Certificate Of Analysis

Analysis Date: 22-07-2025 **Re-test Date:** 25-07-2028

13-O-DESMETHYL TACROLIMUS

Identification

Chemical Name : 15,19-Epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone, 5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26-hexadecahydro-5,16,19-trihydroxy-3-[(1E)-2-[(1R,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethenyl]-14-methoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-, (3S,4R,5S,8R,9E,12S,14S,15S,16S,18R,19R,26aS)

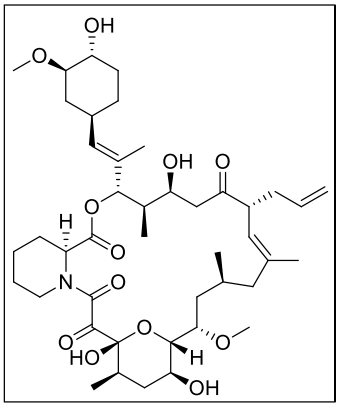
CAT No : ALL-T06874

CAS No : 139958-51-3

Molecular Formula : C43H67NO12

Molecular Weight : 789.99

Exact Mass : 789.47



Analytical Information

Batch No	: ALL-T06874	**HPLC Purity**	: 99.85 %
Solubility	: MeOH: ACN	**Potency**	: 95.90 %
Appearance of Product	: Off White Solid	**Mass**	: Confirm
Long Term Storage	: 2-8 0C	**IR Analysis**	: Confirm
Weight Loss By TGA	: 1.390 %	**1HNMR**	: Confirm
Residue Of Ignition	: 2.558 %		

Additional Information

%Potency = [100 - (Weight Loss By TGA % + Residue Of Ignition %) × Chromatographic Purity%]/100 =

[100 – (1.390 +2.558) × 99.85]/100 = 95.90 %

Recommendation : Released

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

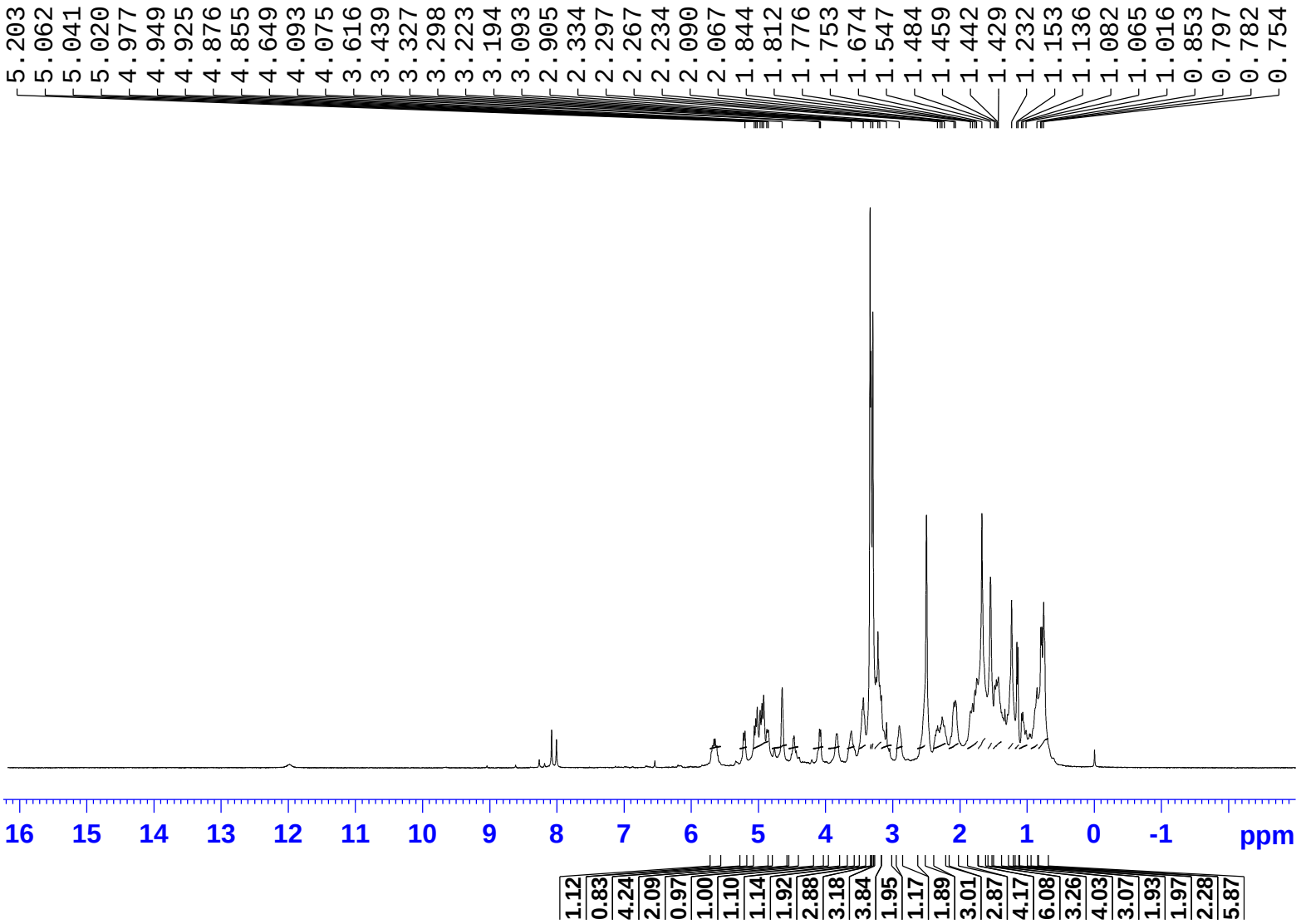
Attachment : HPLC, Mass, 1H NMR, IR, TGA

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

Corporate Office

Allmpus Laboratories Pvt Ltd 38, Solitaire Industrial Estate, Plot No.TS-1, Near Nandi Palace Hotel, MIDC-Phase 1, Dombivli (E),Mumbai, Maharashtra 421203

ALLMPUS LABORATORIES PVT LTD
ALL-T06874
1H-NMR/DMSO
22-JUL-2025



Current Data Parameters

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EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

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PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	8012.820 Hz
FIDRES	0.244532 Hz
AQ	4.0894465 sec
RG	140.13
DW	62.400 usec
DE	17.09 usec
TE	0 K
D1	1.00000000 sec
TD0	1
SFO1	400.0904705 MHz
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P1	14.00 usec
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F2 - Processing parameters

SI	65536
SF	400.0880071 MHz
WDW	EM
SSB	0
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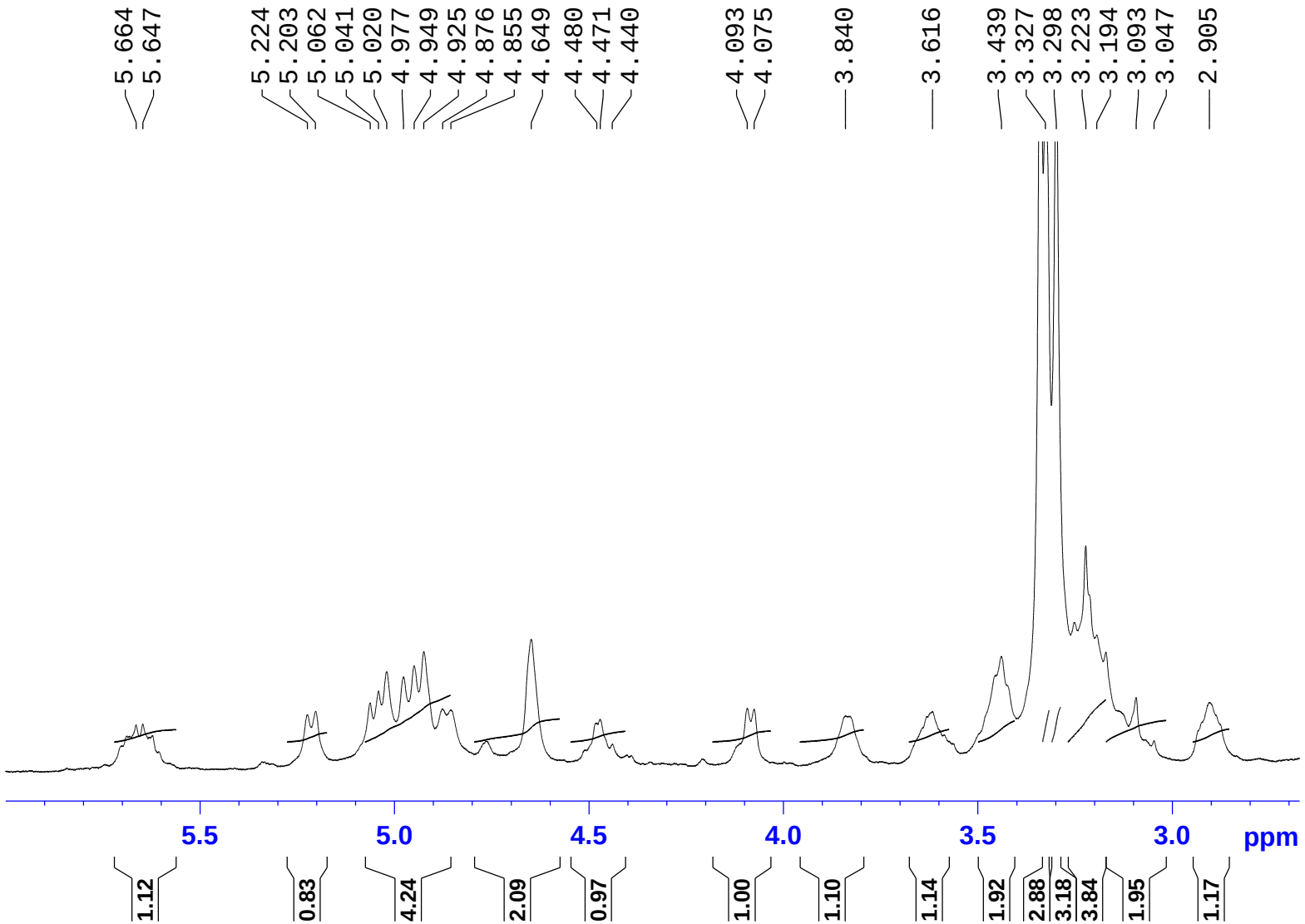
PC	1.00

ALLMPUS LABORATORIES PVT LTD
ALL-T06874
1H-NMR/DMSO
22-JUL-2025



Current Data Parameters

NAME	QC11250722009
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20250722
Time	11.27 h
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PROBHD	Z108618_0828 (
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	8012.820 Hz
FIDRES	0.244532 Hz
AQ	4.0894465 sec
RG	140.13
DW	62.400 usec
DE	17.09 usec
TE	0 K
D1	1.00000000 sec
TD0	1
SFO1	400.0904705 MHz
NUC1	1H
P0	4.67 usec
P1	14.00 usec
PLW1	15.33899975 W

F2 - Processing parameters

SI	65536
SF	400.0880071 MHz
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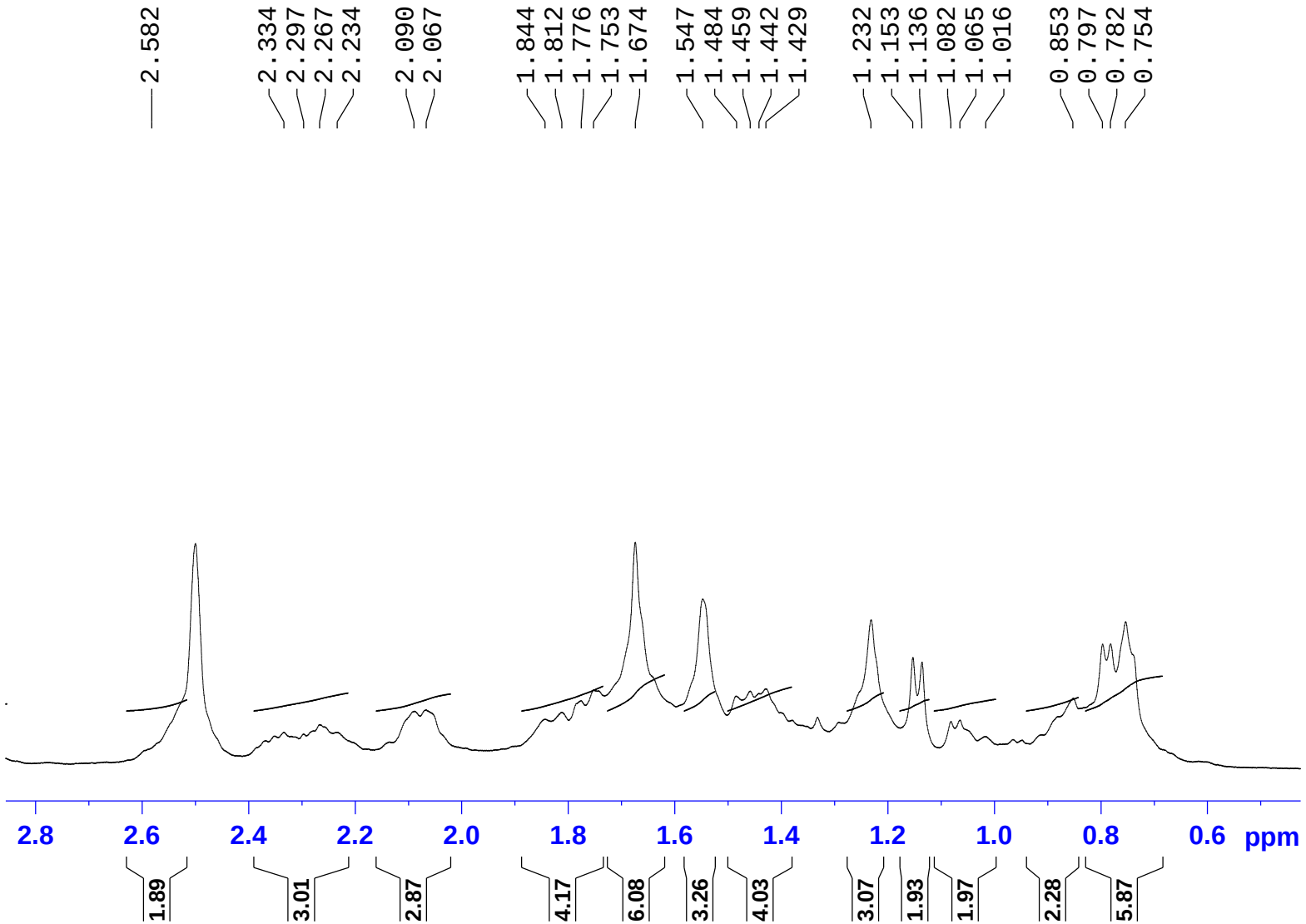
ALLMPUS LABORATORIES PVT LTD
ALL-T06874
1H-NMR/DMSO
22-JUL-2025



Current Data Parameters
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EXPNO 1
PROCNO 1

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PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 140.13
DW 62.400 usec
DE 17.09 usec
TE 0 K
D1 1.00000000 sec
TD0 1
SFO1 400.0904705 MHz
NUC1 1H
P0 4.67 usec
P1 14.00 usec
PLW1 15.33899975 W

F2 - Processing parameters
SI 65536
SF 400.0880071 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

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Sample Name : ALL-T06874

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Acq. Instrument : ALR-QC-LCMS Location : 78
Injection Date : 7/22/2025 5:42:17 PM Inj : 1
Inj Volume : 10.000 µl
Acq. Method : D:\data_ms\JULY-2025\22-07-2025-001 2025-07-22 16-44-32\mass method.M
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(modified after loading)
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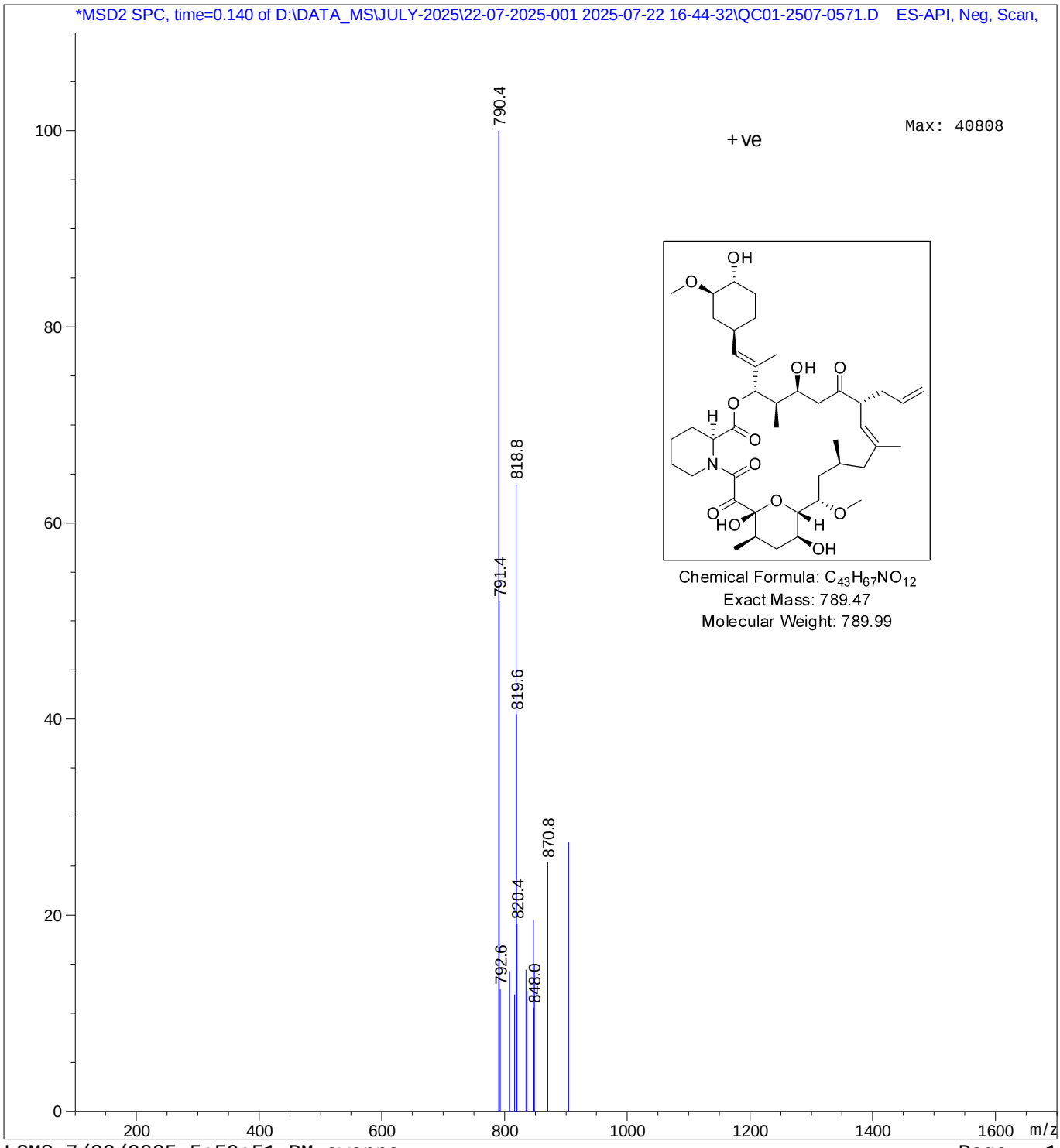
Flow 0.5 ml Fragmentor 110

Sample Info : Diluent : Methanol Expected Mass : 789.99

MS Spectrum



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Sample Name : ALL-T06874

Acq. Operator	: swapna	Seq. Line	: 17
Acq. Instrument	: ALR-QC-LCMS	Location	: 78
Injection Date	: 7/22/2025 5:42:17 PM	Inj	: 1
		Inj Volume	: 10.000 µl
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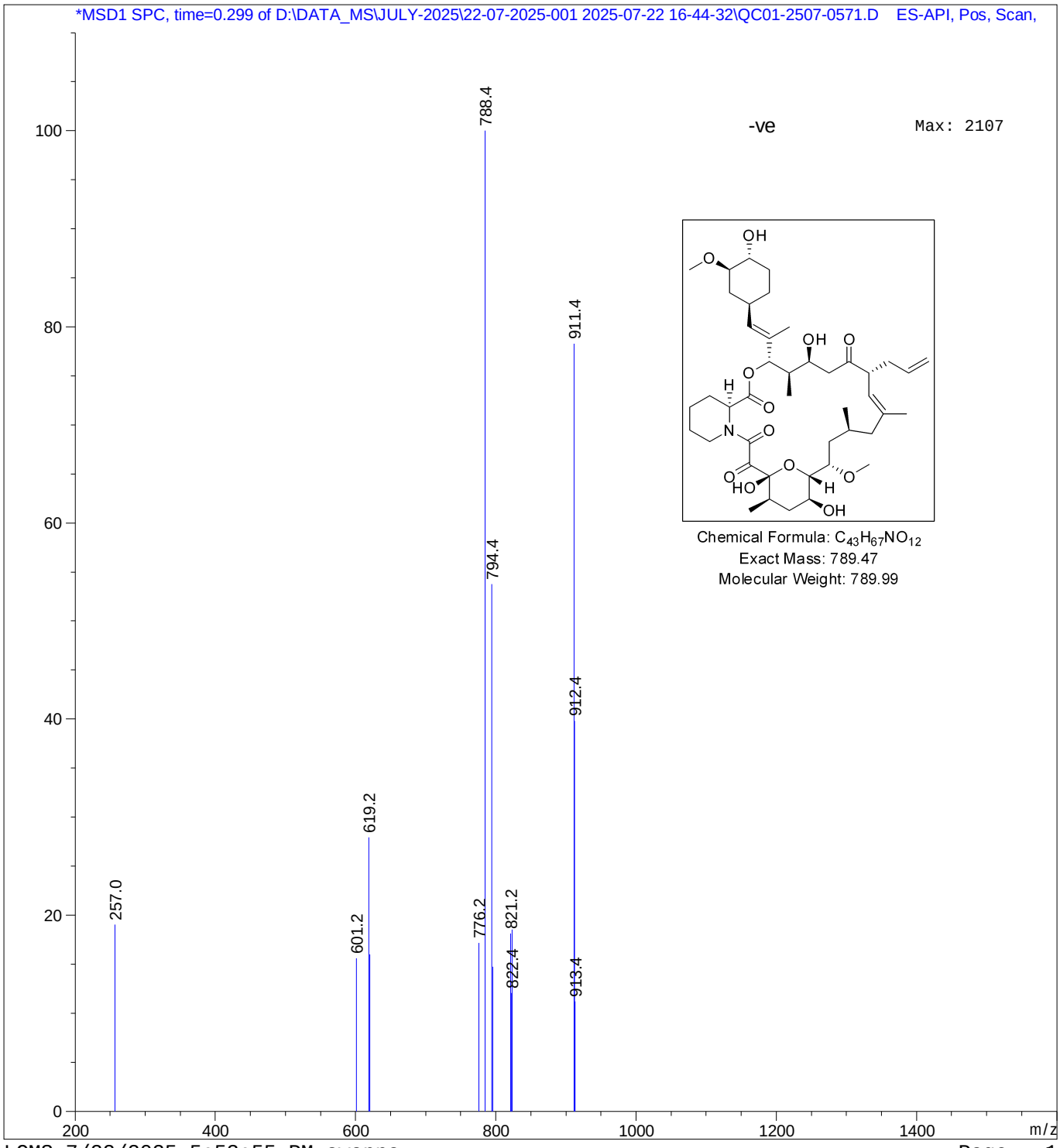
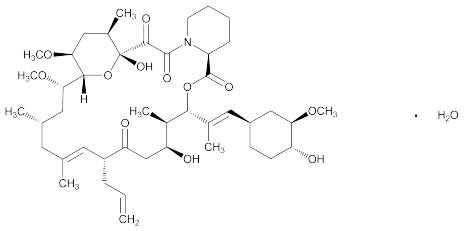
Sample Info	: Diluent : Methanol	Expected Mass	: 789.99

MS Spectrum



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**,3*S**,4*S**)],4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**,18*S**,19*S**,26a*R**]]-;

(–)-(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-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[a]	0.55	16.7	0.2
Tacrolimus diene[b]	0.79	2.2	0.2
Tacrolimus impurity 1[c]	0.96	1.0	0.2
Tacrolimus related compound A[d]	0.96	—	—
Tacrolimus	1.0	1.0	—
Tacrolimus 19-epimer[d, e]	1.1	—	—
Tacrolimus open ring[d, f]	1.3	—	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities[g]	—	—	0.3

[a] (*E*)-3-[[(1*R*,3*R*,4*R*)-4-Hydroxy-3-methoxycyclohexyl]-2-methylacrylaldehyde.
[b] (14*E*,18*E*)-17-Allyl-1-hydroxy-12-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}] octacosa-14,18-diene-2,3,10,16-tetrone.
[c] Specified unidentified impurity.
[d] For informational purposes only; not to be reported.
[e] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-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.
[f] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*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-[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.
[g] 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[a, b]	0.52	—
Ascomycin 19-epimer (if present)[c, d]	0.54	0.1
Tacrolimus 19-epimer[b, e]	0.63	—
Ascomycin[f]	0.87	0.50
Desmethyl tacrolimus (if present)[d, g]	0.94	0.1
Tacrolimus	1.00	—
Tacrolimus 8-epimer[h]	1.28	0.15
Tacrolimus 8-propyl analog[i]	1.33	0.15
Any individual unspecified impurity	—	0.1

Table 3 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities[j]	—	1.0

[a] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*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-[(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.

[b] 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.

[c] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Ethyl-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.

[d] If possible from the manufacturing process.

[e] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-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.

[f] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Ethyl-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.

[g] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-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-4,12,18-trimethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.

[h] (3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-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.

[i] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-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-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

[j] Total impurities limit does not include tacrolimus open ring and tacrolimus 19-epimer.

SPECIFIC TESTS

- **Optical Rotation,** *Specific Rotation* ⟨781S⟩
  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-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-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**,3*S**,4*S**)],4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**, 18*S**,19*S**,26a*R**]]-.
$C_{44}H_{69}NO_{12} \cdot H_2O$ 822.03

USP Tacrolimus Related Compound A RS
(*E*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24, 25,26,26a-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.
$C_{43}H_{69}NO_{12}$ ▲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*,26a*S*)-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.
$C_{44}H_{69}NO_{12}$ ▲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*,26a*S*)-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-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
$C_{44}H_{71}NO_{12}$ 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*,26a*S*)-8-Ethyl-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.
$C_{43}H_{69}NO_{12}$ 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*,26a*S*)-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-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
$C_{44}H_{71}NO_{12}$ 806.03

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

F:\DATA-2025\TACROLIMUS\JULY\22-07-2025\BLANK.lcd

Acquired by	: Admin
Sample Name	: BLANK
Sample ID	: BLANK
Tray#	: 1
Vail #	: 31
Injection Volume	: 20 uL
Data File Name	: BLANK.lcd
Method File Name	: TACROLIMUS 60 MIN.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 22-Jul-25 10:42:34 AM
Data Processed	: 22-Jul-25 11:42:38 AM

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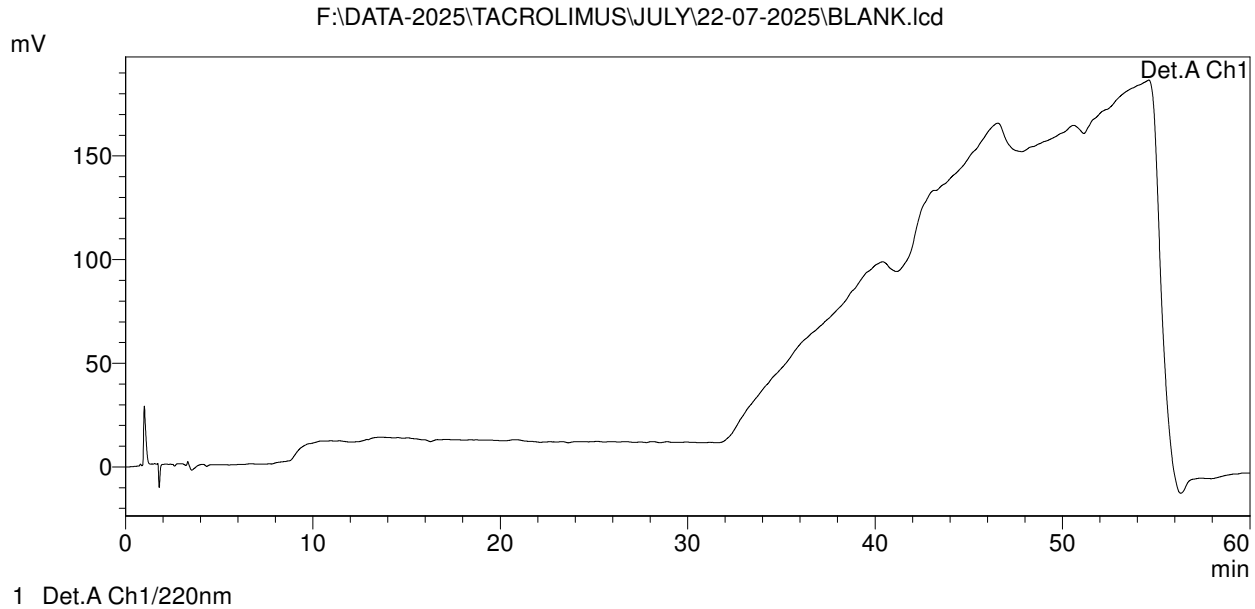

1 Det.A Ch1/220nm

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

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Acquired by	: Admin
Sample Name	: STD
Sample ID	: STD
Tray#	: 1
Vail #	: 32
Injection Volume	: 20 uL
Data File Name	: STD.lcd
Method File Name	: TACROLIMUS 60 MIN.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 22-Jul-25 11:44:29 AM
Data Processed	: 22-Jul-25 12:44:31 PM

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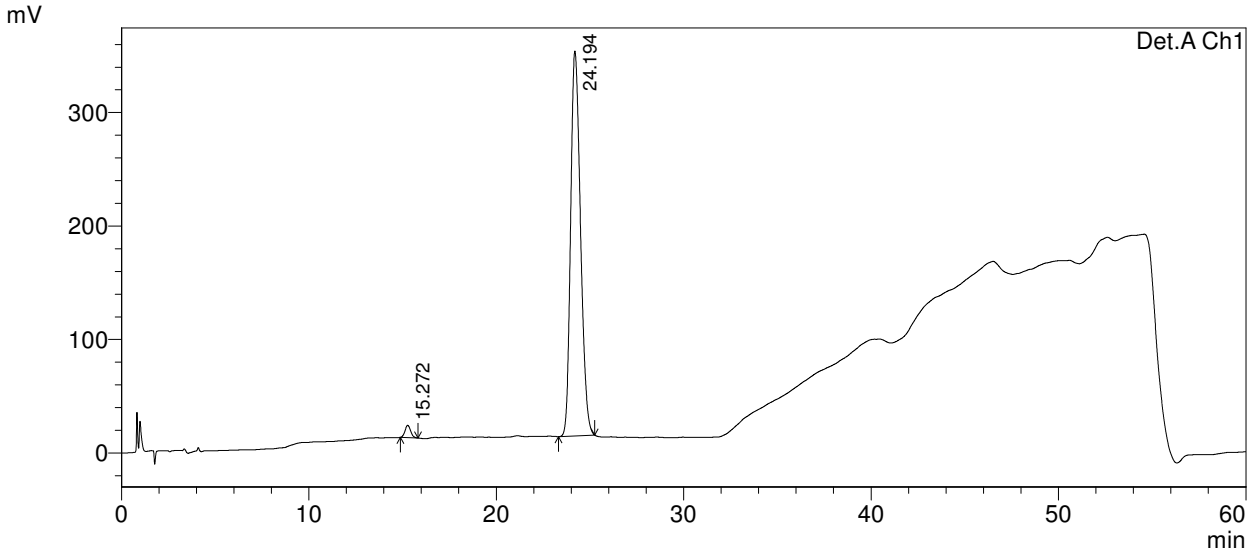

1 Det.A Ch1/220nm

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	15.272	226669	10859	1.793	3.103
2	24.194	12415159	339145	98.207	96.897
Total		12641828	350004	100.000	100.000

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

F:\DATA-2025\TACROLIMUS\JULY\22-07-2025\ALL-T06874.lcd

Acquired by	: Admin
Sample Name	: ALL-T06874
Sample ID	: ALL-T06874
Tray#	: 1
Vail #	: 34
Injection Volume	: 20 uL
Data File Name	: ALL-T06874.lcd
Method File Name	: TACROLIMUS 60 MIN.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 22-Jul-25 1:43:32 PM
Data Processed	: 22-Jul-25 2:43:35 PM

<Chromatogram>

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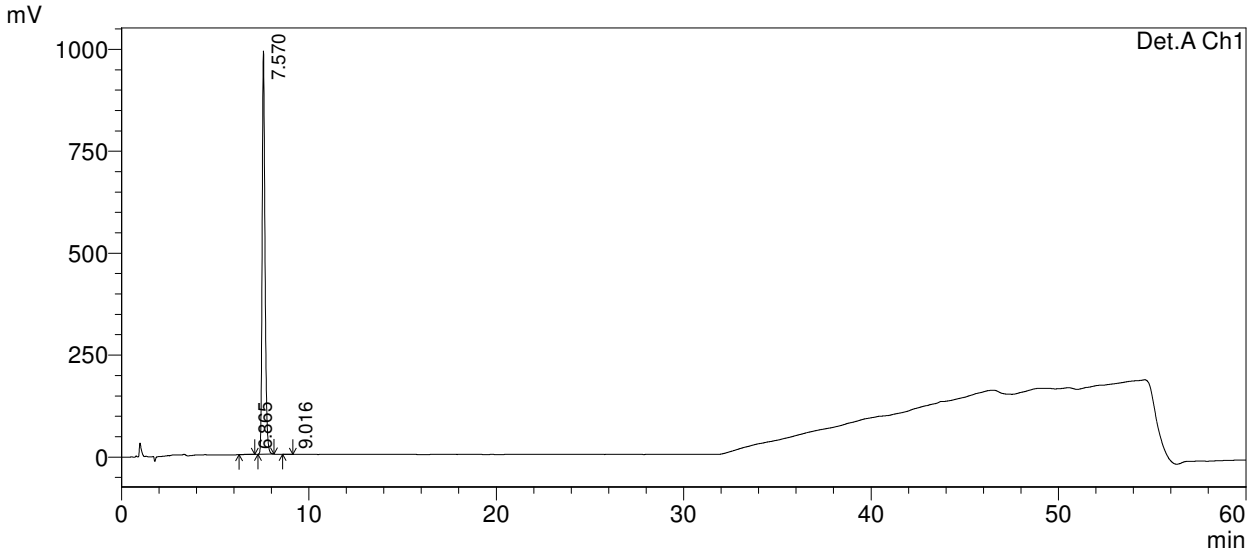


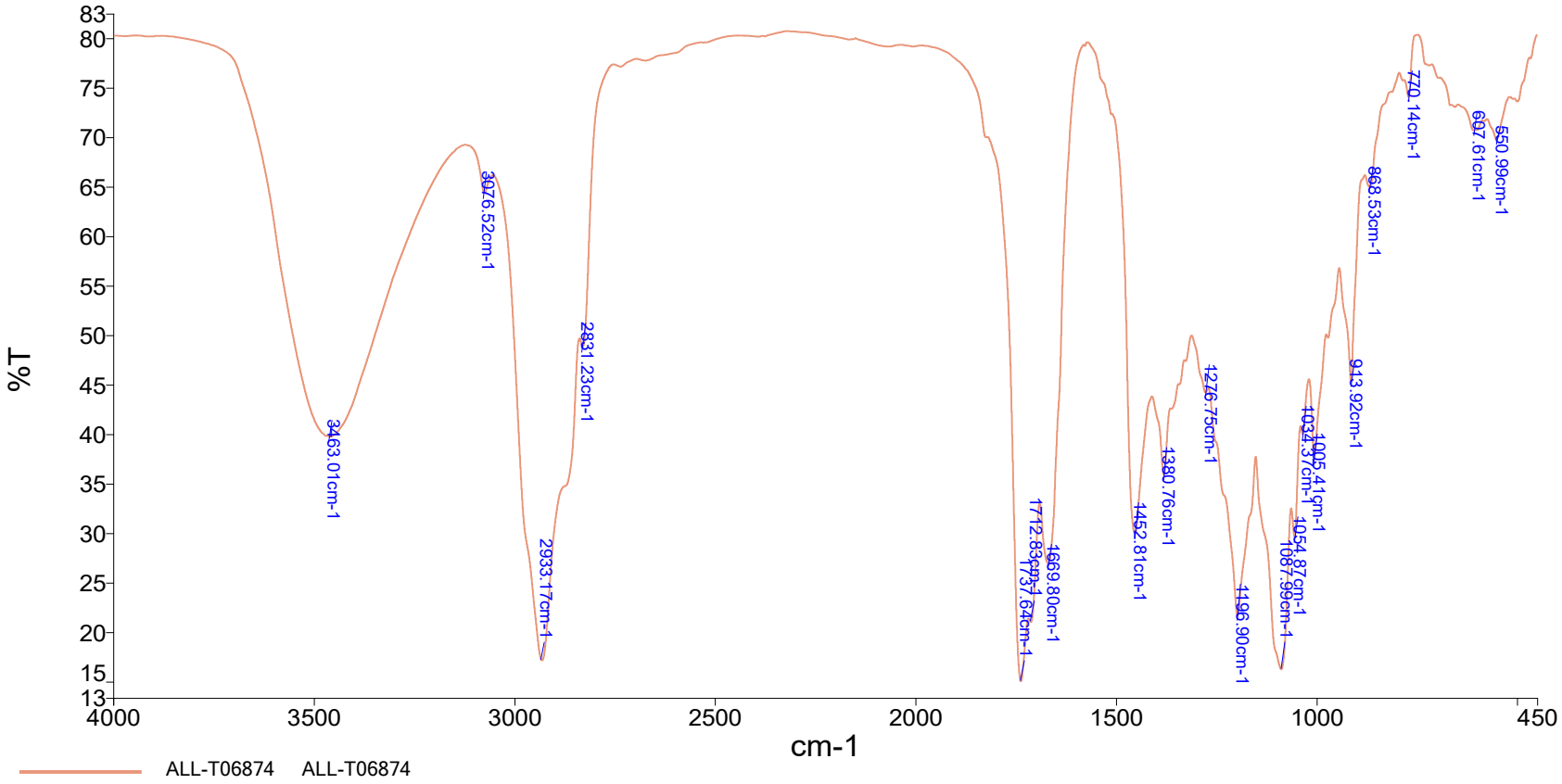
1 Det.A Ch1/220nm

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.865	13771	323	0.129	0.033
2	7.570	10625222	988741	99.851	99.950
3	9.016	2058	174	0.019	0.018
Total		10641051	989238	100.000	100.000

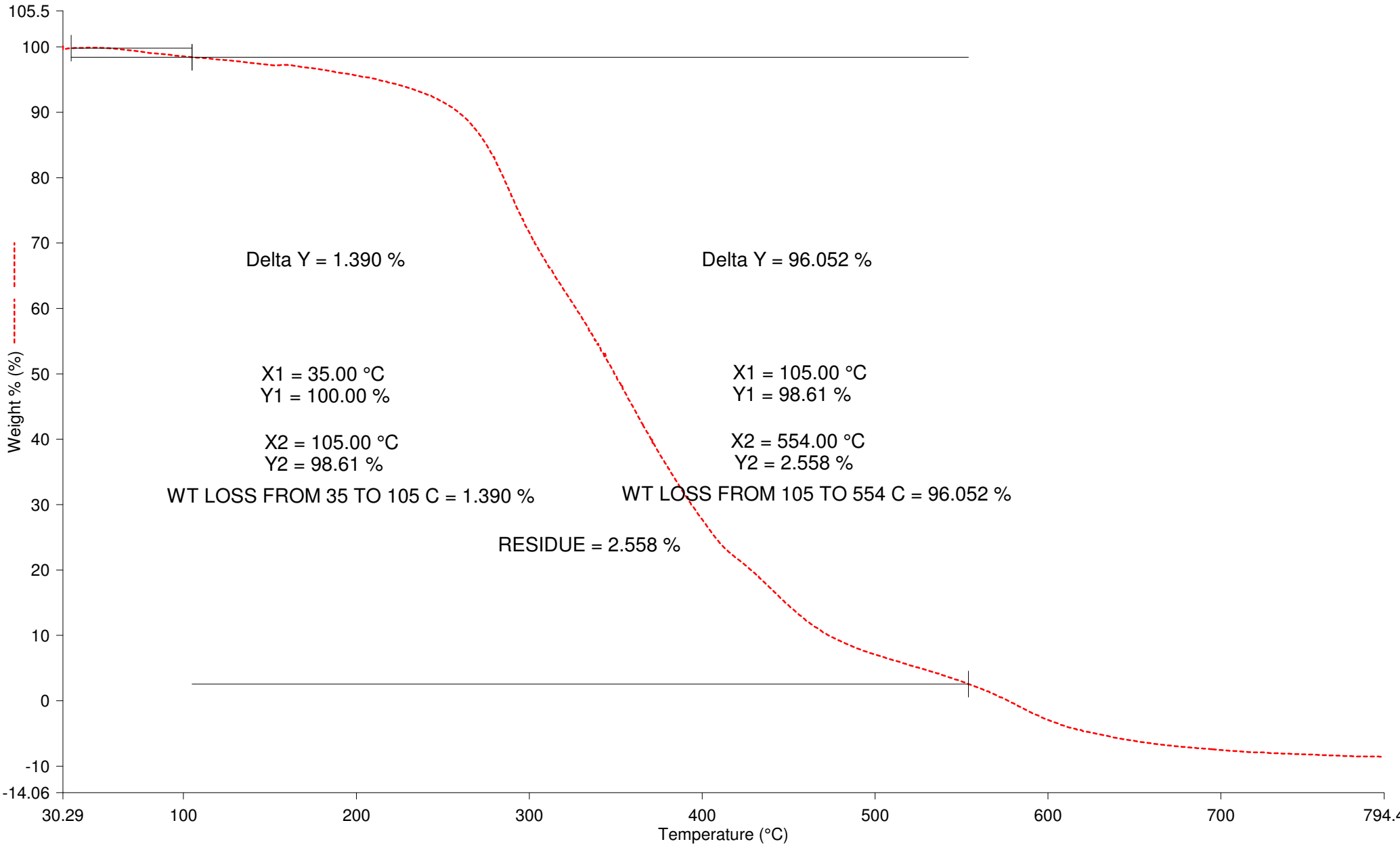
Analyst
Allumpus Laboratories
Date
22 July 2025 15:48



Source Spectra Results	
Spectrum Name	Number Of Peaks
ALL-T06874	20

List of Peak Area/Height		
Peak Number	X (cm-1)	Y (%T)
1	3463.01	39.80
2	3076.52	64.46
3	2933.17	17.16
4	2831.23	48.93
5	1737.64	15.03
6	1712.83	21.04
7	1669.80	26.99
8	1452.81	30.04
9	1380.76	35.94
10	1276.75	44.27
11	1196.90	21.71
12	1087.99	16.25
13	1054.87	29.71
14	1034.37	40.07
15	1005.41	37.89
16	913.92	45.44
17	868.53	65.11
18	770.14	74.20
19	607.61	70.81
20	550.99	69.95

Filename:	D:\TGA Data\ALL-T06874.t6d
Operator ID:	BHARAT
Sample ID:	ALL-T06874
Sample Weight:	1.636 mg
Comment:	



23-07-2025 14:30:22

1) Heat from 30.00°C to 150.00°C at 25.00°C/min

2) Heat from 150.00°C to 800.00°C at 25.00°C/min