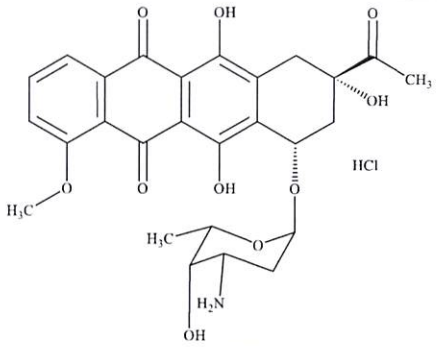
TLC PHARMACEUTICAL STANDARDS

Certificate of Analysis

Certificate No.: **20250701007**

Date: July 1, 2025 Retest date: June 27, 2028

Compound Name:	**Daunorubicin HCl (Epirubicin EP Impurity D HCl)**
Synonyms:	(8S,10S)-8-acetyl-10-(((2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione, hydrochloride (1:1)
TLC Catalogue Number:	D-481
CAS Number:	23541-50-6
Alternate CAS Number:	20830-81-3 (free base)
Molecular Weight:	527.53 36.46
Molecular Formula:	$C_{27}H_{29}NO_{10}$. HCl
Source:	TLC Pharmaceutical Standards
Source Lot No.:	1376-086A1
Storage Conditions:	Store at 2-8 ºC
Solubility:	Methanol, DMSO, Water



Test Description	Specifications	Results
Visual Description	Red solid	**Conforms**
Identification **IR** **MS** **^{1}H NMR**	 Conforms to structure Conforms to structure Conforms to structure	 **Conforms** **Conforms** **Conforms**
Elemental Analysis	Conforms to structure	**Conforms**
Purity (HPLC)	Not less than 95.0%	**99.2%**
Impurity (HPLC)	RT 5.70, 0.28%; RT 13.89, 0.30%	
Water Content (KF)	N/A	**2.4%**
Residue (TGA at 900 ºC)	N/A	**0.4%**
Optical Rotation	$[\alpha]_D^{20.0}$(c=0.05 – 0.10, CH_3OH): +248.0°	$[\alpha]_D^{20.0}$(c=0.11, CH_3OH): **+243.3°**
Residual Solvents (NMR)	No residual solvent	
Assay (%)	Not less than 90.0%	**96.4%**
Recommendation:	**Release.**	

Name	Department	Signature	Date
Reviewed and approved by:	Quality Control		07/03/2025
Approved by:	Quality Assurance		07/03/2025

Attachments: Peak Attribution Table, HPLC, IR, MS, NMR and TGA spectra.

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IR, MS and NMR Peak Attribution Table
lot 1376-086A1

FT-IR	Experimental condition and equipment identification	KBr Thermo Scientific Nicolet iS5
	Description of absorption bands and conclusion	3442.94-3166.11 cm^{-1} -OH, -NH 2981.58-2876.64 cm^{-1} $-CH_2$, $-CH_3$ 1706.02 cm^{-1} C=O Complies.
^1H NMR	Experimental condition and equipment identification	^1H NMR Bruker NMR-400 MHz Solvent: CD_3OD
	Identification and description of all peaks and correlation with respective protons from the structure of molecule and conclusion	δ 7.89 (d, 1H), 7.82 (t, 1H), 7.56 (d, 1H), 5.45 (m, 1H), 5.05 (m, 1H), 4.30 (m, 1H), 4.02 (s, 3H), 3.66 (m, 1H), 3.57 (m, 1H), 2.96 (m, 2H), 2.36-2.29 (m, 4H), 2.15 (m, 1H), 2.04 (m, 1H), 1.89 (m, 1H), 1.29 (d, 3H). Complies.
Mass spectrometry	Experimental condition and equipment identification	ESI Agilent 1260-G6130B
	Identification and characterization of main peak(s) which identify the molecule and conclusion	*m/z*: 526.2, corresponding to $[C_{27}H_{29}NO_{10}-H]^-$ Complies.

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Data File E:\DATA\2025\2025-06-27\1 2025-06-27 17-22-27\1376-086A1 006.D
Sample Name: 1376-086A1

```
==========================================================================
Acq. Operator    : Zhixian Ji                      Seq. Line :   6
Acq. Instrument : LC-1260_6                        Location :   26
Injection Date   : 6/27/2025 8:11:54 PM                Inj :   1
                                                  Inj Volume : 1.200 µl
Acq. Method      : E:\DATA\2025\2025-06-27\1 2025-06-27 17-22-27\9.M
Last changed     : 6/27/2025 5:18:30 PM by Zhixian Ji
Analysis Method : C:\CHEM32\3\METHODS\1.M
Last changed     : 6/30/2025 9:13:31 AM by Zhixian Ji
                   (modified after loading)
Sample Info      : C1097 ZORBAX SB-Aq (4.6*250mm,5um); F=1.0mL/min; T=30 degree;
                   CH3OH/0.1% TFA=65/35(0-6min),65/35-85/15(6-16min),85/15(after 16min)
                   ~1.0mg in 0.5mL CH3OH
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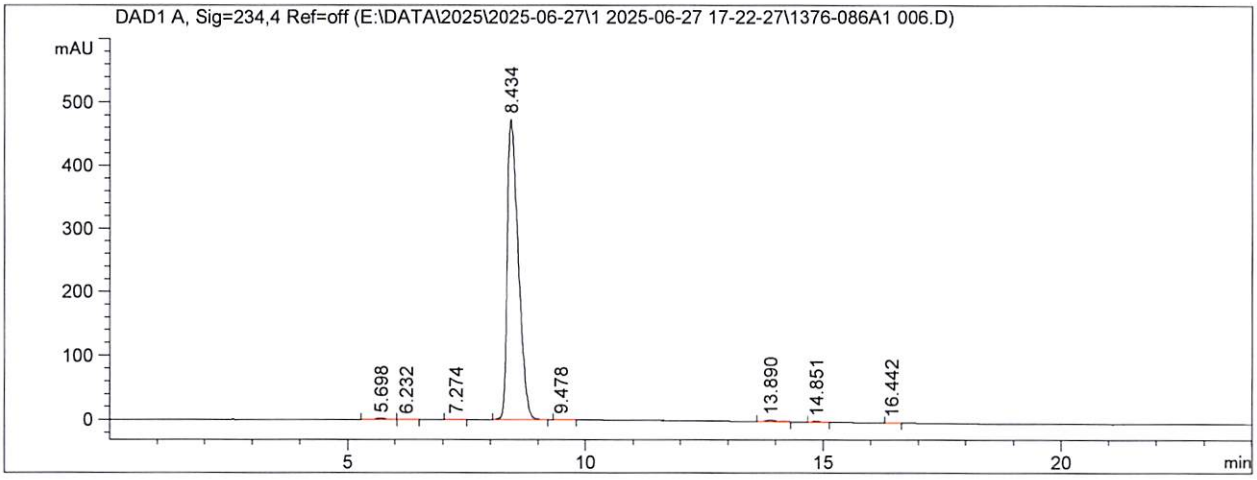


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==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=234,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.698 BB    0.1664   21.37725    2.00556   0.2790
   2   6.232 BV    0.1474    5.05501 4.77819e-1   0.0660
   3   7.274 VV    0.2038    4.50190 2.76685e-1   0.0588
   4   8.434 BBA   0.2455 7595.90869  472.70215  99.1508
   5   9.478 BB    0.1952    2.98099 1.89802e-1   0.0389
   6  13.890 BB    0.1765   22.65366    1.96510   0.2957
   7  14.851 BBA   0.1539    7.47073 7.52045e-1   0.0975
   8  16.442 BBA   0.1145    1.01607 1.17907e-1   0.0133

Totals :                  7660.96431  478.48706
```

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*** End of Report ***

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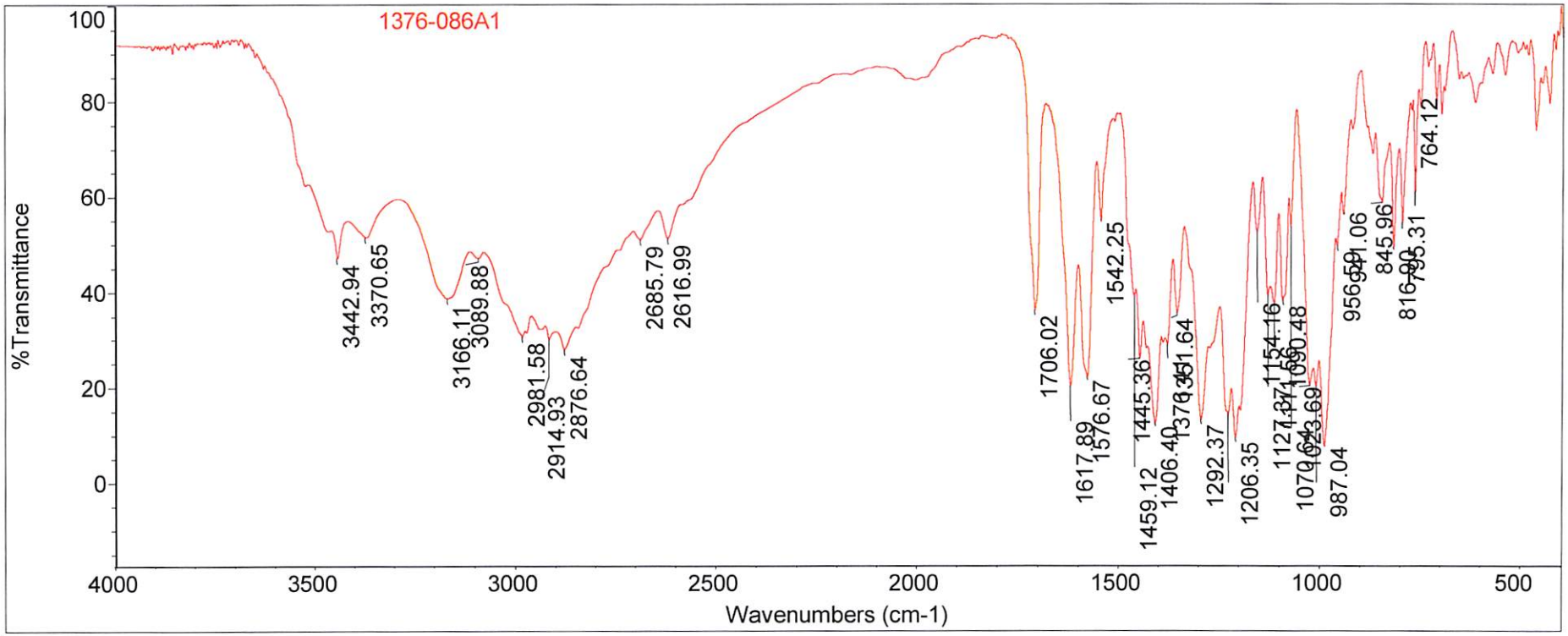
Fri Nov 16 13:43:16 2018 (GMT+08:00)



Collection time: Fri Nov 16 13:41:31 2018 (GMT+08:00)

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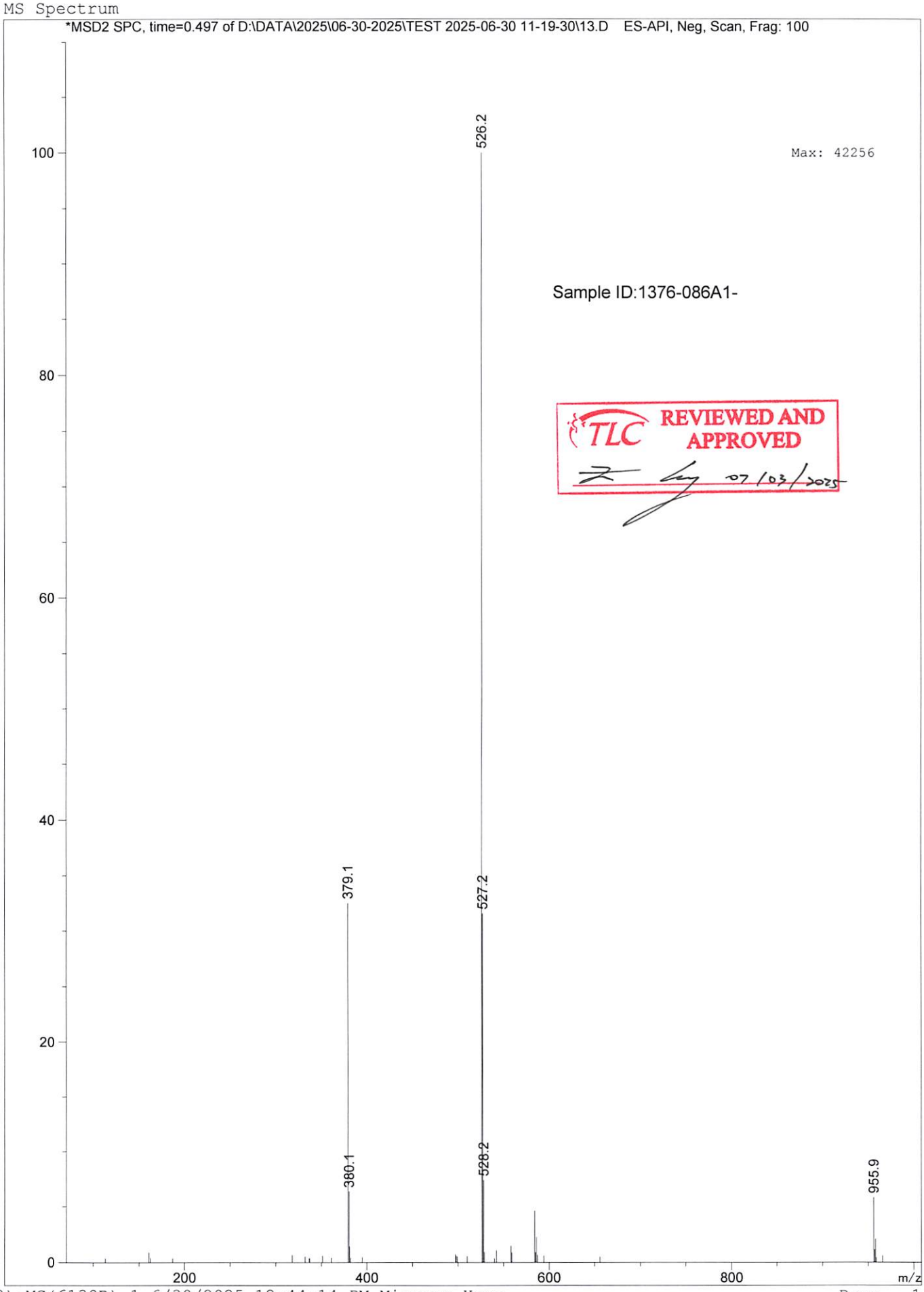
07/03/2025

Fri Nov 16 13:43:01 2018 (GMT+08:00)
FIND PEAKS:
Spectrum: *1376-086A1
Region: 4000.00 400.00
Absolute threshold: 66.016
Sensitivity: 74
Peak list:

Position:	764.12	Intensity:	60.942
Position:	795.31	Intensity:	54.424
Position:	816.90	Intensity:	49.420
Position:	845.96	Intensity:	58.740
Position:	941.06	Intensity:	56.289
Position:	956.59	Intensity:	49.689
Position:	987.04	Intensity:	7.631
Position:	1008.05	Intensity:	20.248
Position:	1023.69	Intensity:	20.600
Position:	1070.64	Intensity:	53.626
Position:	1090.48	Intensity:	38.244
Position:	1111.56	Intensity:	37.571
Position:	1127.37	Intensity:	39.332
Position:	1154.16	Intensity:	52.729
Position:	1206.35	Intensity:	9.629
Position:	1224.63	Intensity:	14.502
Position:	1292.37	Intensity:	13.232
Position:	1351.64	Intensity:	35.746
Position:	1376.41	Intensity:	29.145
Position:	1406.40	Intensity:	12.561
Position:	1445.36	Intensity:	26.216
Position:	1459.12	Intensity:	39.015
Position:	1542.25	Intensity:	55.150
Position:	1576.67	Intensity:	22.144
Position:	1617.89	Intensity:	20.352
Position:	1706.02	Intensity:	36.246
Position:	2616.99	Intensity:	51.182
Position:	2685.79	Intensity:	50.970
Position:	2876.64	Intensity:	27.946
Position:	2914.93	Intensity:	30.071
Position:	2981.58	Intensity:	30.613
Position:	3089.88	Intensity:	47.102
Position:	3166.11	Intensity:	38.585
Position:	3370.65	Intensity:	51.467
Position:	3442.94	Intensity:	47.087

Signature: GYJ, 11-16-2018 13:43:13 (GMT+08:00), Authorship - signifies ownership
1376-086A1

MS Spectrum



Sample ID:1376-086A1-

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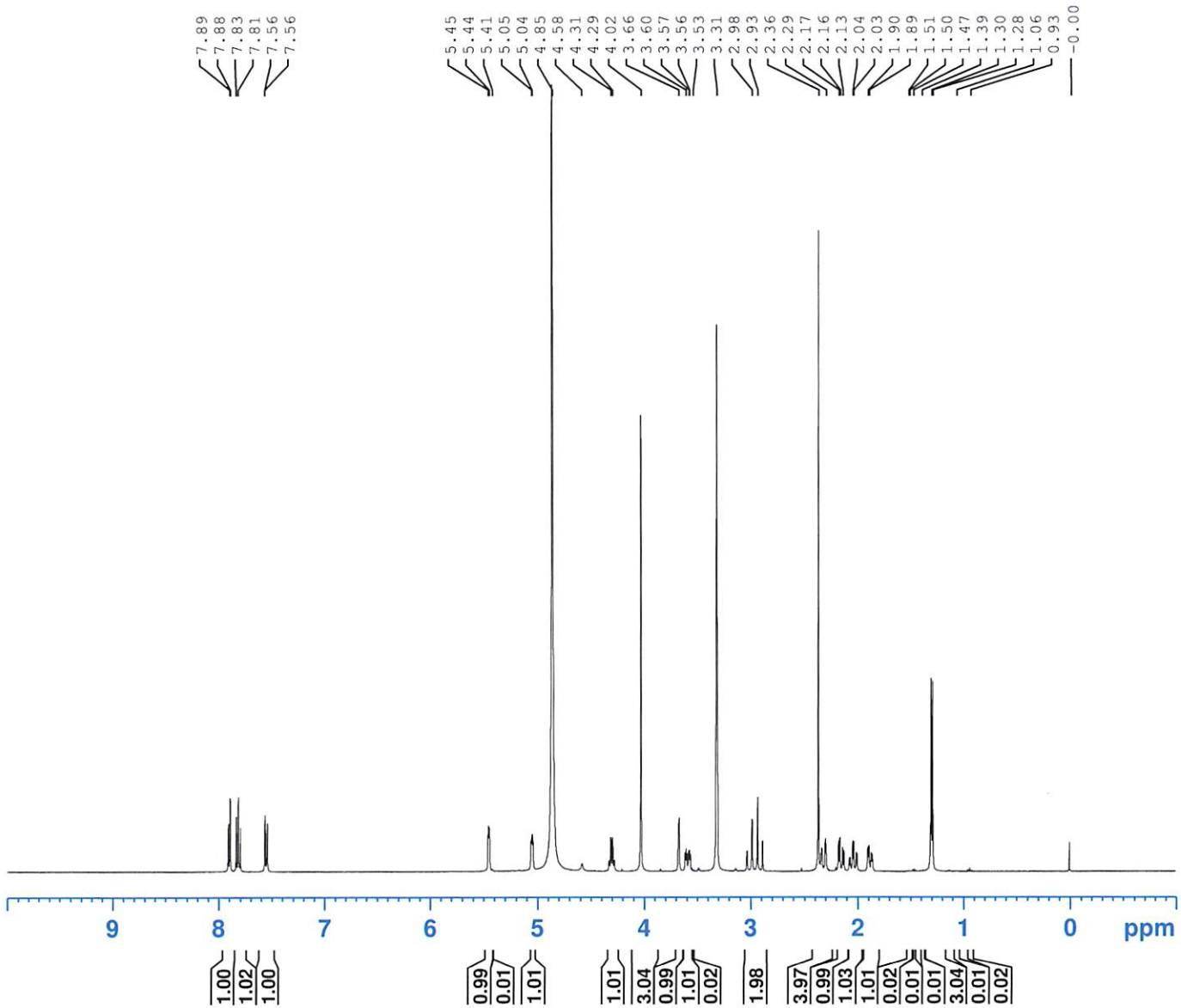
1376-086A1 1H NMR in CD3OD



BRUKER

Current Data Parameters
NAME 2025-06-27(NMR-400_2)
EXPNO 147
PROCNO 1

F2 - Acquisition Parameters
Date_ 20250627
Time 16.16 h
INSTRUM spect
PROBHD Z116098_0656 (
PULPROG zg30
TD 65536
SOLVENT CD3OD
NS 16
DS 0
SWH 9615.385 Hz
FIDRES 0.293438 Hz
AQ 3.4078720 sec
RG 71.77
DW 52.000 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.1836016 MHz
NUC1 1H
P1 10.00 usec
PLW1 18.13100052 W

F2 - Processing parameters
SI 65536
SF 400.1800092 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 0.50

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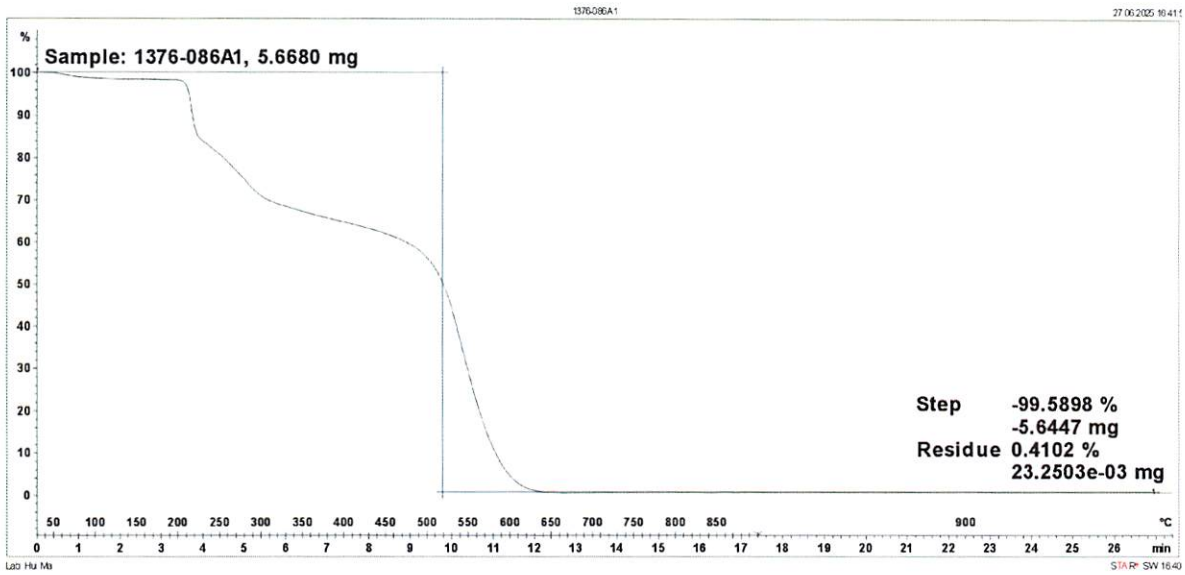
TGA



Sample:	1376-086A1, 5.6680 mg
Module:	TGA 2 SF/1100/1354, 30.08.2022 03:25:58
Method:	30-900-50K-O2-ISO-10min-Pt
User:	Hui Ma
Experiment name:	1376-086A1, 27.06.2025 16:10:24
Performed:	27.06.2025 16:40:29

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