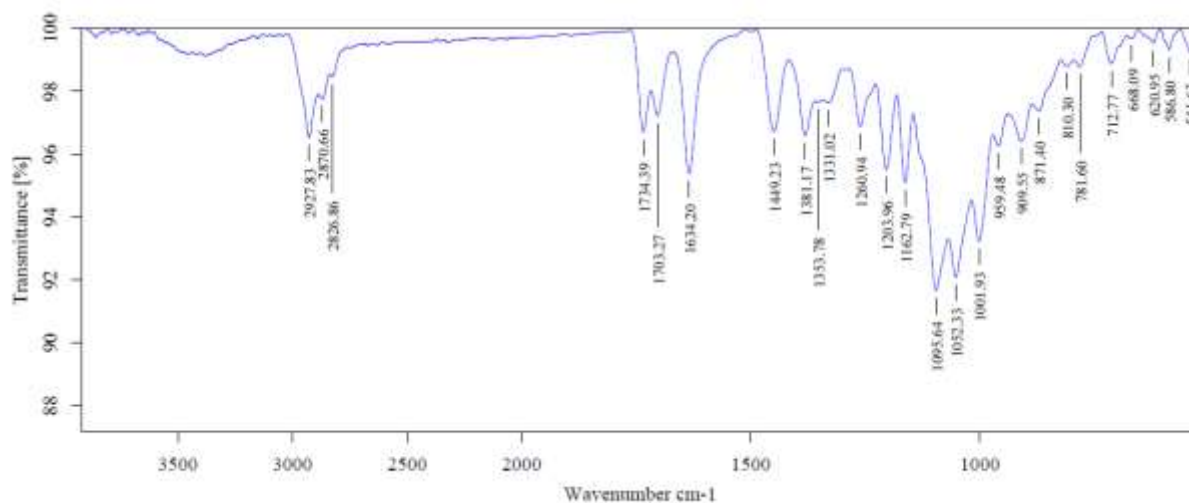
Sr.No	Assignment of C	C <sup>13</sup> (δ=0.000 ppm)
1	C-40,10,26,19	9.73-15.76
2	C-28,2,38,5,39,4,39,48,50,21,4, 27,29,49,5,4	20.09-37.05
3	C-33,56,25,14,51	41.81-57.83
4	C-6,34,13,47,30,32	69.89-78.25
5	C-1	84.11
6	C-9	97.60
7	C-24,22,18,7,8,23	123.76-138.99
8	C-43,42	169.79

11	C-15,52	214.04
----	---------	--------

### 3. IR Spectrum

**Method:** Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy.

**Instrument:** BRUKER Model ALPHA



IR Stretching	Observed Frequency	Reported Frequency
<b>Aliphatic</b>		
C-H	2870.66	3000-2840
C-O	1052.33	1124-1087
<b>Alcohol</b>		
-OH	-----	3700-3584
<b>Ketone</b>		
C=O	1703.27	1725-1705
<b>Amine</b>		
C-N	2927.83	3300-3000

#### **4. Mass Spectral Data**

The impurity was analyzed using Waters 3100 mass instrument.

Chemical Formula: C<sub>43</sub>H<sub>67</sub>NO<sub>12</sub>

Molecular Weight: 789.99

The Molecular Weight of product was detected as 808.5 corresponding to (M+1+18) in positive mode and 786.6 (M-1) in negative mode spectrum.

#### **5. HPLC Purity**

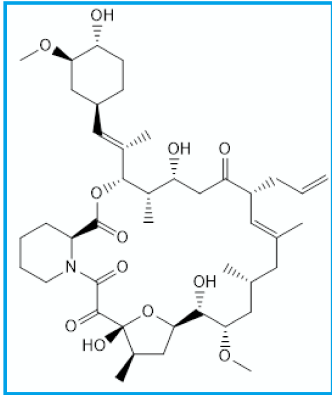
The purity of compound was determined using area normalization method. The purity of product was found to be 96.39 % at 22.949 RT value.

#### **6. Conclusion**

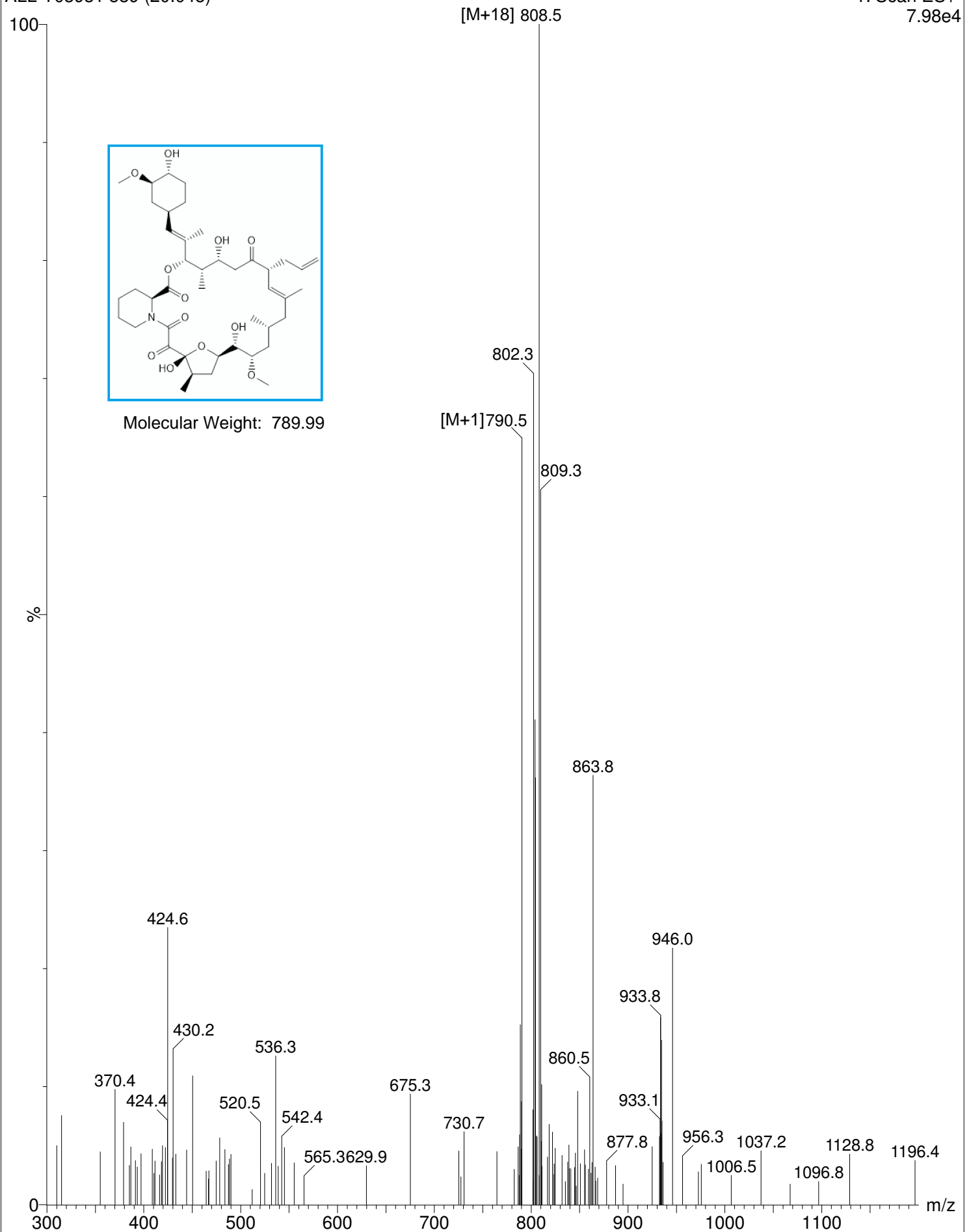
The results from the analytical techniques mass, HPLC and NMR confirmed the molecular structure of the DESMETHYL TACROLIMUS / TACROLIMUS 13 DMT.

ALL-T03931 589 (20.048)

1: Scan ES+  
7.98e4

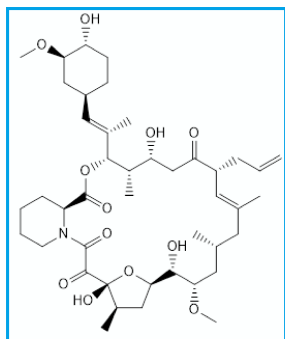


Molecular Weight: 789.99

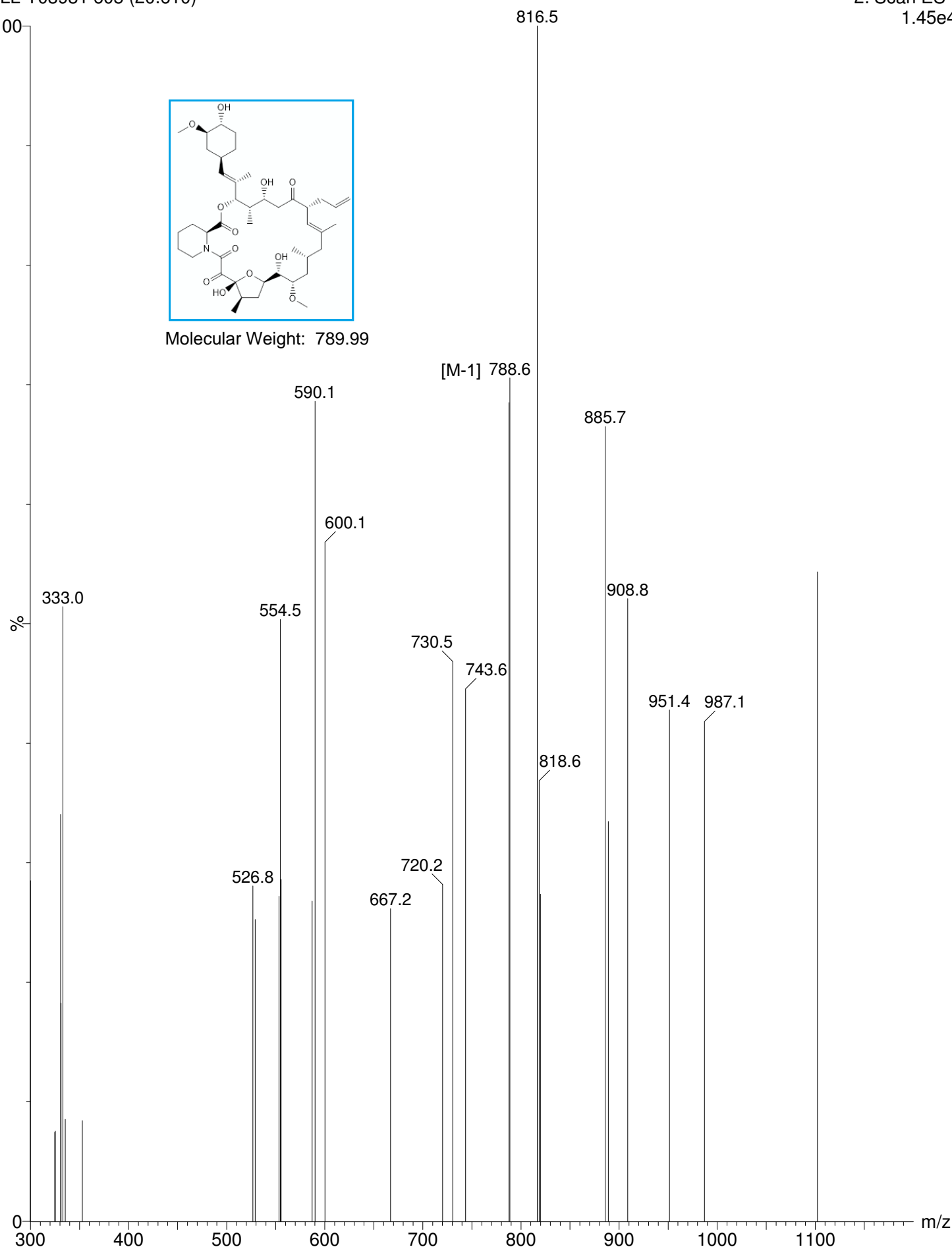


ALL-T03931 605 (20.610)

2: Scan ES-  
1.45e4

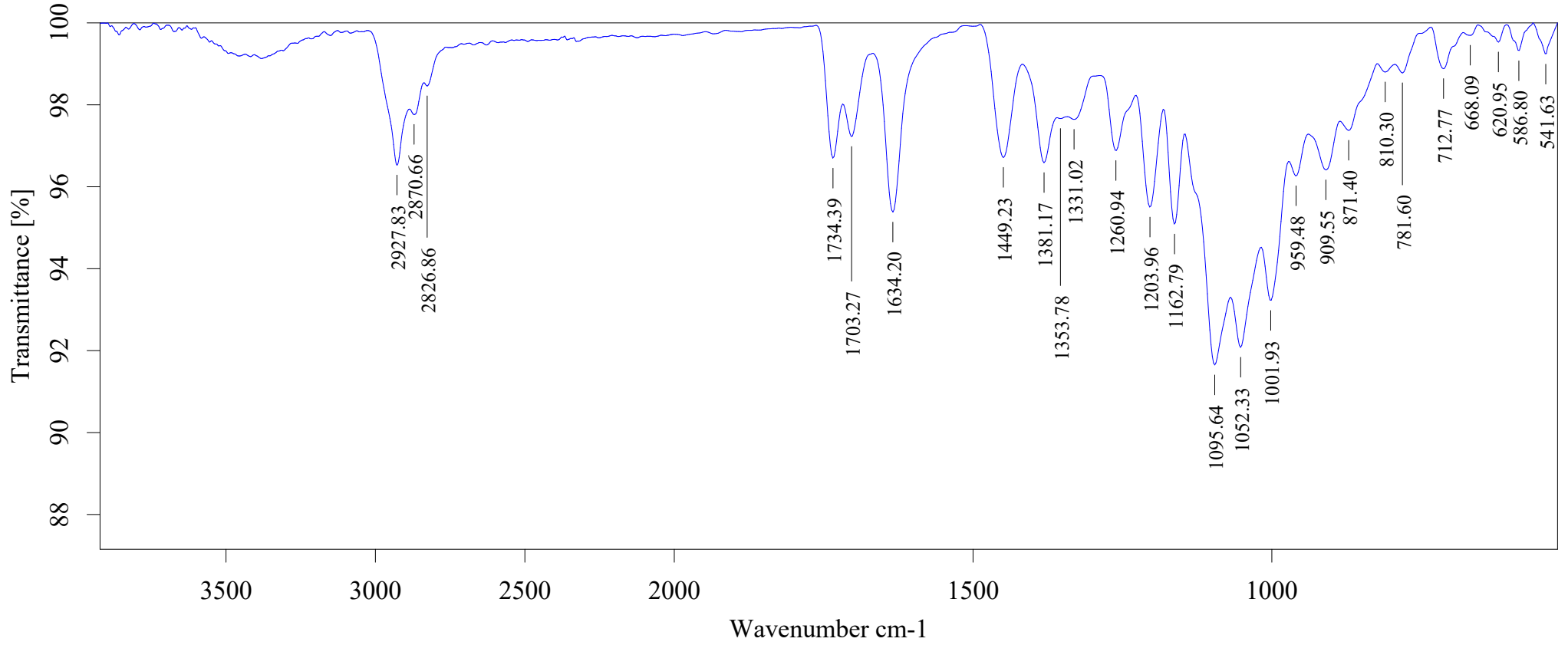


Molecular Weight: 789.99



Instrument ID No: AA-ID-002

# INFRARED SPECTRUM



Sample Name:ALL-T03931

Experiment:ACCU ANALYTICAL-131.xpm

Lot No./Batch No:

Resolution:4

Date & Time:18-04-2023,10:07:15

Sample Scans:16

Operator Name:Accu Chemist

Frequency Range:4000 to 500

Analysed by:

Checked by:

Date:

18-04-2023 10:09:39

Date:

"C:\Users\Public\Documents\Bruker\OPUS\_8.7.31\MEAS\ALL-T03931.0

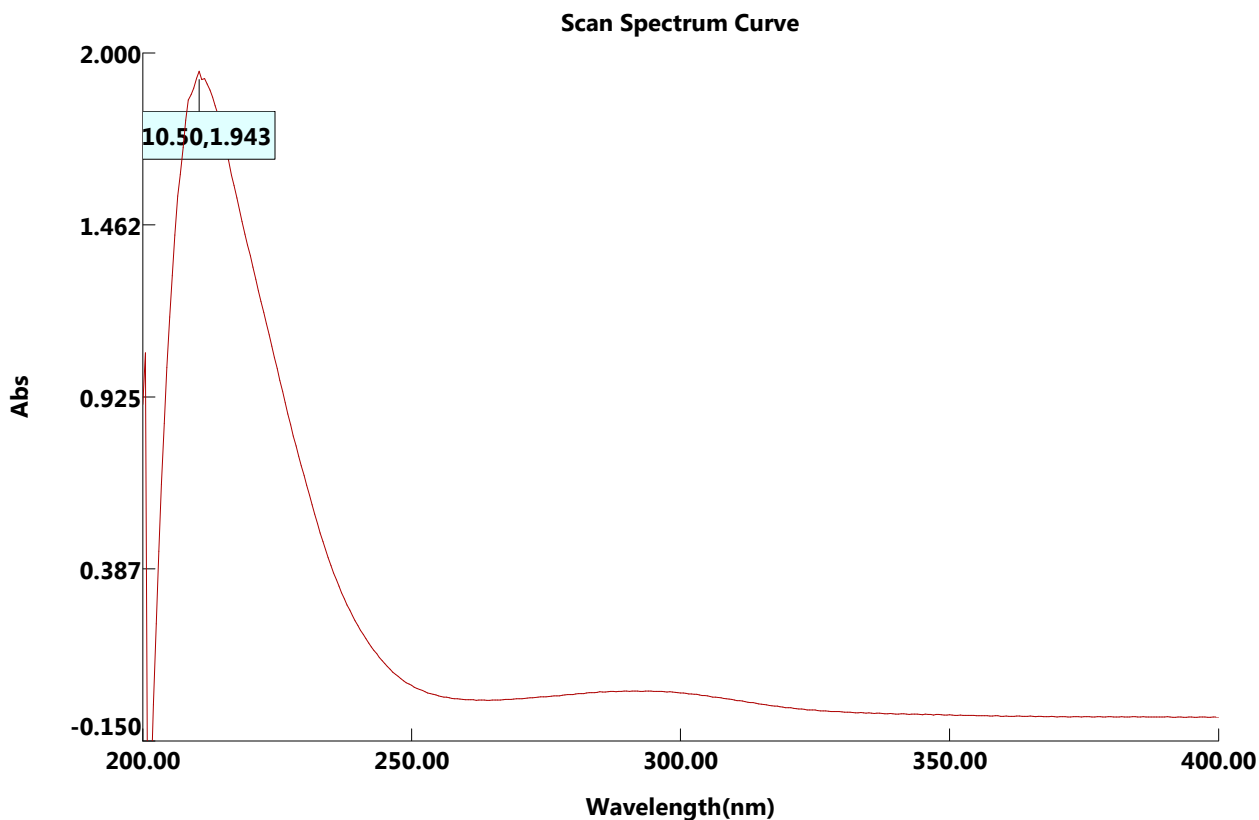
- Peak Table TR
  - Peak Picking

Peak Picking Values  
 Method: Standard  
 Searched for minima: Yes  
 Number of peaks: 26  
 Sensitivity > [%]: 1.000000  
 From: 4000.000000  
 to: 400.000000  
 Absolute peak height > 0.000000  
 Relative peak height < [%] 0.000000  
 Absolute peak height < 0.000000

Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold <	Shoulder
2927.8299	0.965	0.034	117.1621	40.873253	0
2870.6564	0.978	0.002	18.5118	1.597216	0
1734.3859	0.967	0.030	57.9138	30.711824	0
1703.2717	0.972	0.012	52.7256	9.551462	0
1634.1996	0.954	0.046	30.6051	54.907810	0
1449.2319	0.967	0.027	32.7267	27.284069	0
1381.1701	0.966	0.027	84.4563	25.502136	0
1260.9364	0.969	0.016	26.3162	16.213760	0
1203.9633	0.955	0.026	23.2396	28.726118	0
1162.7942	0.951	0.022	16.6629	26.508444	0
1095.6382	0.916	0.084	145.5716	100.005142	0
1052.3261	0.921	0.014	18.6311	14.657585	0
1001.9276	0.932	0.015	18.3115	15.658182	0
959.4763	0.963	0.005	14.6606	4.275040	0
909.5520	0.964	0.011	27.4744	10.500599	0
871.3963	0.974	0.003	361.2304	2.718293	0
810.3005	0.988	0.002	15.3346	2.233447	0
781.5973	0.988	0.004	325.8653	2.742132	0
712.7705	0.989	0.010	25.1894	12.194527	0
620.9495	0.995	0.004	20.7129	5.068223	0
586.8013	0.993	0.007	18.3476	7.679455	0

## Peak Picking Values

Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold <	Shoulder
541.6257	0.992	0.007	16.4810	7.375900	0
2826.8620	0.985	0.001	11.9659	4.232205	0
1331.0175	0.976	0.003	133.7701	3.061247	0
668.0895	0.997	0.002	62.7688	6.544214	0
1353.7799	0.977	0.000	8.0176	1.013995	0



● **Instrument Performance**

Model : UV-VIS Spectrophotometer  
 Number : 23-1950-01-0032  
 Spectral Bandwidth : 2.00 nm

● **Scan Spectrum Performance**

Scan Range : 200.00 to 400.00 nm  
 Measure Mode : Abs  
 Interval : 0.50 nm  
 Speed : Medium  
 Data File : QC08-2404-021.spd  
 Create Date/Time : Monday, April 22, 2024 2:51:13 PM  
 Data Type : Original  
 Method File:

● **Analyse Note**

Analyser : Swapna  
 Sample Name : ALL-T03931  
 Comment : ALL-T03931

No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	210.50	1.943	

Filename: D:\TGA Data\ALL-T03931.t6d  
Operator ID: BHARAT  
Sample ID: ALL-T03931  
Sample Weight: 1.810 mg  
Comment:

PerkinElmer Thermal Analysis

