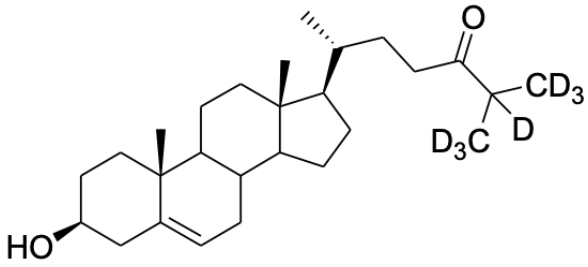
Certificate of Analysis



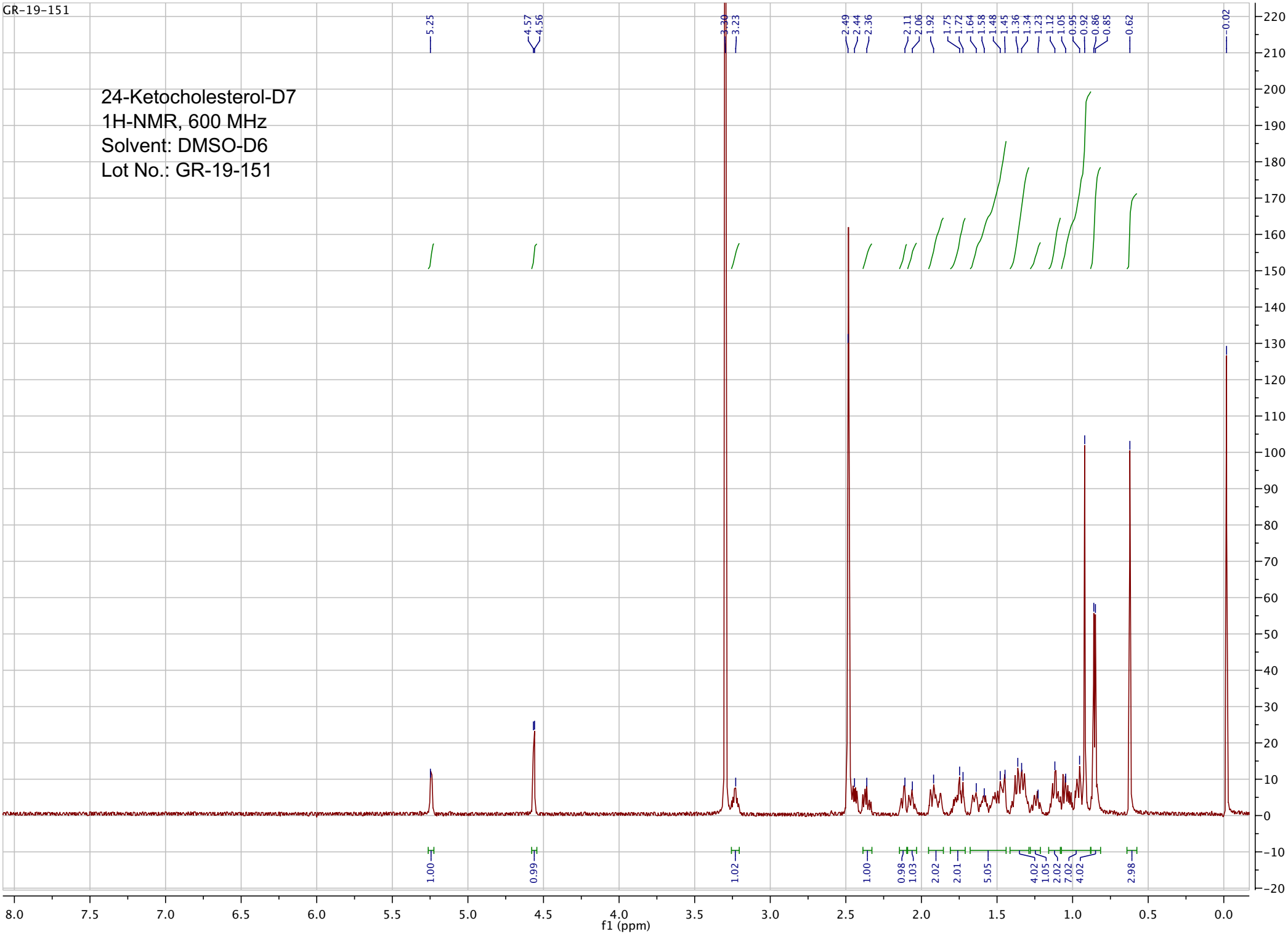
Name:	**24-Ketocholesterol-D7**
Lot#:	GR-19-151
Test Date:	02/03/2025 (re-test date:02/03/2030)
CAS No.:	17752-16-8 (unlabeled)
MF:	$C_{27}H_{37}D_7O_2$
MW:	407.68
Appearance:	White Solid
Purity:	97.5% by HPLC (average of two sample preparations); 99% atom D
^{1}H-NMR:	Conforms
MS-ESI (+)	Conforms (shows peaks at m/z = 408.39 $[M+H]^+$ and 390.38 $[M-H_2O+H]^+$)
Storage	Store at -18^{o}C in a dry place away from direct sunlight

Approved by:

Date: 02/04/2025

Viorica Rusu, QC/QA Manager

700, Collip Circle, The Research Park, London, ON, N6G 4X8
Ph: (647)4079078, e-mail: grusu@esschemco.com, www.esschemco.com


GR-19-151
24-Ketocholesterol-D7
1H-NMR, 600 MHz
Solvent: DMSO-D6
Lot No.: GR-19-151
5.25
4.57
4.56
3.30
3.23
2.49
2.44
2.36
2.11
2.06
1.92
1.75
1.72
1.64
1.58
1.48
1.45
1.36
1.34
1.23
1.12
1.05
0.95
0.92
0.86
0.85
0.62
-0.02
1.00
0.99
1.02
1.00
0.98
1.03
2.02
2.01
5.05
4.02
1.05
2.02
7.02
4.02
2.98
f1 (ppm)

24.06.21.0858

MASS SPECTROMETRY

Sample Submission Form 2022-2023

The AIMS Mass Spectrometry Laboratory has no Biocontainment Level designation.
All samples of biological origin must therefore be inactivated and free from pathogens.

	Yes	No
Is sample of biological origin?	○	⊘
If yes, has sample been treated to inactivate pathogens?	○	○

Authorized Signature: [signature]

P.I. Name: Expert Synthesis Solutions

Account:

Name: Gheradie Rusu

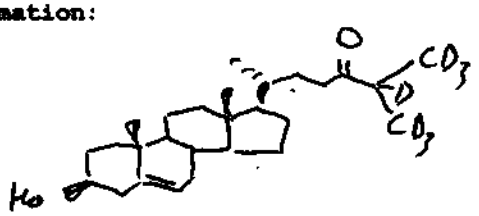
Email (Please type): grusu@csschemco.com

Sample ID: GR-19-151

Molecular Formula: $C_{27}H_{37}D_7O_2$ Molecular Mass: 407.7

Solubility: H_2O ☐ CH_3OH ☐ CH_3CN ☒ CH_2Cl_2 ☐ Other

Structure / Sample information:



Please, integrate the peaks at M ± 10 Da

Thank

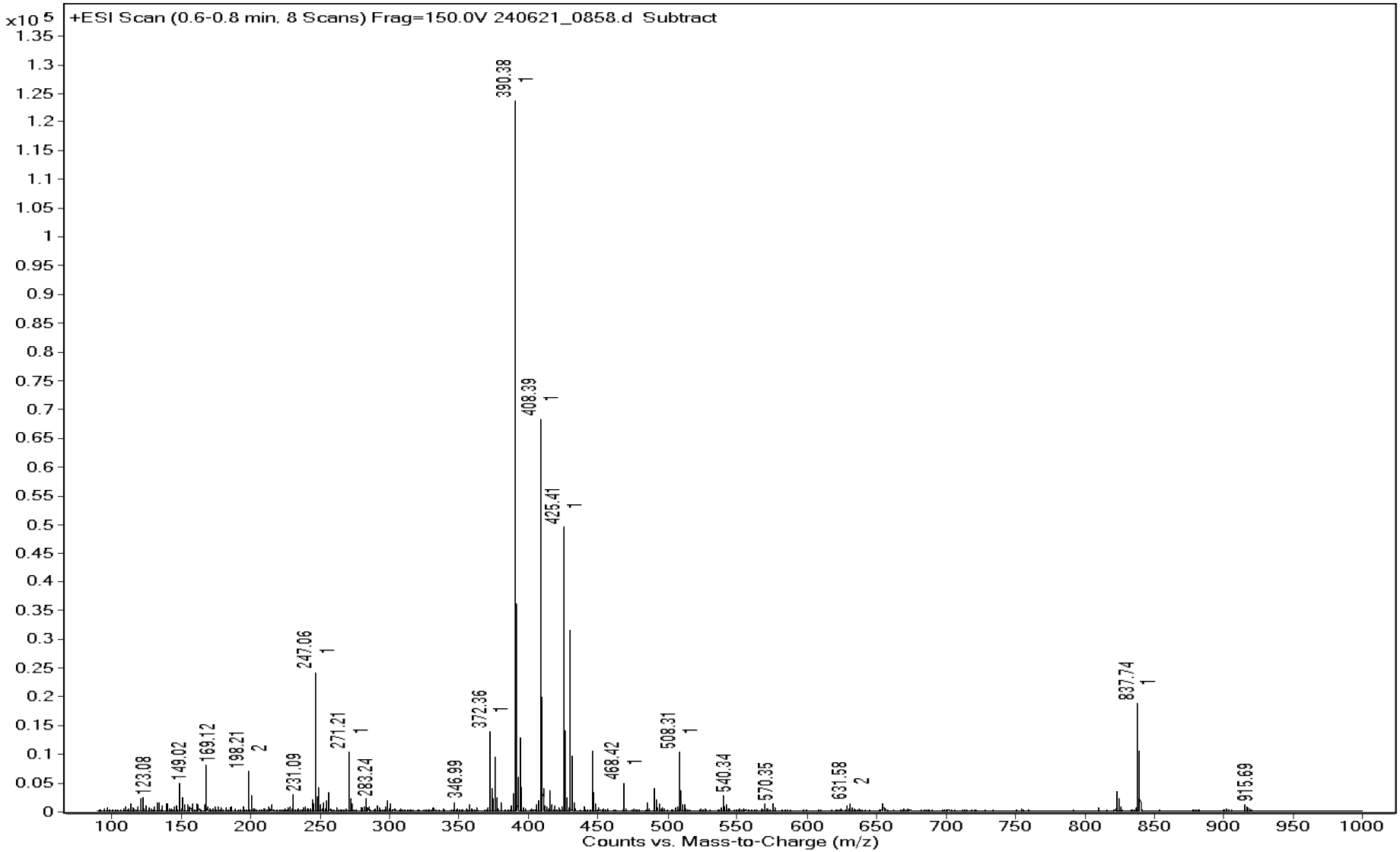
Alternative to MALDI a low energy ESI could be used.

Attach Sample Here

Ionisation/Analysis:	EI	☐	ESI	☐	DART	☐	MALDI ☒
Polarity:	+	☒	-	☐			
Accurate Mass:		☐					

ESI Mass Spectrum

Sample Name	GR-19-151	**Data File**	240621_0858.d	**Acq Method**	HRMS.m
DA Method	AIMS_Accurate_Mass.m	**Instrument**	Agilent 6538 UHD	**Acq Date, Time**	21/06/2024 10:49:41 AM
Comment	ESI+				



ESI-MS Report
Molecular Formula Generation (MFG)

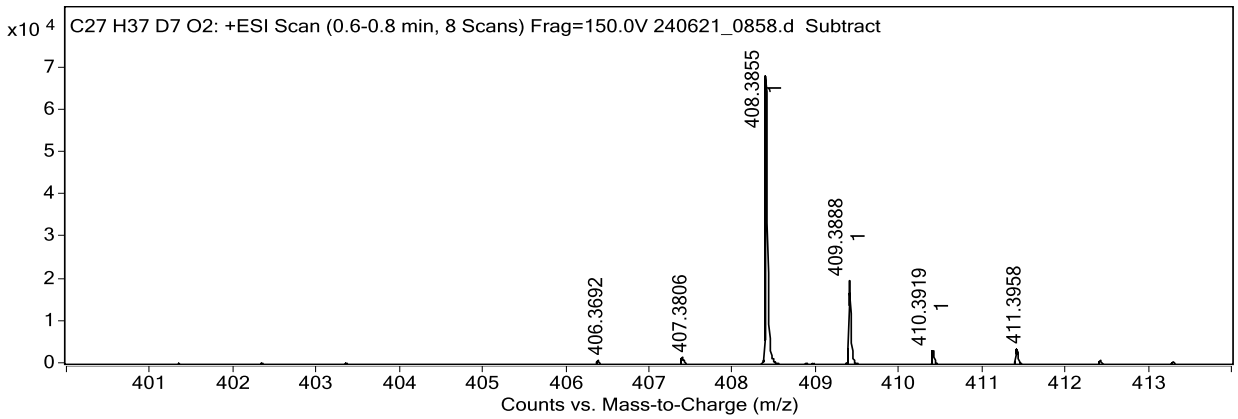
Sample Name	GR-19-151	**Data File**	240621_0858.d
Acq Method	HRMS.m	**DA Method**	AIMS_Accurate_Mass.m
Instrument	Agilent 6538 UHD	**Acq Date, Time**	21/06/2024 10:49:41 AM
Comment	ESI+		

Target Ion Species

Ion Species	*m/z*	*Ionic Formula*
(M+H)+	408.3855	C27 H38 D7 O2

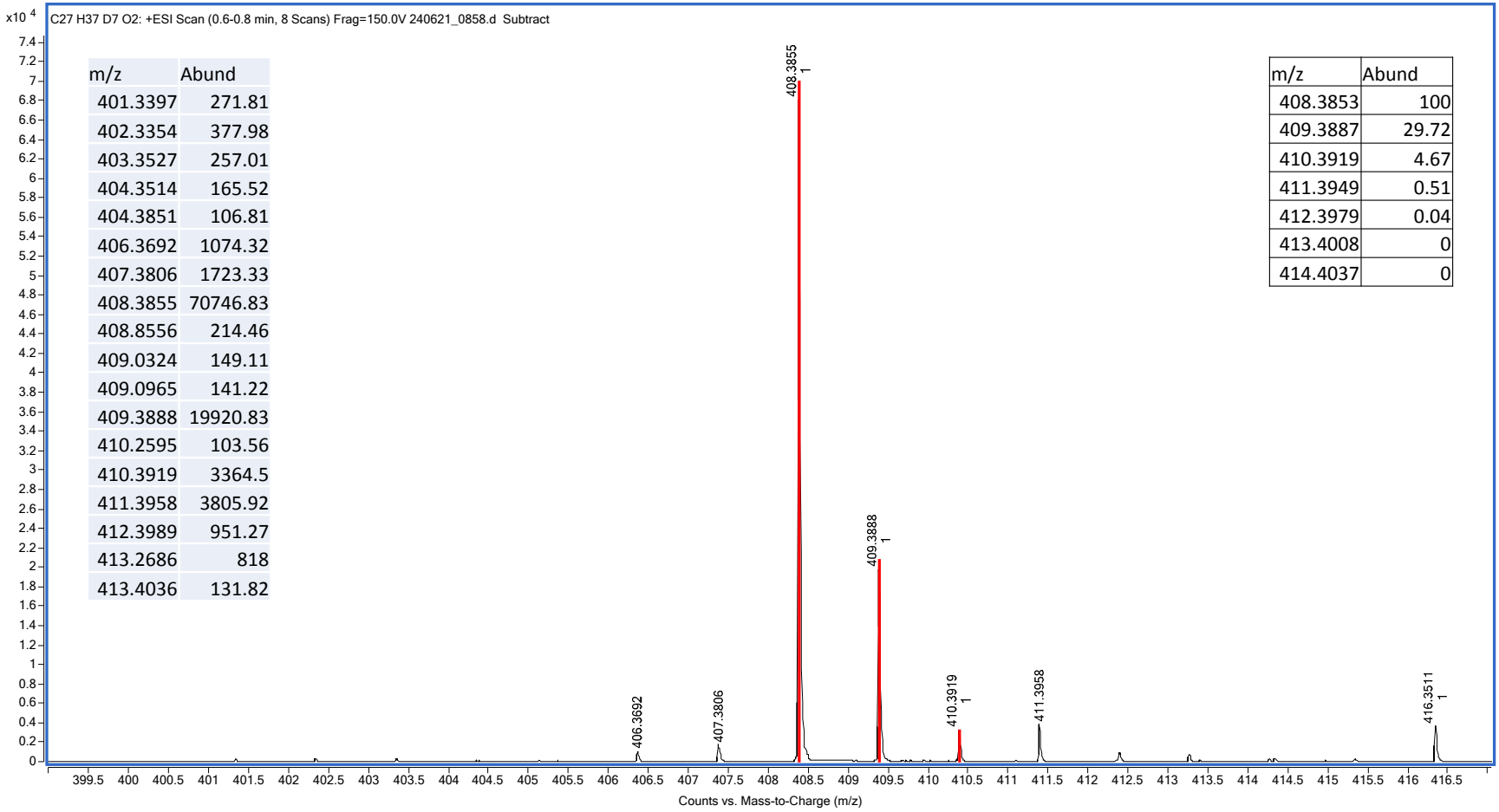
MFG Calculator Results

Target m/z	*Ionic Formula*	*Calc m/z*	*+/- (mDa)*	*+/- (ppm)*	*DBE*	*MFG Score*
408.3855	C25 H38 D6 N3 O	408.3855	0.0	0.0	6.0	99.76
408.3855	C27 H38 D7 O2	408.3853	0.2	0.5	6.0	99.53
408.3855	C23 H38 D5 N6	408.3858	-0.3	-0.7	6.0	99.29
408.3855	C27 H51 D P	408.3864	-0.9	-2.2	3.0	96.98
408.3855	C22 H42 D5 N2 O4	408.3844	1.1	2.7	1.0	95.37
408.3855	C20 H42 D4 N5 O3	408.3846	0.9	2.2	1.0	94.42
408.3855	C21 H39 D7 N4 O P	408.3843	1.2	2.9	2.0	93.75
408.3855	C18 H42 D3 N8 O2	408.3848	0.7	1.7	1.0	92.70
408.3855	C19 H39 D6 N7 P	408.3845	1.0	2.4	2.0	92.43
408.3855	C26 H50 N O2	408.3836	1.9	4.7	3.0	91.74



Predicted Isotope Match Table

Isotope	*m/z*	*Calc m/z*	Diff (mDa)	Abund (%)	Calc Abund (%)	+/-
1	408.3855	408.3853	0.2	100.0	100.0	0.0
2	409.3888	409.3887	0.1	28.2	29.7	1.5
3	410.3919	410.3919	0.0	4.8	4.7	-0.1


x10 4
C27 H37 D7 O2: +ESI Scan (0.6-0.8 min, 8 Scans) Frag=150.0V 240621_0858.d Subtract
m/z Abund
401.3397 271.81
402.3354 377.98
403.3527 257.01
404.3514 165.52
404.3851 106.81
406.3692 1074.32
407.3806 1723.33
408.3855 70746.83
408.8556 214.46
409.0324 149.11
409.0965 141.22
409.3888 19920.83
410.2595 103.56
410.3919 3364.5
411.3958 3805.92
412.3989 951.27
413.2686 818
413.4036 131.82
m/z Abund
408.3853 100
409.3887 29.72
410.3919 4.67
411.3949 0.51
412.3979 0.04
413.4008 0
414.4037 0
408.3855
1
409.3888
1
406.3692
407.3806
410.3919
1
411.3958
416.3511
1
Counts vs. Mass-to-Charge (m/z)

Data File C:\CHEM32\1\DATA\VR011825\SIG13050.D
Sample Name: GR-19-151

```
==========================================================================
Acq. Operator   : vrusu
Acq. Instrument : Instrument 1                    Location : Vial 10
Injection Date  : 2/3/2025 9:41:29 AM
                                                  Inj Volume : 10.0 µl
Acq. Method     : C:\CHEM32\1\METHODS\VR020325_151.M
Last changed    : 2/3/2025 9:40:28 AM by vrusu
Analysis Method : C:\CHEM32\1\METHODS\VR020325_151PM.M
Last changed    : 2/3/2025 3:27:28 PM
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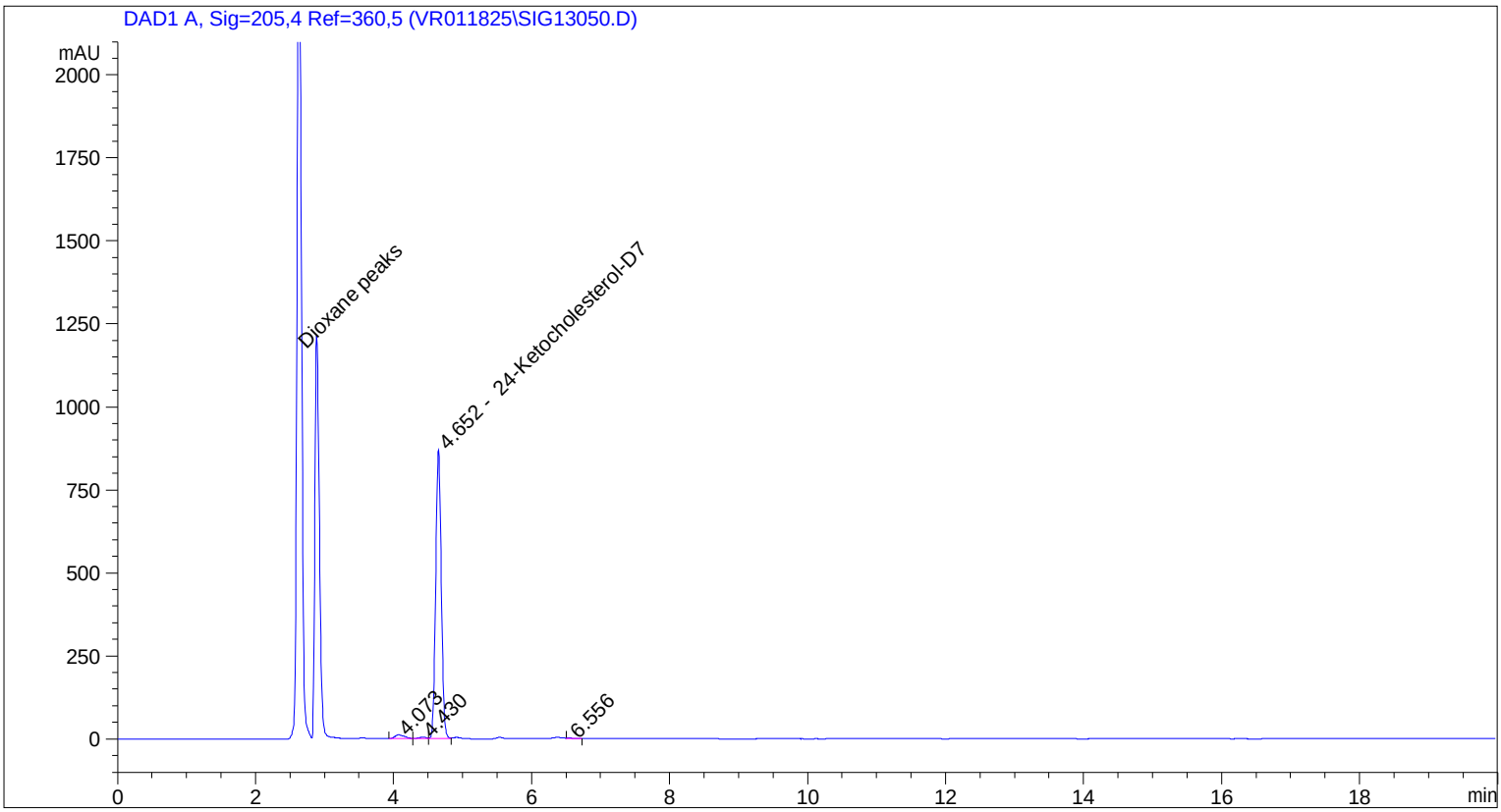


```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/3/2025 3:25:45 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=205,4 Ref=360,5

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   [mAU*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   4.073 BB      0.1310   96.11428   1.9076 ?
   2   4.430 BV      0.1027   26.48152   0.5256 ?
   3   4.652 VV      0.0877 4903.18359  97.3128 24-Ketocholesterol-D7
   4   6.556 VB      0.0990   12.80146   0.2541 ?

Totals :                   5038.58085
```

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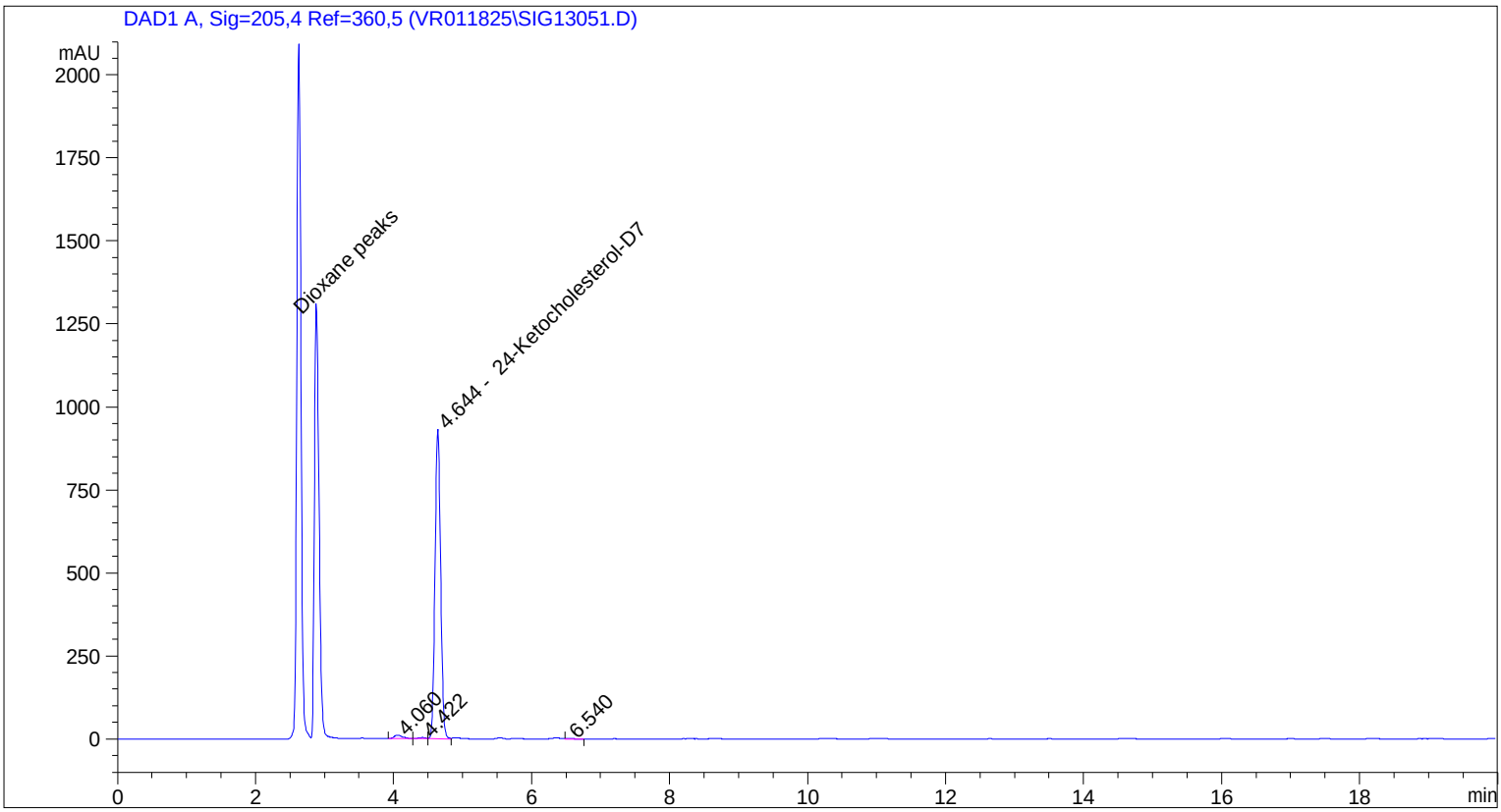
*** End of Report ***

Data File C:\CHEM32\1\DATA\VR011825\SIG13051.D
Sample Name: GR-19-151 spl-2

```
==========================================================================
Acq. Operator   : vrusu
Acq. Instrument : Instrument 1                    Location : Vial 11
Injection Date  : 2/3/2025 10:03:22 AM
                                                  Inj Volume : 10.0 µl
Acq. Method     : C:\CHEM32\1\METHODS\VR020325_151.M
Last changed    : 2/3/2025 10:02:23 AM by vrusu
Analysis Method : C:\CHEM32\1\METHODS\VR020325_151PM.M
Last changed    : 2/3/2025 3:27:28 PM
```




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

Sorted By             :      Signal
Calib. Data Modified  :      2/3/2025 3:25:45 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=205,4 Ref=360,5

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   [mAU*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   4.060 BB      0.1257   86.45611   1.6013 ?
   2   4.422 BV      0.1120   24.92353   0.4616 ?
   3   4.644 VV      0.0880 5274.57959  97.6962 24-Ketocholesterol-D7
   4   6.540 VB      0.1145   13.00346   0.2409 ?

Totals :                   5398.96268
```

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*** End of Report ***