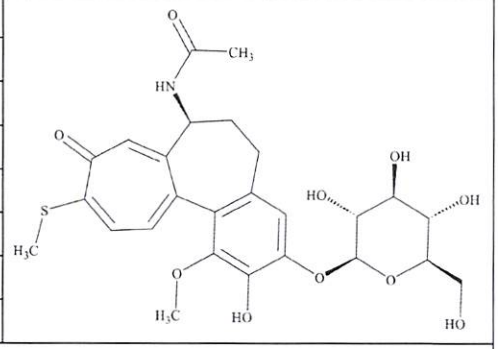
TLC PHARMACEUTICAL STANDARDS

# Certificate of Analysis

Certificate No.: **20250509013**

Date: May 9, 2025 Retest date: March 28, 2028

| | |
|---|---|
| **Compound Name:** | **Thiocolchicoside EP Impurity K (2-Hydroxy)** |
| **Synonyms:** | *N*-((S)-2-hydroxy-1-methoxy-10-(methylthio)-9-oxo-3-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl)acetamide |
| **TLC Catalogue Number:** | T-7310 |
| **CAS Number:** | N/A |
| **Alternate CAS Number:** | N/A |
| **Molecular Weight:** | 549.59 |
| **Molecular Formula:** | $C_{26}H_{31}NO_{10}S$ |
| **Source:** | TLC Pharmaceutical Standards |
| **Source Lot No.:** | 6732-068A4 |
| **Storage Conditions:** | Hygroscopic, store at 2-8 °C |
| **Solubility:** | Methanol, DMSO, DMF |



| **Test Description** | **Specifications** | **Results** |
|---|---|---|
| **Visual Description** | Yellow solid | **Conforms** |
| **Identification**<br>**IR**<br>**MS**<br>**$^1$H NMR** | <br>Conforms to structure<br>Conforms to structure<br>Conforms to structure | <br>**Conforms**<br>**Conforms**<br>**Conforms** |
| **Purity (HPLC)** | Not less than 95.0% | **96.5%** |
| **Impurity (HPLC)** | RT 9.89, 0.50%; RT 14.31, 0.75%; RT 14.54, 1.79% | |
| **Residue (TGA at 900 °C)** | N/A | **0.2%** |
| **Residual Solvents (NMR)** | 2.8% methanol | |
| **Assay (qNMR)** | Not less than 90.0% | **92.0%** |
| **Recommendation:** | **Release.** The compound contains 1.8% M-14 impurity (RT 14.54) according to its HPLC and LC-MS. | |

| **Name** | **Department** | **Signature** | **Date** |
|---|---|---|---|
| Reviewed and approved by: | Quality Control | | 05/09/2025 |
| Approved by: | Quality Assurance | | 05/09/2025 |

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

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# IR, MS and NMR Peak Attribution Table
## lot 6732-068A4

| | | |
|---|---|---|
| FT-IR | Experimental condition and equipment identification | KBr<br>Thermo Scientific Nicolet iS5 |
| | Description of absorption bands and conclusion | 3369.74 $cm^{-1}$ -NH, -OH<br>2919.81 $cm^{-1}$ $-CH_2$, $-CH_3$<br>1656.07-1606.75 $cm^{-1}$ C=O, C=C<br>Complies. |
| $^1H$ NMR | Experimental condition and equipment identification | $^1H$ NMR<br>Bruker NMR-400 MHz<br>Solvent: $CD_3OD$ |
| | Identification and description of all peaks and correlation with respective protons from the structure of molecule and conclusion | δ 7.36 (m, 2H), 7.18 (s, 1H), 6.90 (s, 1H), 4.88 (d, 1H), 4.50 (m, 1H), 3.90 (d, 1H), 3.70 (m, 1H), 3.56 (s, 3H), 3.55-3.35 (m, 4H), 2.56 (m, 1H), 2.47 (s, 3H), 2.30 (m, 1H), 2.19 (m, 1H), 1.98 (s, 3H), 1.89 (m, 1H).<br>Complies. |
| Mass spectrometry | Experimental condition and equipment identification | ESI<br>Agilent 1260-G6130B |
| | Identification and characterization of main peak(s) which identify the molecule and conclusion | *m/z*: 550.2, corresponding to $[C_{26}H_{31}NO_{10}S+H]^+$<br>Complies. |

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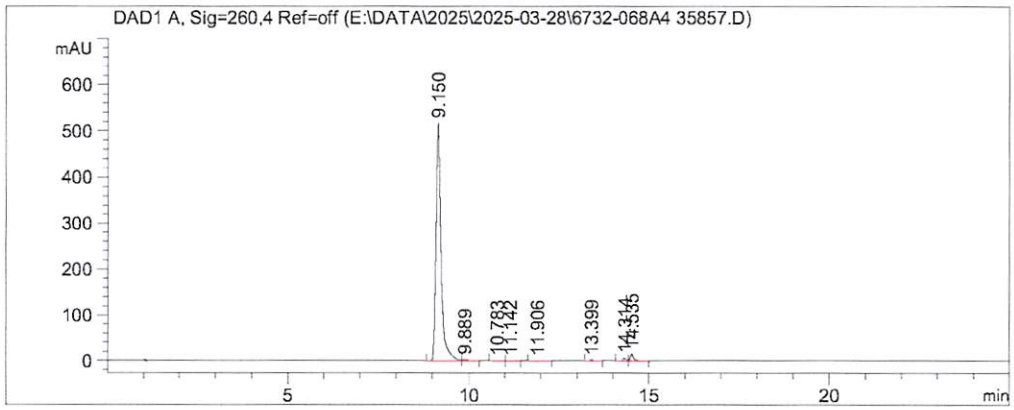
Data File E:\DATA\2025\2025-03-28\6732-068A4 35857.D
Sample Name: 6732-068A4

```
==========================================================================
Acq. Operator    : Min Zhang
Sample Operator  : Min Zhang
Acq. Instrument  : LC-1260_4                        Location :   33
Injection Date   : 3/28/2025 11:03:44 AM
                                                  Inj Volume : 1.500 µl
Acq. Method      : C:\CHEM32\1\METHODS\1.M
Last changed     : 3/28/2025 11:00:12 AM by Min Zhang
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\5.M
Last changed     : 3/28/2025 12:41:14 PM by Min Zhang
                   (modified after loading)
Sample Info      : C1122 Poroshell 120 SB-C18 (4.6*100mm,2.7um); F=1.0mL/min; T=30 degree;
                   CH3OH/10mmol/L NaH2PO4 (adjusted to pH 2.6 with H3PO4)=25/75(0-6min),25/75-
                   45/55(6-16min),45/55(after 16min)
                   ~0.9mg in 0.5mL CH3OH
```




```
==========================================================================
                          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=260,4 Ref=off
```

| Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|---|---|---|---|---|---|---|
| 1 | 9.150 | BV | 0.1501 | 5148.05957 | 517.19543 | 96.5172 |
| 2 | 9.889 | VBA | 0.1453 | 26.77800 | 2.66265 | 0.5020 |
| 3 | 10.783 | BV | 0.1186 | 6.03254 | 7.77158e-1 | 0.1131 |
| 4 | 11.142 | VB | 0.1392 | 1.33305 | 1.32684e-1 | 0.0250 |
| 5 | 11.906 | VV | 0.1536 | 2.37669 | 2.10767e-1 | 0.0446 |
| 6 | 13.399 | VBA | 0.0986 | 13.58878 | 2.06867 | 0.2548 |
| 7 | 14.314 | BV | 0.0880 | 40.01868 | 6.86668 | 0.7503 |
| 8 | 14.535 | VBA | 0.0984 | 95.63892 | 14.60211 | 1.7931 |
| Totals : | | | | 5333.82622 | 544.51617 | |

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Data File E:\DATA\2025\2025-03-28\6732-068A4 35857.D
Sample Name: 6732-068A4

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*** End of Report ***

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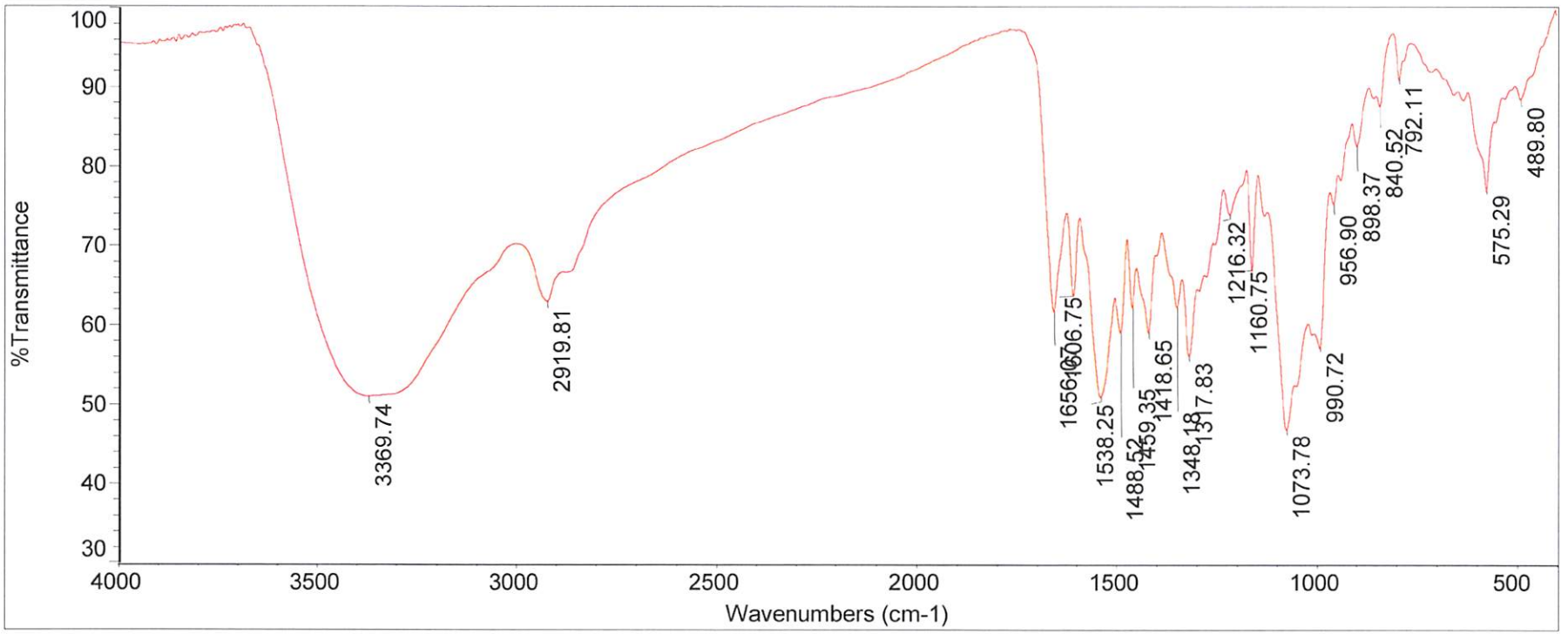
6732-068A4




Collection time: Fri Mar 28 15:00:38 2025 (GMT+08:00)

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Fri Mar 28 15:02:10 2025 (GMT+08:00)
FIND PEAKS:
Spectrum: 6732-068A4
Region: 4000.00 400.00
Absolute threshold: 97.077
Sensitivity: 60
Peak list:

| | | | |
|---|---|---|---|
| Position: | 489.80 | Intensity: | 88.476 |
| Position: | 575.29 | Intensity: | 76.928 |
| Position: | 792.11 | Intensity: | 91.015 |
| Position: | 840.52 | Intensity: | 87.606 |
| Position: | 898.37 | Intensity: | 82.658 |
| Position: | 956.90 | Intensity: | 75.297 |
| Position: | 990.72 | Intensity: | 57.135 |
| Position: | 1073.78 | Intensity: | 46.834 |
| Position: | 1160.75 | Intensity: | 66.895 |
| Position: | 1216.32 | Intensity: | 73.926 |
| Position: | 1317.83 | Intensity: | 56.117 |
| Position: | 1348.18 | Intensity: | 62.250 |
| Position: | 1418.65 | Intensity: | 59.063 |
| Position: | 1459.35 | Intensity: | 62.244 |
| Position: | 1488.52 | Intensity: | 59.087 |
| Position: | 1538.25 | Intensity: | 50.921 |
| Position: | 1606.75 | Intensity: | 63.748 |
| Position: | 1656.07 | Intensity: | 61.721 |
| Position: | 2919.81 | Intensity: | 62.968 |
| Position: | 3369.74 | Intensity: | 50.975 |

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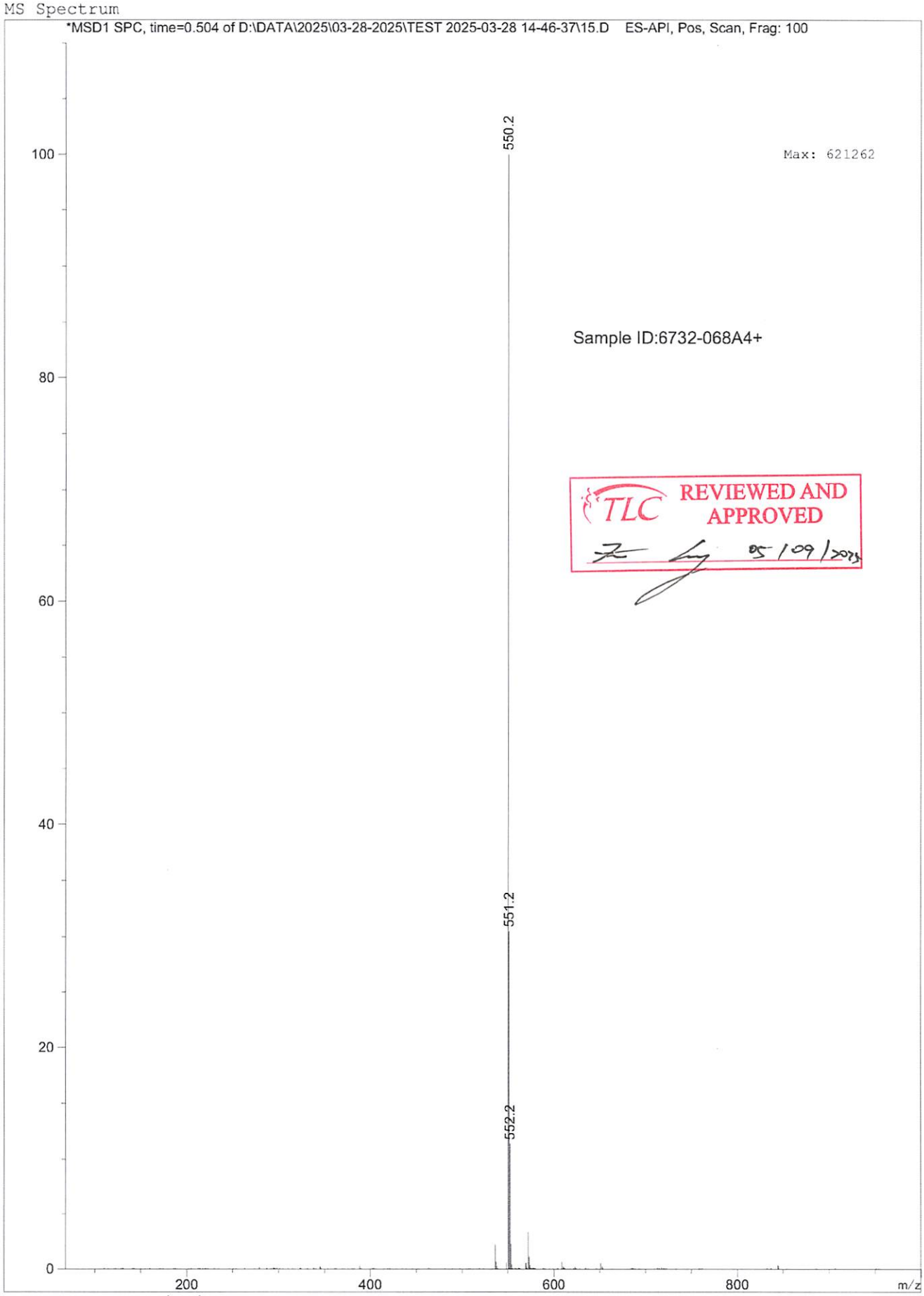
Signature: Lulu Zhang, 03-28-2025 15:02:23 (GMT+08:00), Authorship - signifies ownership
6732-068A4

MS Spectrum

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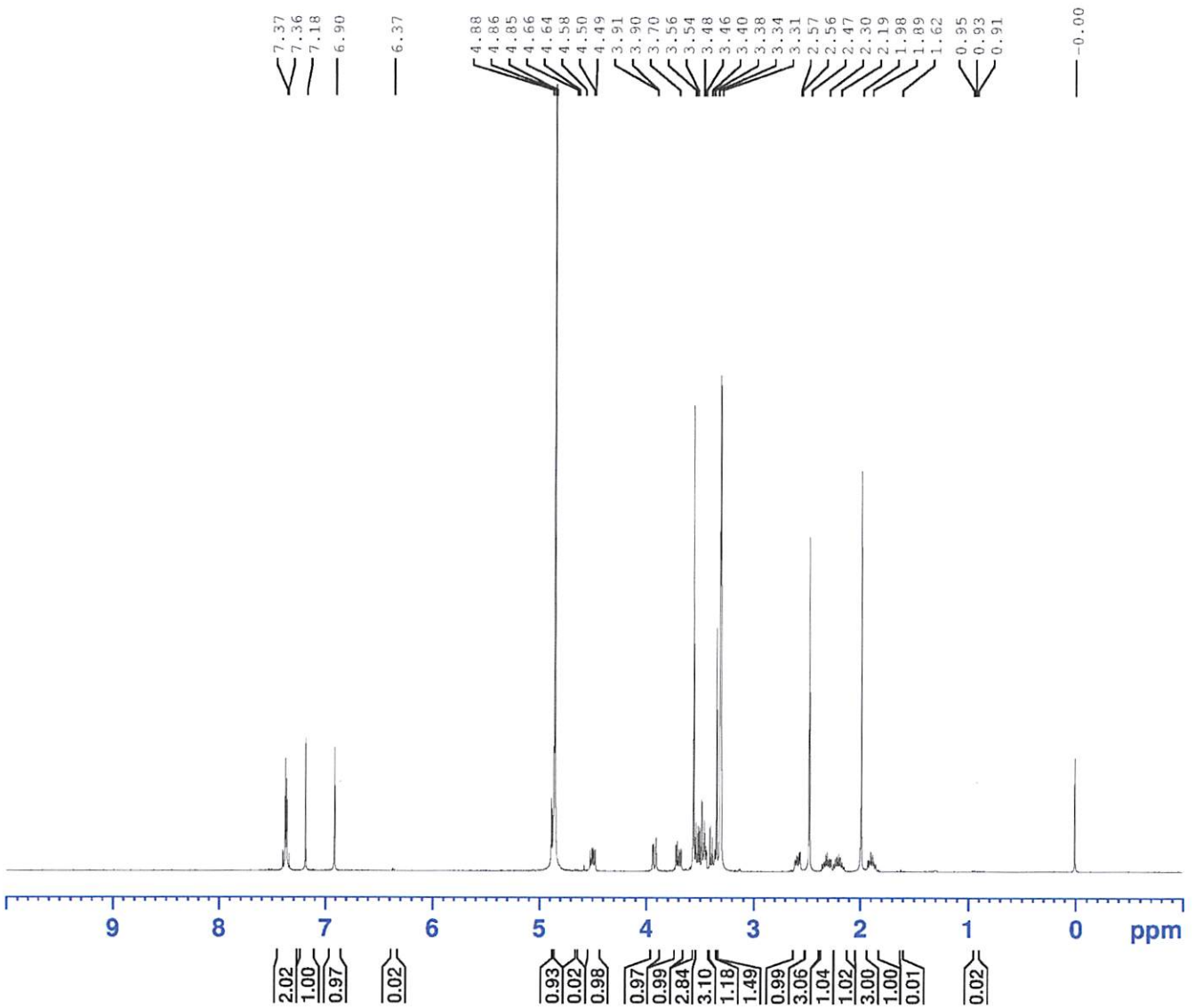
6732-068A4 1H NMR in CD3OD




BRUKER

```
Current Data Parameters
NAME      2025-03-31(NMR-400_2)
EXPNO                         9
PROCNO                        1

F2 - Acquisition Parameters
Date_              20250331
Time                   10.01 h
INSTRUM                spect
PROBHD    Z116098_0656 (
PULPROG                 zg30
TD                     65536
SOLVENT                CD3OD
NS                         4
DS                         0
SWH                 9615.385 Hz
FIDRES              0.293438 Hz
AQ                  3.4078720 sec
RG                     109.98
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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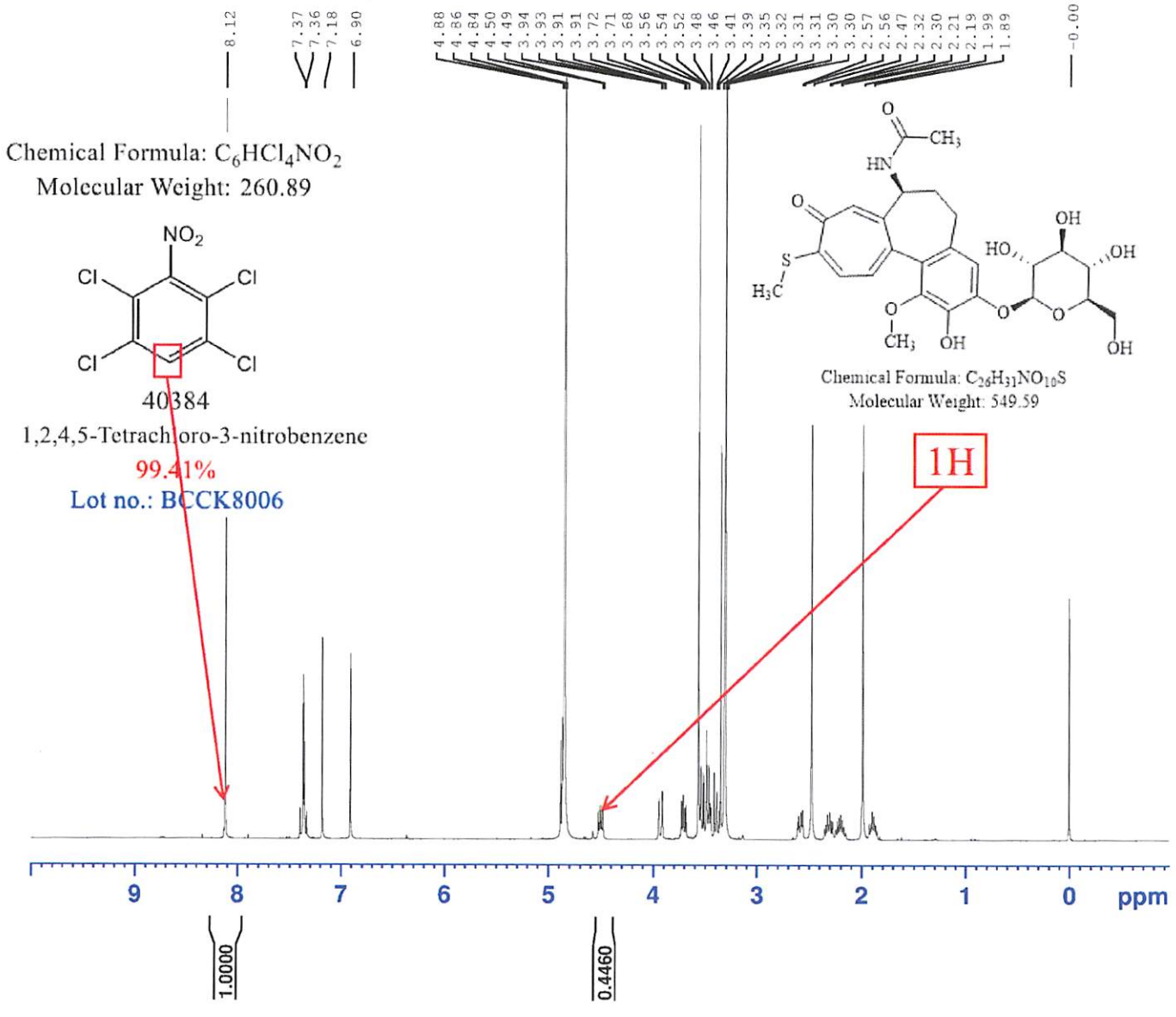
6732-068A4(5.604mg) and 40384(5.520mg) 1H NMR in CD3OD




BRUKER

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Current Data Parameters
NAME      2025-03-31(NMR-400_1)
EXPNO                 16
PROCNO                 1

F2 - Acquisition Parameters
Date_           20250331
Time              14.34 h
INSTRUM           spect
PROBHD   Z116098_0293 (
PULPROG             zg30
TD                65536
SOLVENT           CD3OD
NS                    8
DS                    0
SWH            4807.692 Hz
FIDRES        0.146719 Hz
AQ            6.8157439 sec
RG               115.63
DW               104.000 usec
DE                 6.50 usec
TE                298.0 K
D1           50.00000000 sec
TD0                   1
SFO1         400.1320007 MHz
NUC1                 1H
P1                10.11 usec
PLW1         16.24900055 W

F2 - Processing parameters
SI                65536
SF           400.1300088 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

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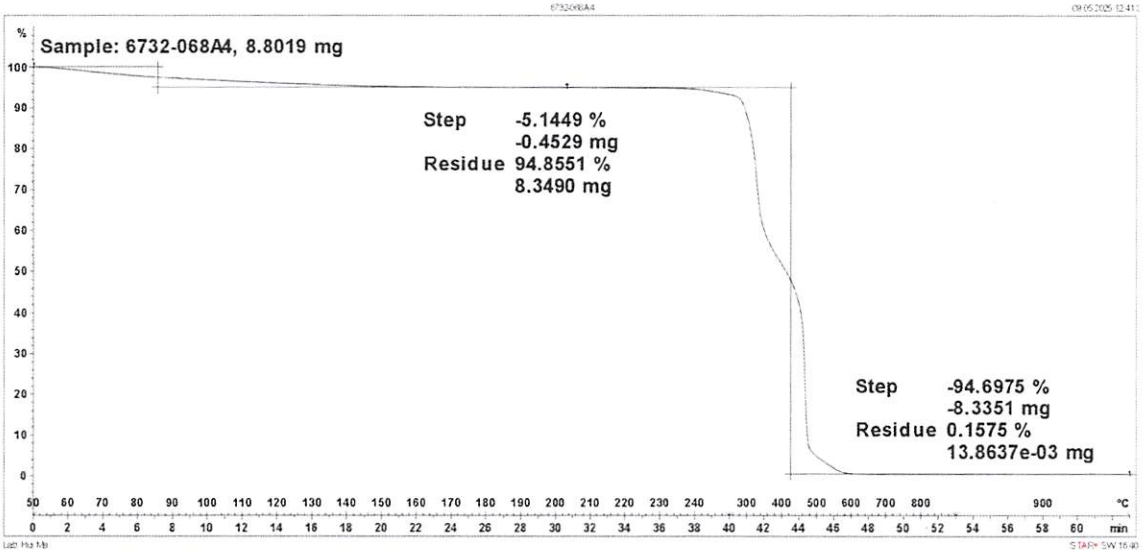
# TGA




| | |
|---|---|
| **Sample:** | 6732-068A4, 8.8019 mg |
| **Module:** | TGA 2 SF/1100/1354, 30.08.2022 03:25:58 |
| **Method:** | 50-250-5K-N2-900-50K -O2-ISO-10min-Pt |
| **User:** | Hui Ma |
| **Experiment name:** | 6732-068A4, 09.05.2025 10:33:55 |
| **Performed:** | 09.05.2025 11:46:58 |

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