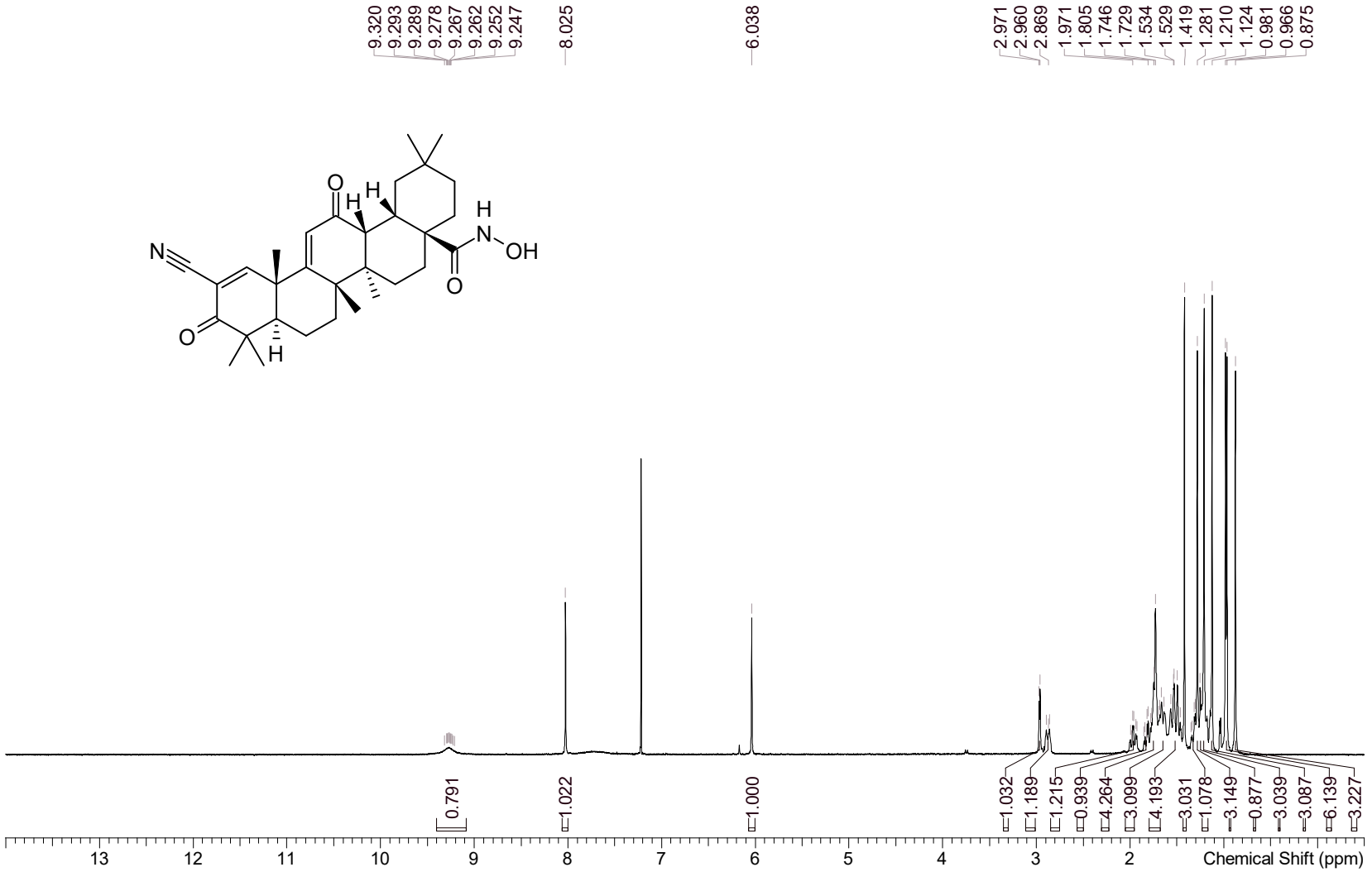
**Compound ID: 51**

EW53902-30-P1A CDCl3 ZKNJ_018_400MHz




| | |
|---|---|
| Acquisition Time (sec) | 3.0002 |
| Comment | EW53902-30-P1A CDCl3 ZKNJ_018_400MHz |
| Date | 04 Mar 2024 03:00:42 (GMT) |
| Frequency (MHz) | 399.9340 |
| Nucleus | 1H |
| Number of Transients | 8 |
| Origin | QUANTUM-I |
| Original Points Count | 24038 |
| Owner | admin |
| Points Count | 65536 |
| Pulse Sequence | s1pul30 |
| Receiver Gain | 70.28 |
| SW(cyclical) (Hz) | 8012.00 |
| Solvent | CHLOROFORM-d |
| Spectrum Offset (Hz) | 2399.6038 |
| Spectrum Type | undefined |
| Sweep Width (Hz) | 8011.88 |
| Temperature (degree C) | 25.550 |

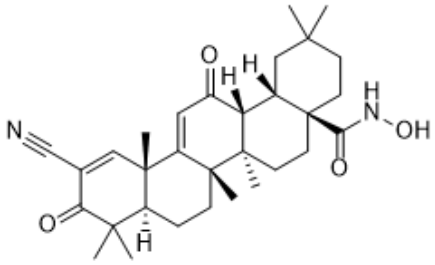


Operator:

Date:

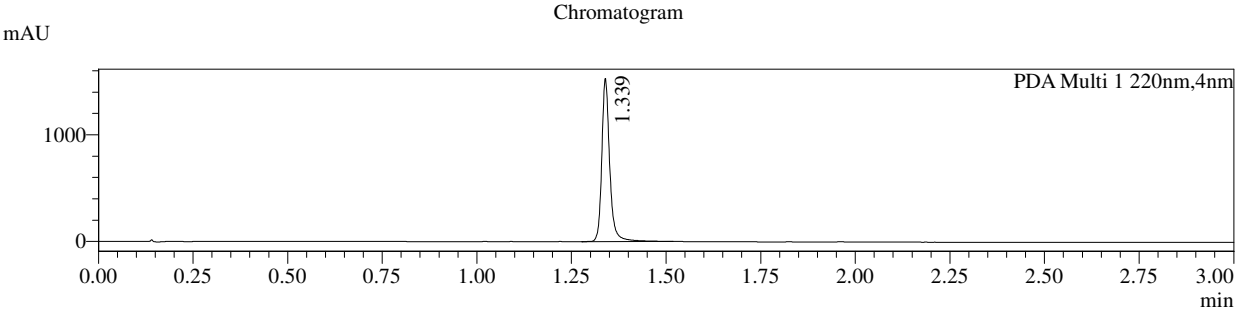
# LCMS REPORT

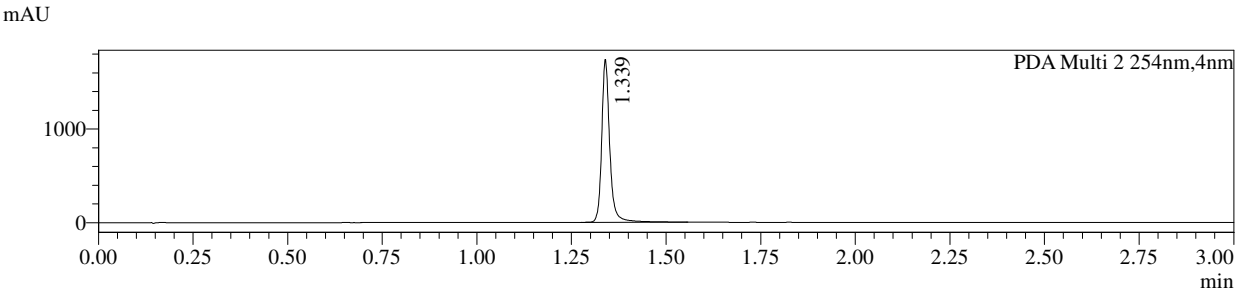
Compound ID : 51
Sample ID : EW53902-30-P1B
Injection Vol : 3ul
Location : vial34
Tray Name :2
Acq Method : D:\method\5-95AB_3min_220&254.lcm
Org DataFile : D:\DATA\2024\2403\240304\EW53902-30-P1B.lcd
Injection Date : 3/4/2024 11:26:47
Instrument :LCMS-070




Exact Mass: 506.31

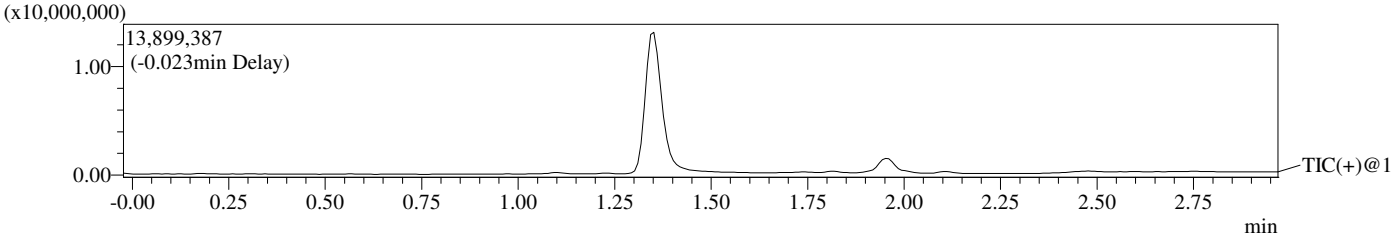
Chromatogram




1 PDA Multi 1 / 220nm,4nm
2 PDA Multi 2 / 254nm,4nm

MS Chromatogram

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Integration Result

==========

Peak Table

PDA Ch1 220nm

| Peak# | Ret. Time | Height | Height% | USP Width | Area | Area% |
|---|---|---|---|---|---|---|
| 1 | 1.339 | 1529746 | 100.000 | 0.037 | 2235521 | 100.000 |

Peak Table

PDA Ch2 254nm

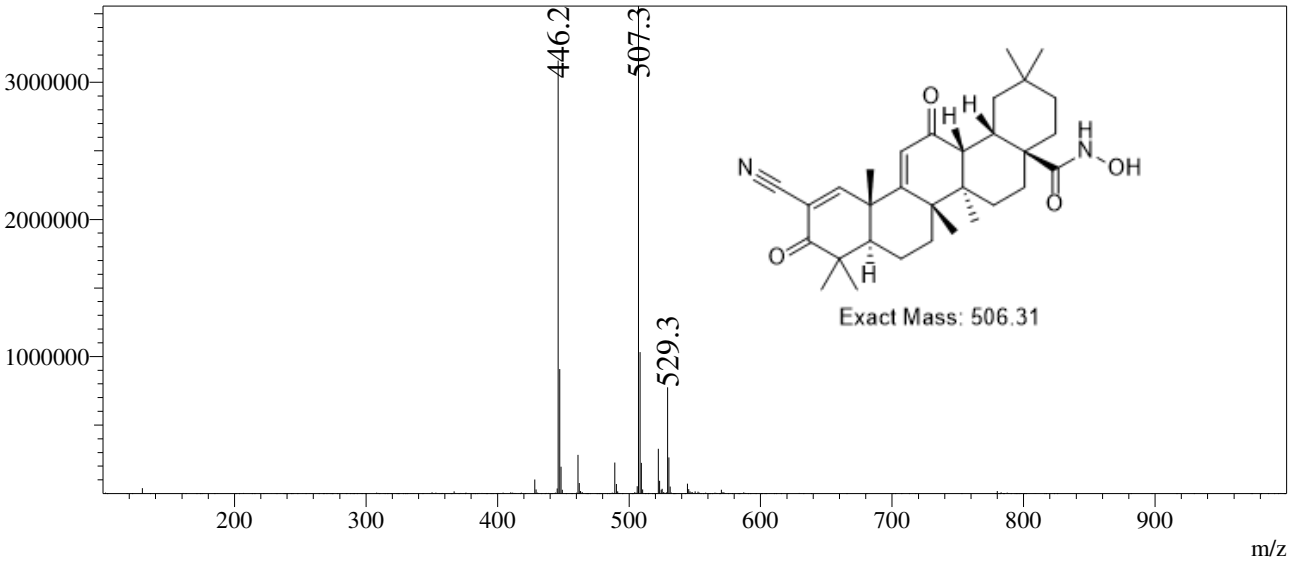
| Peak# | Ret. Time | Height | Height% | USP Width | Area | Area% |
|---|---|---|---|---|---|---|
| 1 | 1.339 | 1739577 | 100.000 | 0.038 | 2582084 | 100.000 |

Operator:_____

Date:_____

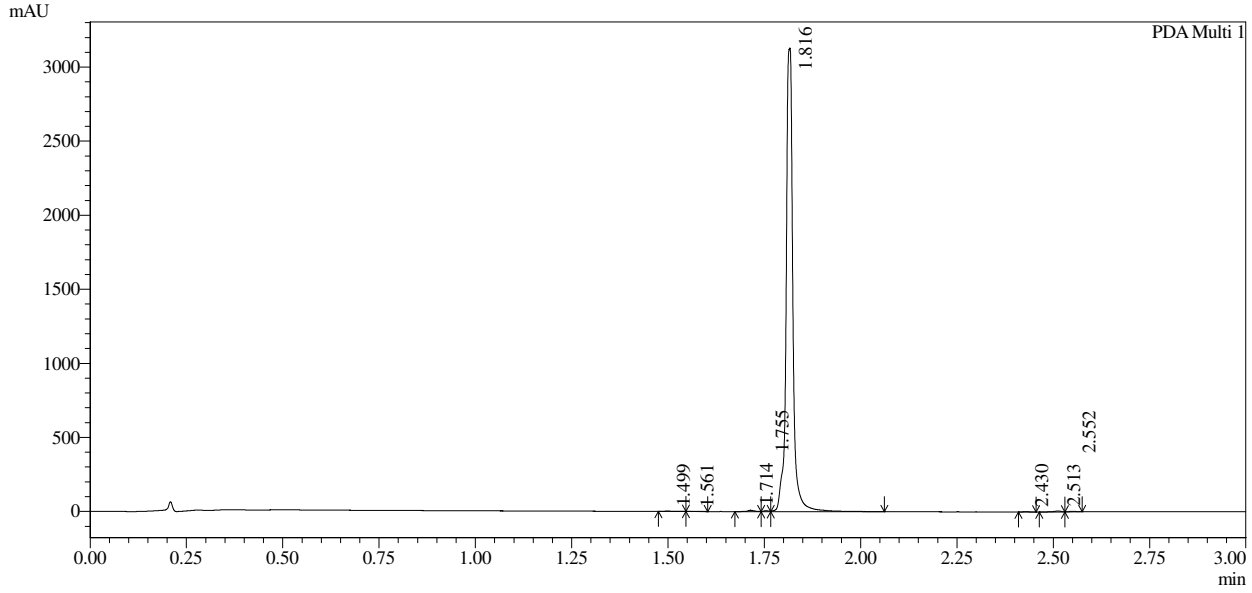
Mass Spectrum

RetTime: 1.343 Datafile: D:\DATA\2024\2403\240304\EW53902-30-P1B.lcd



# HPLC REPORT

Compound ID : 51
Sample ID : EW53902-30-P1D
Vial# : 27
Injection Volumn : 3
Filename : D:\DATA\2024\2403\240304\EW53902-30-P1D.lcd
Method Name : D:\method\10-80AB_3min.lcm
Instrument : HPLC-087
Run time : 03/04/2024 10:22:13




1 PDA Multi 1 / 220nm,4nm

---

Integration result

---

PeakTable

PDA Ch1 220nm

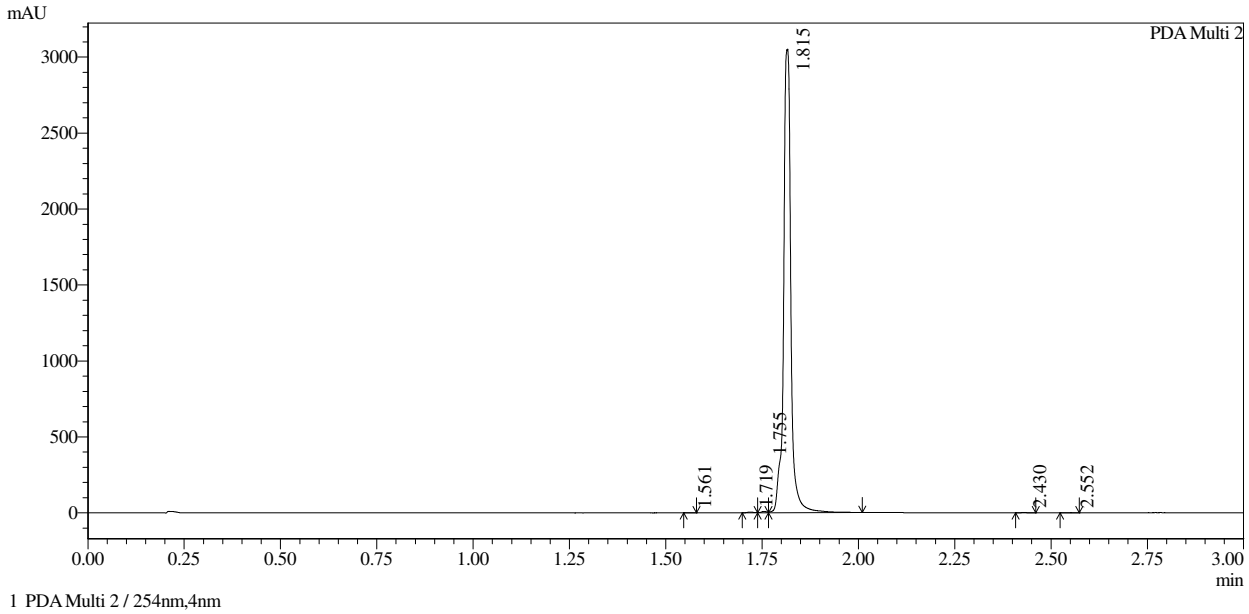
| Peak# | Ret. Time | USP Width | Resolution | Height | Area | Area % |
|---|---|---|---|---|---|---|
| 1 | 1.499 | 0.026 | 0.000 | 2529 | 3106 | 0.080 |
| 2 | 1.561 | 0.024 | 2.467 | 1311 | 1267 | 0.033 |
| 3 | 1.714 | 0.029 | 5.822 | 9116 | 11295 | 0.292 |
| 4 | 1.755 | 0.027 | 1.435 | 5943 | 5716 | 0.148 |
| 5 | 1.816 | 0.026 | 2.300 | 3127796 | 3832109 | 99.062 |
| 6 | 2.430 | 0.023 | 25.097 | 1555 | 1496 | 0.039 |
| 7 | 2.513 | 0.030 | 3.126 | 6536 | 8508 | 0.220 |
| 8 | 2.552 | 0.031 | 1.297 | 3982 | 4895 | 0.127 |
| Total | | | | 3158768 | 3868394 | 100.000 |

Operater : _____

Date : _____

# HPLC REPORT

Compound ID : 51
Sample ID : EW53902-30-P1D
Filename : D:\DATA\2024\2403\240304\EW53902-30-P1D.lcd
Method Name : D:\method\10-80AB_3min.lcm
Instrument : HPLC-087
Run time : 03/04/2024 10:22:13




1 PDA Multi 2 / 254nm,4nm

---

Integration result

---

PeakTable

PDA Ch2 254nm

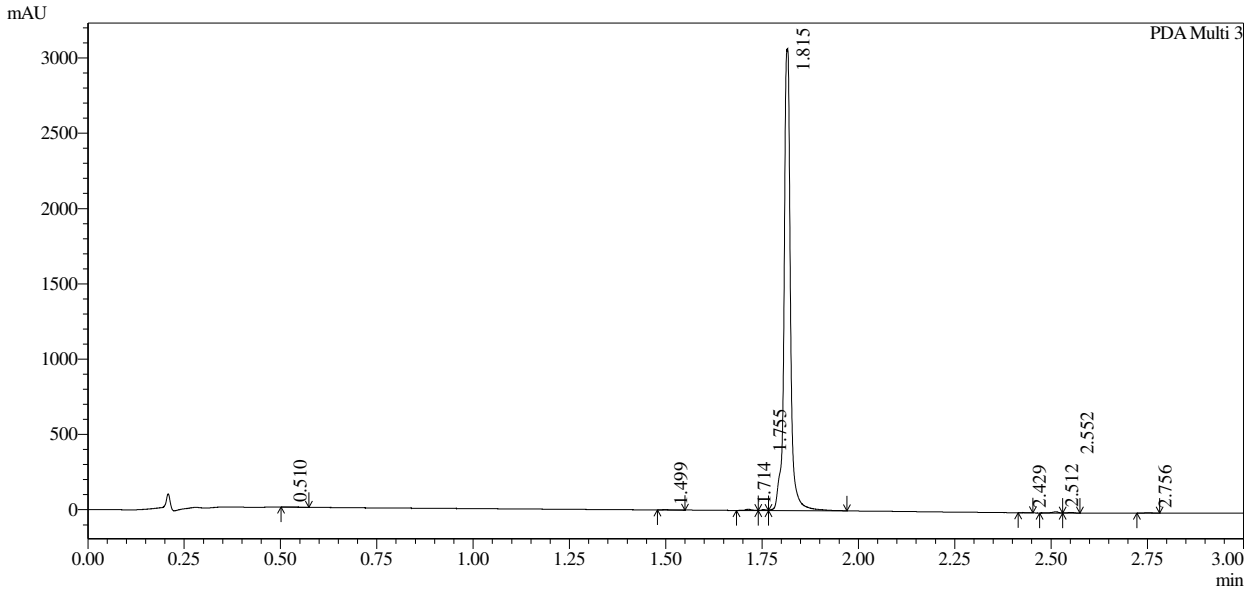
| Peak# | Ret. Time | USP Width | Resolution | Height | Area | Area % |
|---|---|---|---|---|---|---|
| 1 | 1.561 | 0.023 | 0.000 | 1421 | 1272 | 0.031 |
| 2 | 1.719 | 0.032 | 5.749 | 5338 | 6424 | 0.159 |
| 3 | 1.755 | 0.027 | 1.219 | 9355 | 9014 | 0.223 |
| 4 | 1.815 | 0.027 | 2.249 | 3048181 | 4029562 | 99.508 |
| 5 | 2.430 | 0.024 | 24.239 | 2005 | 1940 | 0.048 |
| 6 | 2.552 | 0.025 | 5.031 | 1138 | 1267 | 0.031 |
| Total | | | | 3067439 | 4049480 | 100.000 |

Operater : _____

Date : _____

# HPLC REPORT

Compound ID : 51
Sample ID : EW53902-30-P1D
Filename : D:\DATA\2024\2403\240304\EW53902-30-P1D.lcd
Method Name : D:\method\10-80AB_3min.lcm
Instrument &Column : HPLC-087
Run time : 03/04/2024 10:22:13




1 PDA Multi 3 / 215nm,4nm

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Integration result

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PeakTable

PDA Ch3 215nm

| Peak# | Ret. Time | USP Width | Resolution | Height | Area | Area % |
|---|---|---|---|---|---|---|
| 1 | 0.510 | 0.055 | 0.000 | 357 | 1060 | 0.029 |
| 2 | 1.499 | 0.026 | 24.421 | 2401 | 2935 | 0.081 |
| 3 | 1.714 | 0.028 | 7.981 | 7700 | 8845 | 0.245 |
| 4 | 1.755 | 0.025 | 1.537 | 5764 | 5304 | 0.147 |
| 5 | 1.815 | 0.025 | 2.408 | 3059282 | 3570172 | 99.006 |
| 6 | 2.429 | 0.021 | 26.900 | 1191 | 1064 | 0.030 |
| 7 | 2.512 | 0.029 | 3.337 | 7963 | 9930 | 0.275 |
| 8 | 2.552 | 0.034 | 1.268 | 4535 | 5338 | 0.148 |
| 9 | 2.756 | 0.033 | 6.067 | 957 | 1365 | 0.038 |
| Total | | | | 3090150 | 3606013 | 100.000 |

Operater : _____

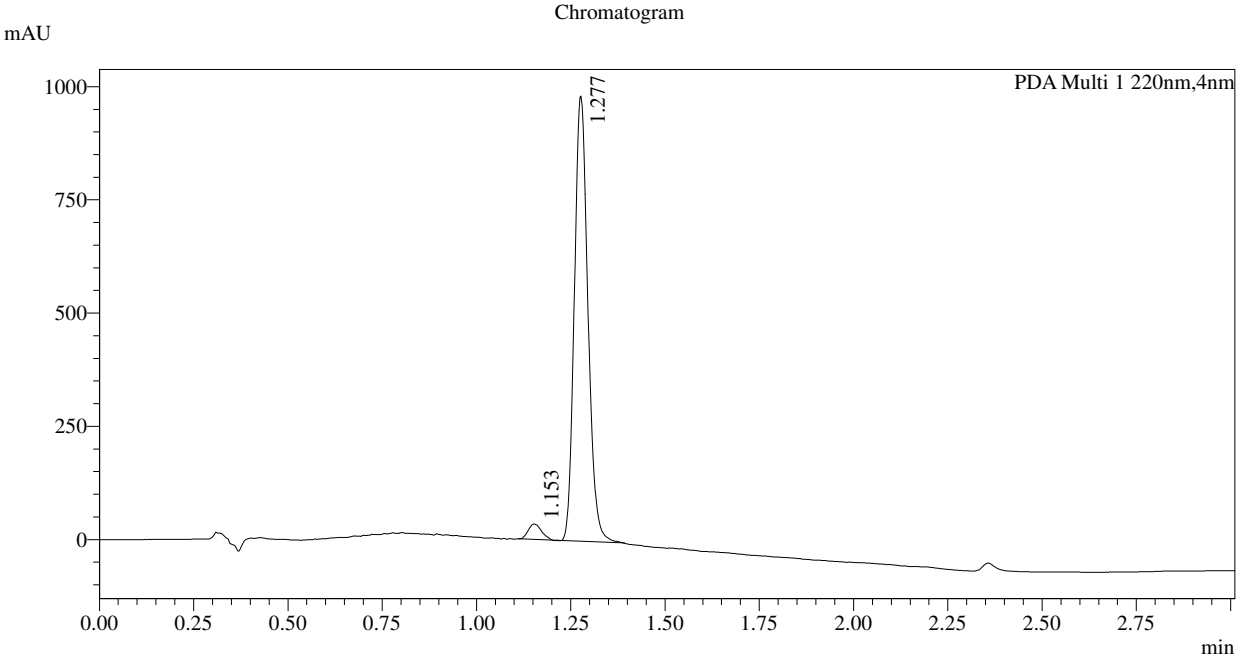
Date : _____

# Chiral SFC Report

Compound ID : 51
Sample ID : EW53902-30-P1A_I1601
Injection Vol : 5ul
Location :Tray1 vial5
Acq Method : D:\METHODS\IC-3-IPA+ACN(DEA)-20-60-3ML-35T.lcm
Raw Data : D:\DATA\2024\202403\20240304\EW53902-30-P1A_I1601.lcd
Injection Date : 3/4/2024 4:54:14 PM
Instrument : CAS-WH-ANA-SFC-I(SHIMADZU LC-30ADsf)

Method details: "Column:Chiralpak IC-3 50*4.6mm I.D.,3um
Mobile phase:Phase A for CO2,and Phase B for IPA+ACN(0.05%DEA);
Gradient elution: From 20% to 60% of IPA+ACN(0.05%DEA) in CO2 ,
Flow rate:3mL/min;Detector:PDA;
Column Temp:35C;Back Pressure:100Bar"

Chromatogram




1 PDA Multi 1 / 220nm,4nm

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Integration Result

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Peak Table

PDA Ch1 220nm

| Peak# | Ret. Time | Height | Height% | Resolution(USP) | Area | Area% |
|---|---|---|---|---|---|---|
| 1 | 1.153 | 33036 | 3.279 | -- | 83232 | 3.273 |
| 2 | 1.277 | 974431 | 96.721 | 1.797 | 2459655 | 96.727 |

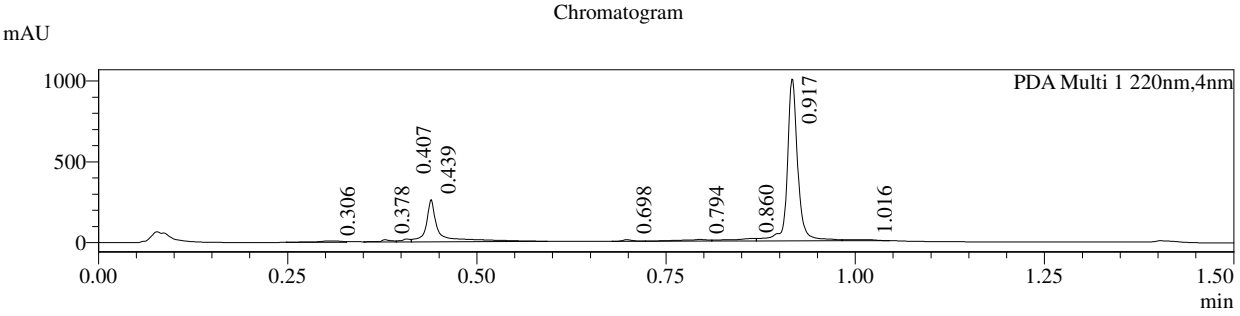
Operator:_____

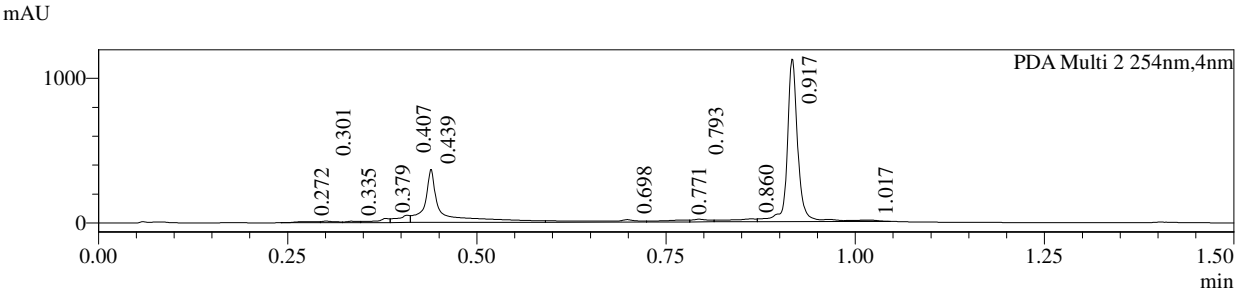
Date:_____

# LCMS REPORT

Compound ID : 1
Sample ID : EW53902-29-P1A
Injection Vol : 3ul
Location : vial02
Tray Name :2
Acq Method : D:\method\5-95CD_1.5min_220&254_POS.lcm
Org DataFile : D:\DATA\2024\2402\240229\EW53902-29-P1A.lcd
Injection Date : 02/29/2024 12:13:39
Instrument : LCMS-053 2-109

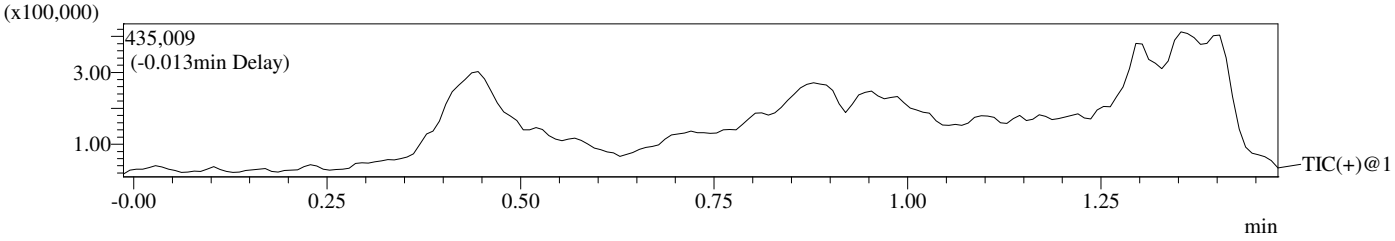
Chromatogram




1 PDA Multi 1 / 220nm,4nm
2 PDA Multi 2 / 254nm,4nm

MS Chromatogram




Integration Result

Peak Table

PDA Ch1 220nm

| Peak# | Ret. Time | Height | Height% | USP Width | Area | Area% |
|---|---|---|---|---|---|---|
| 1 | 0.306 | 8241 | 0.613 | 0.051 | 18296 | 1.299 |
| 2 | 0.378 | 14750 | 1.097 | 0.024 | 14364 | 1.020 |
| 3 | 0.407 | 17695 | 1.316 | 0.059 | 15138 | 1.075 |
| 4 | 0.439 | 259802 | 19.317 | 0.022 | 298568 | 21.196 |
| 5 | 0.698 | 9760 | 0.726 | 0.026 | 9412 | 0.668 |
| 6 | 0.794 | 10436 | 0.776 | 0.060 | 28023 | 1.989 |
| 7 | 0.860 | 15102 | 1.123 | 0.000 | 36080 | 2.561 |
| 8 | 0.917 | 1001985 | 74.500 | 0.025 | 969333 | 68.814 |
| 9 | 1.016 | 7169 | 0.533 | 0.191 | 19422 | 1.379 |

Peak Table

PDA Ch2 254nm

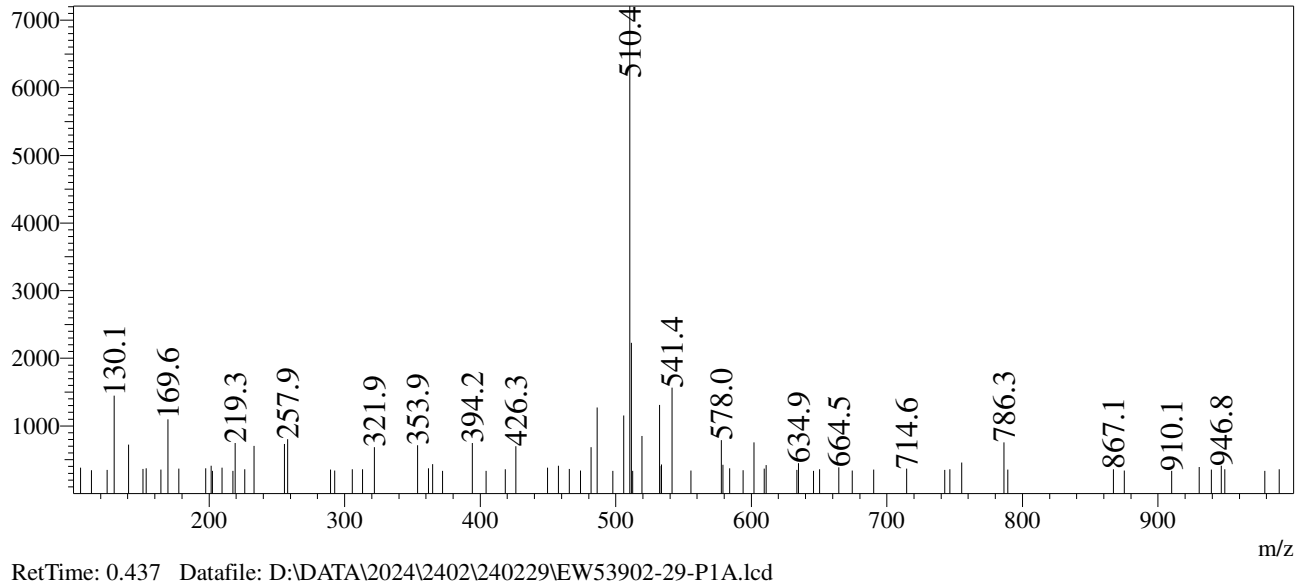
| Peak# | Ret. Time | Height | Height% | USP Width | Area | Area% |
|---|---|---|---|---|---|---|
| 1 | 0.272 | 7560 | 0.453 | 0.151 | 15931 | 0.809 |
| 2 | 0.301 | 10610 | 0.635 | 0.043 | 13142 | 0.668 |
| 3 | 0.335 | 9811 | 0.587 | 0.052 | 11046 | 0.561 |
| 4 | 0.379 | 27971 | 1.675 | 0.095 | 33984 | 1.726 |
| 5 | 0.407 | 49609 | 2.970 | 0.127 | 57820 | 2.937 |
| 6 | 0.439 | 365682 | 21.894 | 0.024 | 542425 | 27.553 |
| 7 | 0.698 | 16636 | 0.996 | 0.039 | 66536 | 3.380 |
| 8 | 0.771 | 12440 | 0.745 | 0.786 | 32800 | 1.666 |
| 9 | 0.793 | 18686 | 1.119 | 0.063 | 28184 | 1.432 |
| 10 | 0.860 | 19409 | 1.162 | 0.684 | 51490 | 2.616 |
| 11 | 0.917 | 1124282 | 67.312 | 0.025 | 1103405 | 56.049 |
| 12 | 1.017 | 7557 | 0.452 | 0.044 | 11887 | 0.604 |

Operator:_____

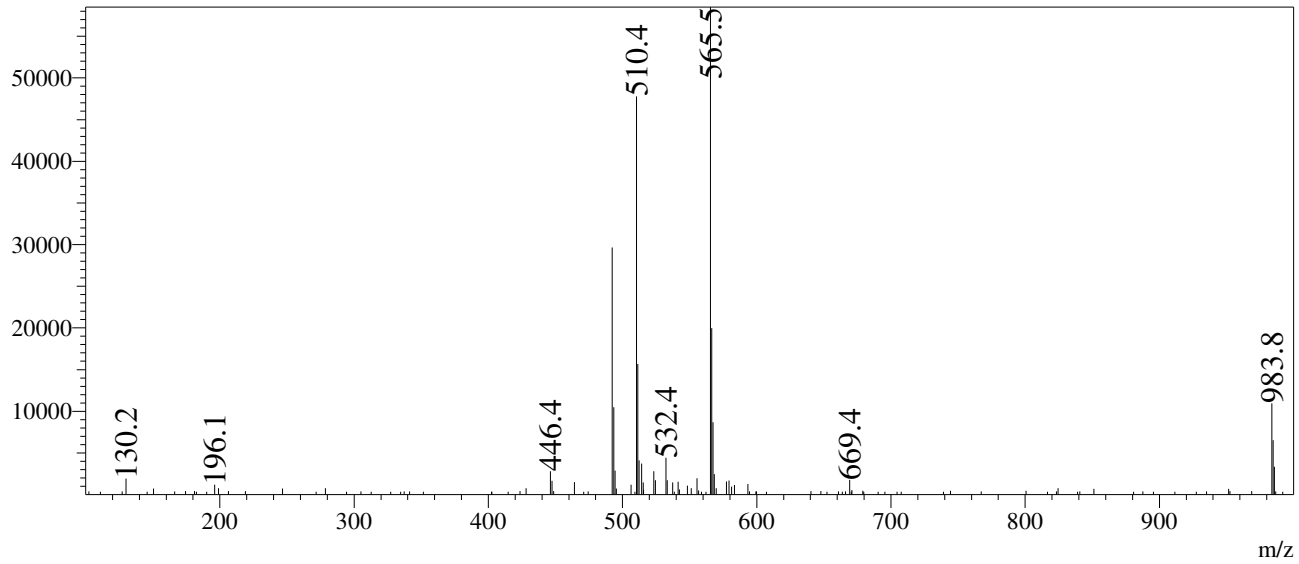
Date:_____

Mass Spectrum

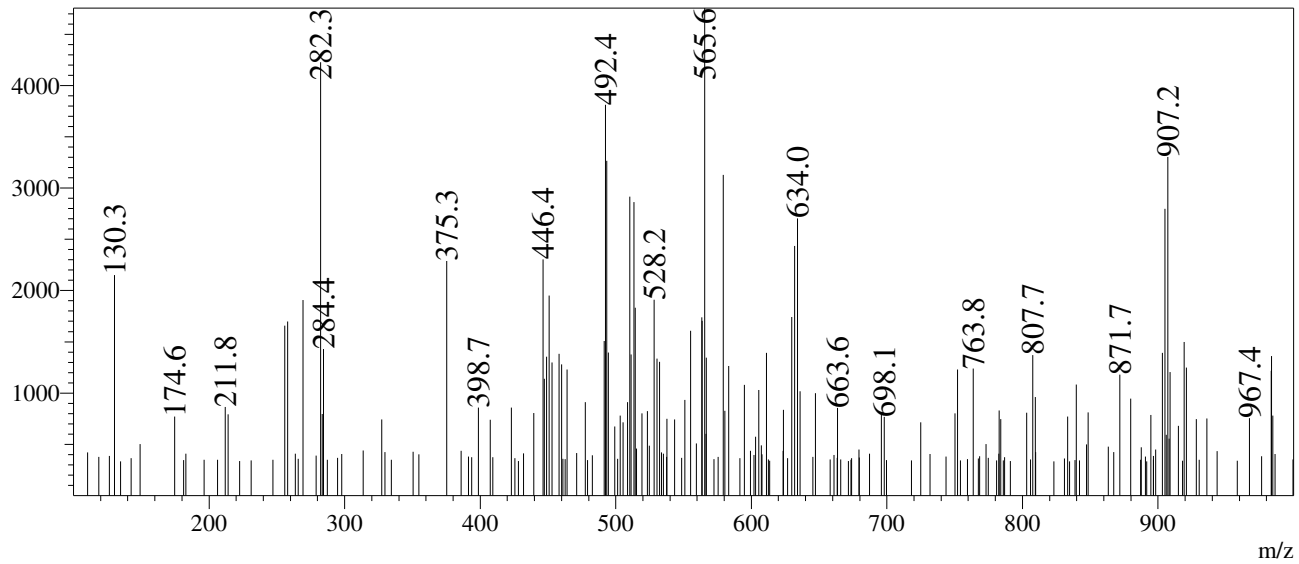
RetTime: 0.303 Datafile: D:\DATA\2024\2402\240229\EW53902-29-P1A.lcd



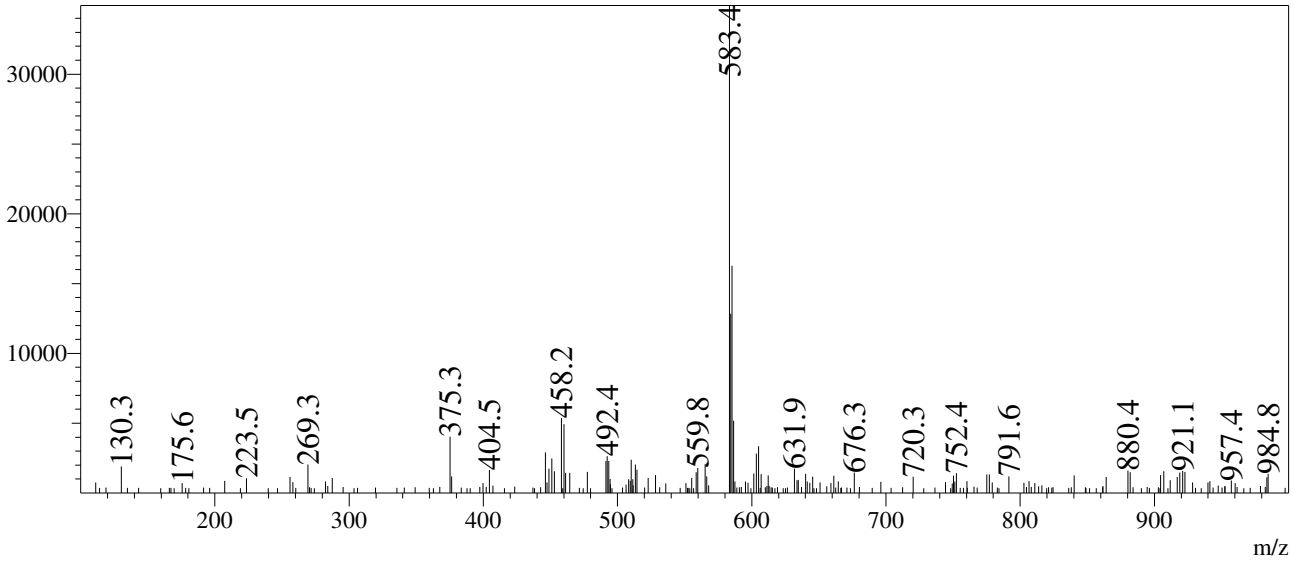

RetTime: 0.437 Datafile: D:\DATA\2024\2402\240229\EW53902-29-P1A.lcd



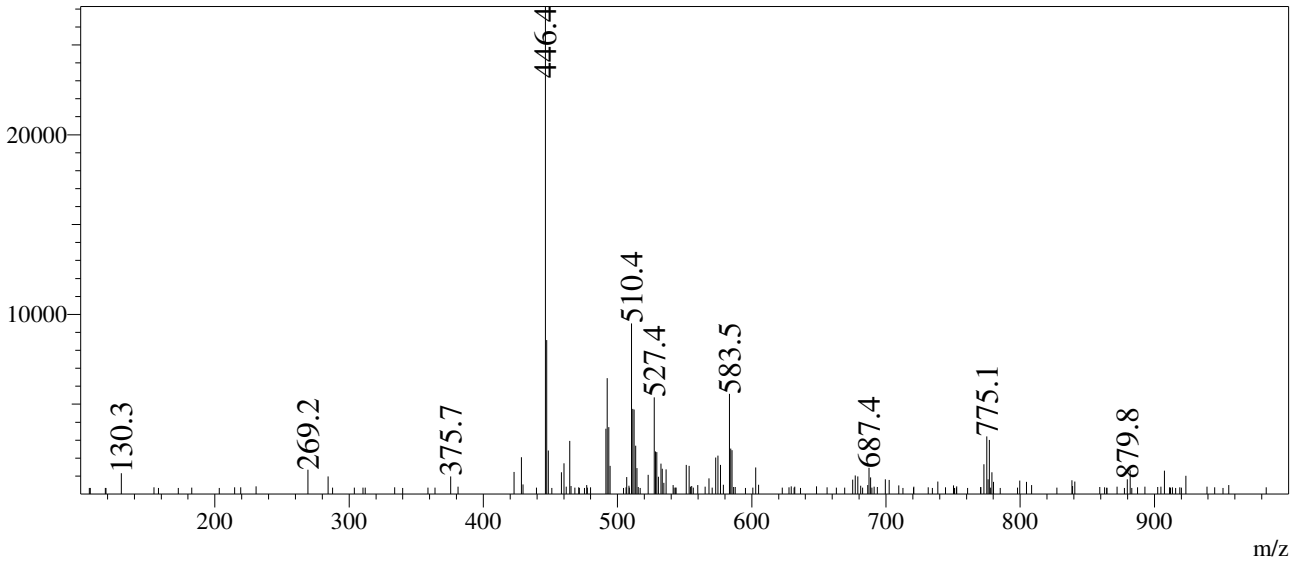

RetTime: 0.795 Datafile: D:\DATA\2024\2402\240229\EW53902-29-P1A.lcd



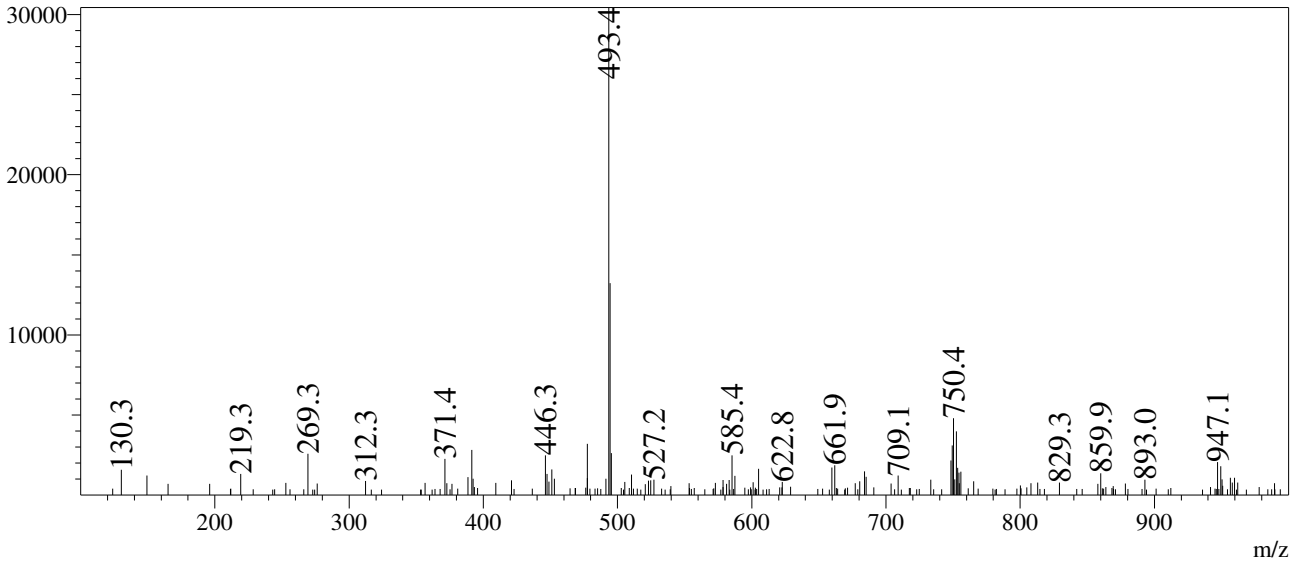
RetTime: 0.862 Datafile: D:\DATA\2024\2402\240229\EW53902-29-P1A.lcd




RetTime: 0.920 Datafile: D:\DATA\2024\2402\240229\EW53902-29-P1A.lcd




RetTime: 1.020 Datafile: D:\DATA\2024\2402\240229\EW53902-29-P1A.lcd



# Metabolite profiling in human liver microsomes for Cpd.51

| | |
|---|---|
| **Test article** | Cpd.51 |
| **Purpose** | To identify major metabolites of test compound in human liver microsomes |
| **Study director(s)** | Jinyu Ba |
| **Major contributor(s)** | Haihong Li |
| **Effective date** | December 24th, 2025 |
| **Sponsor** | 烟台大学 |
| **Study number** | CPB-P25-66382 |

## Corresponding address

Shanghai ChemPartner Co., Ltd.

2727 Jinke Rd, Block A, Pudong, Shanghai, 201203, China

To the best of our knowledge this report has been reviewed for completeness, accuracy, and compliance with the protocol.

**Study director:**

Date

Jinyu Ba, M.S.
Study Director of Metabolite ID
Shanghai ChemPartner Co., Ltd.
Tel: (86) 21-51320088 Ext. 3073
Email: jyba@chempartner.com

**Reviewed and approved by:**

Date

Haihong Li, M.S.
Senior Research Scientist of Metabolite ID
Shanghai ChemPartner Co., Ltd.
Tel: (86) 21-51320088 Ext. 3074
Email: hhli1@chempartner.com

# CONTENTS

# INTRODUCTION AND OBJECTIVE

The objective of this study is to identify major metabolites of Cpd.51 generated in human liver microsomes.

*In vitro* metabolites identification was conducted after incubating Cpd.51 (parent compound, final concentration 10 μM) with human liver microsomes (final protein concentration, 1 mg/mL) at 37 °C in 100 mM potassium phosphate buffer containing 5 mM $MgCl_2$ in the presence of NADPH or without NADPH. The samples taken at 0 minute (min) and 5 min were quenched by methanol and analyzed using Waters G2-XS Q-Tof UPLC-MS system with positive-ion and negative-ion electrospray ionization. LC-MS extract ion chromatograms (EIC) of the $T_5$ and $T_0$ were compared to identify the major putative metabolites. The MS/MS spectra of Cpd.51 and the metabolites were obtained during positive-ion and negative-ion electrospray. The possible chemical structures of the metabolites were deduced based on their MS/MS spectra and retention times. The metabolic pathways of Cpd.51 in human liver microsomes were proposed.

# MATERIALS AND REAGENTS

Cpd.51 (Purity: 99.1%), provided by 烟台大学
Acetonitrile, SIGMA-ALDRICH (Cat. # 34851-4L, Lot. # WXBD3947V)
Methanol (MeOH), SIGMA-ALDRICH (Cat. # 34860-4L-R, Lot. # WXBD3647V)
$K_2HPO_4 \cdot 3H_2O$, Titan (Lot. # P2128180)
$KH_2PO_4$, Titan (Lot. # P1343518)
$MgCl_2 \cdot 6H_2O$, Titan (Lot. # P1040534)
Human liver microsomes (HLM), XENOTECH (Cat. # H0610, Lot. # 2110263)
NADPH, MCE (Cat. # HY-F0003, Lot. # 728189)

# EXPERIMENTAL PROCEDURES

1. Assay buffer: 100 mM potassium phosphate buffer ($K^+/Mg^{2+}$ buffer, pH 7.4):

| Reagent | $K_2HPO_4 \cdot 3H_2O$ (g) | $KH_2PO_4$ (g) | $MgCl_2 \cdot 6H_2O$ (g) | $H_2O$ (mL) |
|---|---|---|---|---|
| | FW: 228.22 | FW: 136.09 | FW: 203.3 | |
| 100 mM | 9.240265 | 1.297376 | 0.509460 | 500 |

2. Preparation of cofactor solution in the $K^+/Mg^{2+}$ Buffer:

| Reagent | $K^+/Mg^{2+}$ buffer (mL) | NADPH (mg) | Total (mL) |
|---|---|---|---|
| 8 mM NADPH | 1.049 | 7.00 | 1.049 |

3. Assay procedure:
   1) $T_0$: add 20 μL of 20 mg/mL liver microsomes (LM) stock solution, 100 μL of cofactor solution and 279 μL of $K^+/Mg^{2+}$ buffer to the $T_0$ vial, and quench the mixed solution by adding 1200 μL of MeOH, vortex at 1000 rpm for 5 min, and then add 1 μL of 4 mM compound solution.
   2) $T_5$: add 20 μL of 20 mg/mL liver microsomes (LM) stock solution, 100 μL of cofactor solution and 279 μL of $K^+/Mg^{2+}$ buffer to the $T_5$ vial, and then start the reaction by adding 1 μL of 4 mM compound solution. After 5 min incubation, quench the reaction by adding 1200 μL of MeOH and vortex at 1000 rpm for 5 min.
   3) $T_{5\text{-w/o}}$: add 20 μL of 20 mg/mL liver microsomes (LM) stock solution and 379 μL of $K^+/Mg^{2+}$ buffer to the $T_{5\text{-w/o}}$ vial, and then start the reaction by adding 1 μL of 4 mM compound solution. After 5 min incubation, quench the reaction by adding 1200 μL of

MeOH and vortex at 1000 rpm for 5 min.

4) Protein precipitation: centrifuge quenched samples at 14000 rpm for 5 min.

5) 100 μL of the supernatant was diluted with 50 μL of water and the mixture was vortexed for 5 min, and centrifuged at 14000 rpm for 5 min. Then 10 μL of the supernatant was injected onto LC-UV-MS for analysis.

## LC-UV-MS CONDITIONS

1) LC condition:

Column: Acquity UPLC®BEH C18 (2.1 × 50 mm, 1.7 μm);

Mobile Phase: A ($H_2O$ with 0.1% formic acid);
B (ACN with 0.1% formic acid);

Gradient Program:

| Time (min) | Flow Rate (μL/min) | A (%) | B (%) |
|---|---|---|---|
| 0.00 | 400 | 98 | 2 |
| 0.34 | 400 | 98 | 2 |
| 2.00 | 400 | 80 | 20 |
| 7.00 | 400 | 30 | 70 |
| 11.00 | 400 | 5 | 95 |
| 12.00 | 400 | 5 | 95 |
| 12.10 | 400 | 98 | 2 |
| 13.00 | 400 | 98 | 2 |

2) MS condition:

UPLC-UV-G2-XS Q-TOF: $MS^E$ Centroid ESI (+/-)

**Scan Mode:** $MS^E$ Centroid
Source
Capillary (kV): 3.00 (+) / 2.50 (-)
Sampling Cone: 40
Source Offset: 80
Temperature (°C)
Source: 120
Desolvation: 350
Gas Flows (L/h)
Cone Gas: 50
Desolvation Gas: 600

## SUMMARY

Cpd.51 was transformed into 10 major metabolites in this study, which were named as $M_{E.M.}$ according to the exact mass of metabolites and the retention time in the current LC condition.

LC-UV chromatograms of Cpd.51 and its metabolites incubated in human liver microsomes are shown in **Figure 1**, while the LC-MS chromatograms are shown in **Figure 2**. Proposed metabolic pathways of Cpd.51 in human liver microsomes are shown in **Figure 4**. A summary, including observed *m/z* value, retention time, relative UV abundance, and MS peak area of Cpd.51 and its metabolites in human liver microsomes is presented in **Table 1**.

M508 was also detected in $T_0$, $T_{5\text{-w/o}}$ and neat solution, while the LC-MS chromatograms are shown in **Figure 3.**

**Figure 1.** LC-UV (λ = 250-260 nm) chromatograms of Cpd.51 and its metabolites incubated in human liver microsomes with or without NADPH for 0 min and 5 min

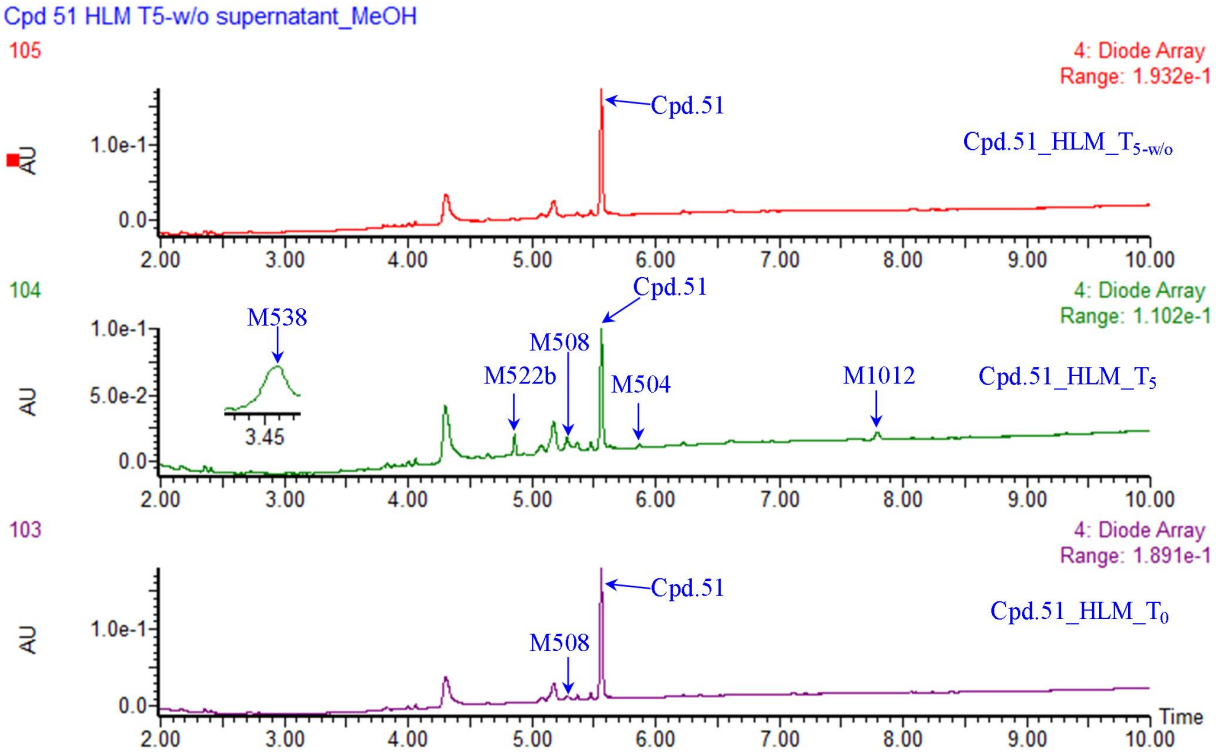
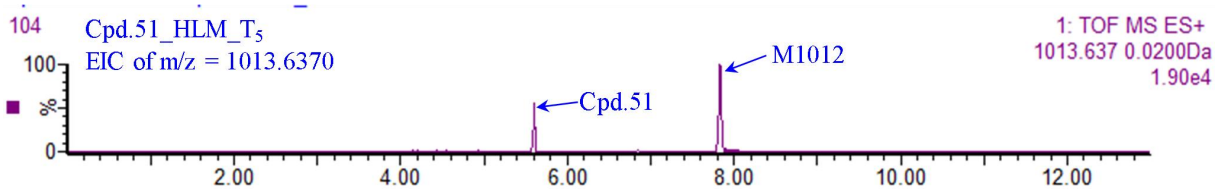
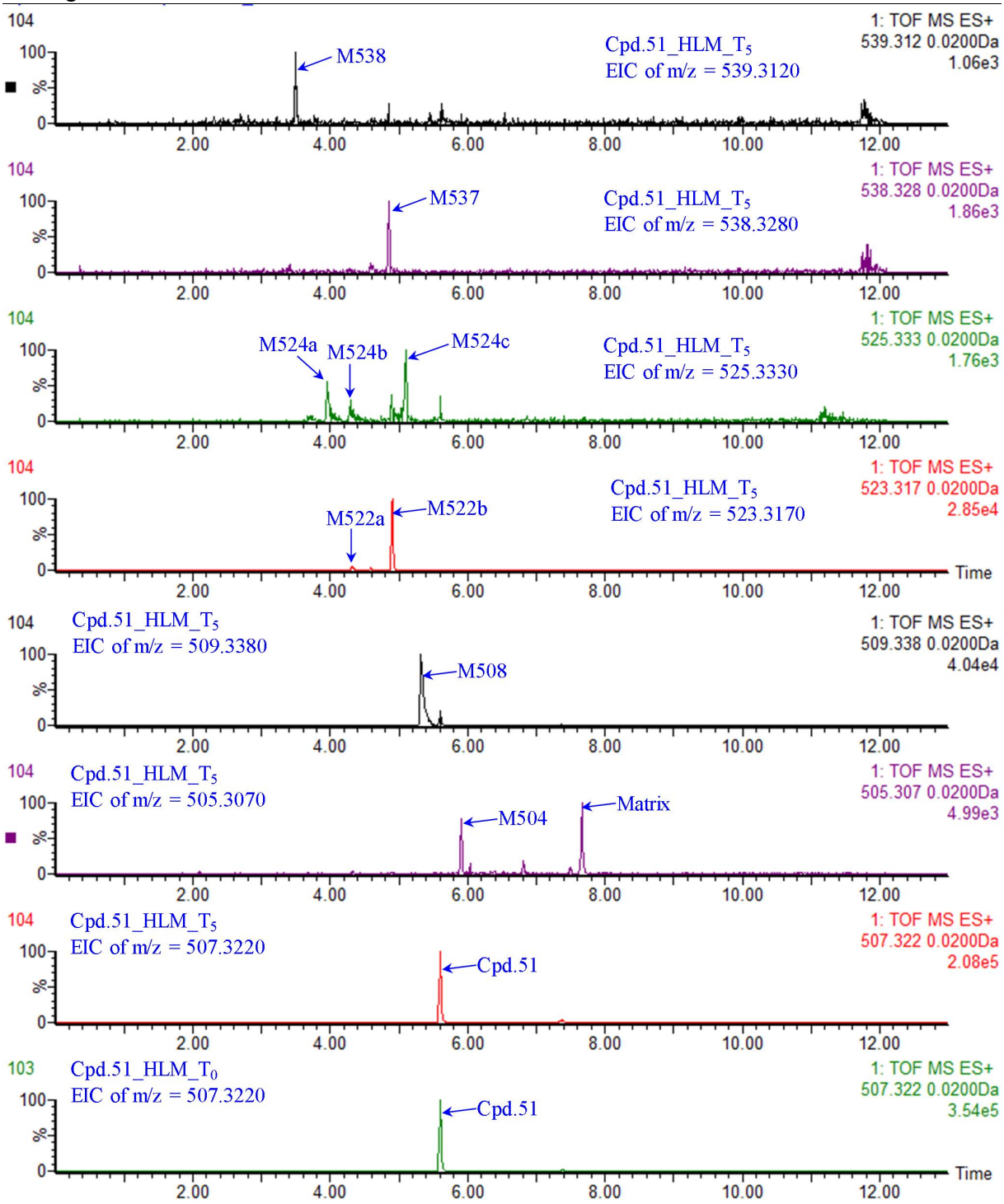



**Figure 2.** LC-MS EIC of Cpd.51 and its metabolites incubated in human liver microsomes with NADPH for 0 min and 5 min.

Positive-ion mode:






Negative-ion mode:

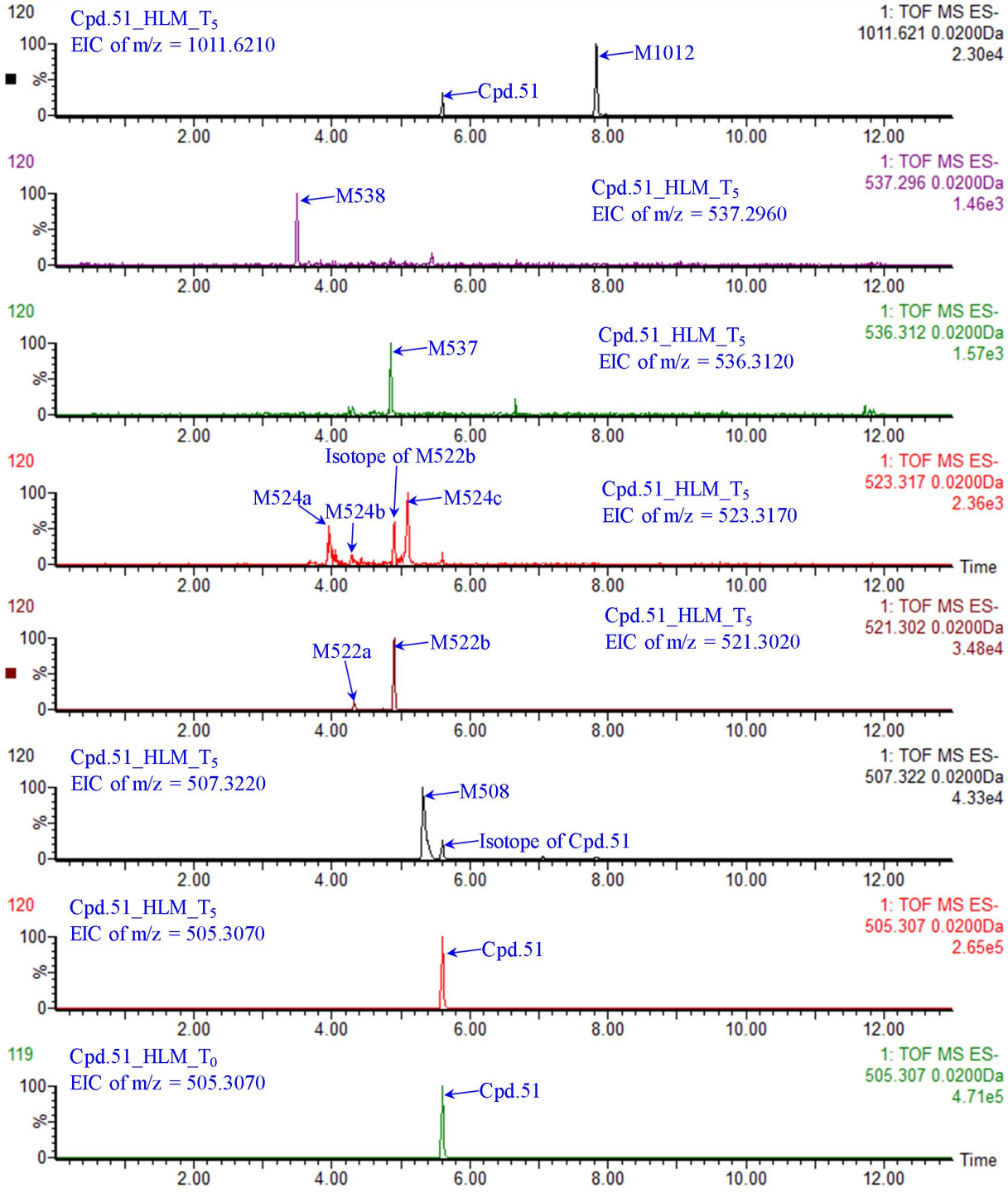

Cpd.51_HLM_T5
EIC of m/z = 1011.6210
1: TOF MS ES-
1011.621 0.0200Da
2.30e4
M1012
Cpd.51
M538
Cpd.51_HLM_T5
EIC of m/z = 537.2960
1: TOF MS ES-
537.296 0.0200Da
1.46e3
M537
Cpd.51_HLM_T5
EIC of m/z = 536.3120
1: TOF MS ES-
536.312 0.0200Da
1.57e3
Isotope of M522b
M524a
M524b
M524c
Cpd.51_HLM_T5
EIC of m/z = 523.3170
1: TOF MS ES-
523.317 0.0200Da
2.36e3
M522a
M522b
Cpd.51_HLM_T5
EIC of m/z = 521.3020
1: TOF MS ES-
521.302 0.0200Da
3.48e4
M508
Isotope of Cpd.51
Cpd.51_HLM_T5
EIC of m/z = 507.3220
1: TOF MS ES-
507.322 0.0200Da
4.33e4
Cpd.51
Cpd.51_HLM_T5
EIC of m/z = 505.3070
1: TOF MS ES-
505.307 0.0200Da
2.65e5
Cpd.51
Cpd.51_HLM_T0
EIC of m/z = 505.3070
1: TOF MS ES-
505.307 0.0200Da
4.71e5
Time
2.00
4.00
6.00
8.00
10.00
12.00

**Figure 3.** LC-MS EIC of M508 in $T_0$, $T_5$, $T_{5\text{-w/o}}$ and neat solution.

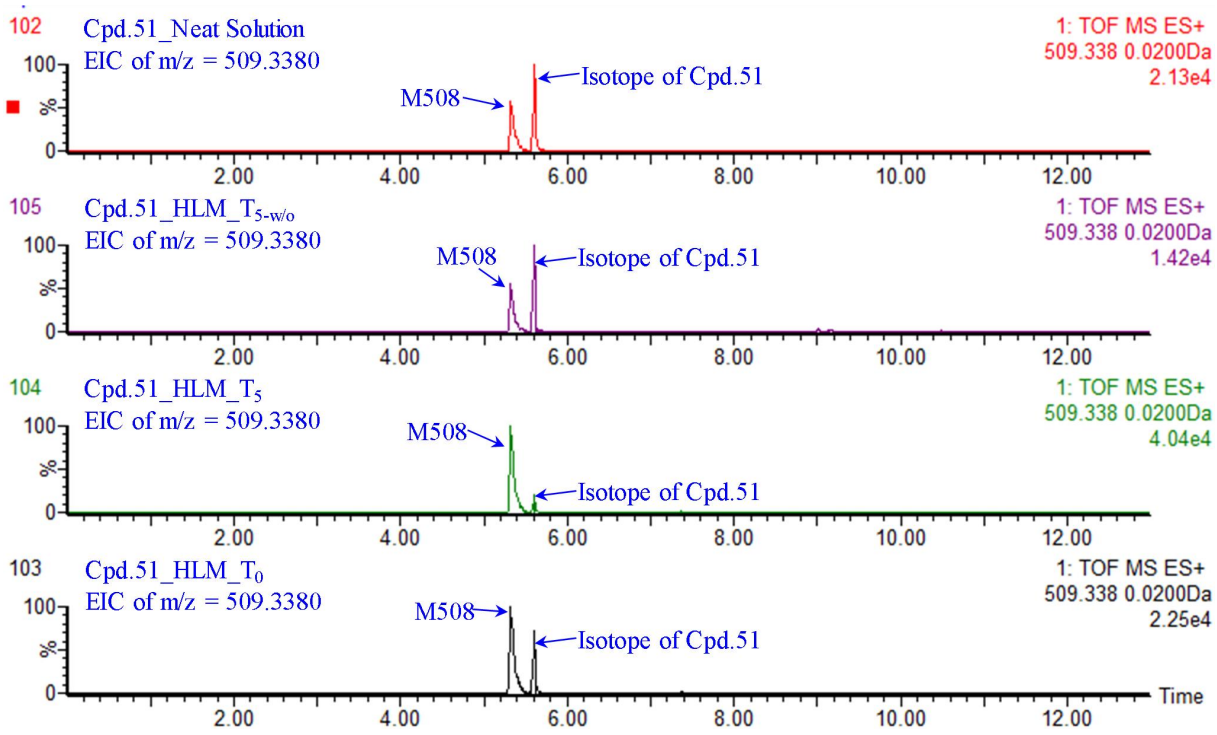


**Figure 4.** Proposed metabolic pathways of Cpd.51 incubated in human liver microsomes in the presence of NADPH

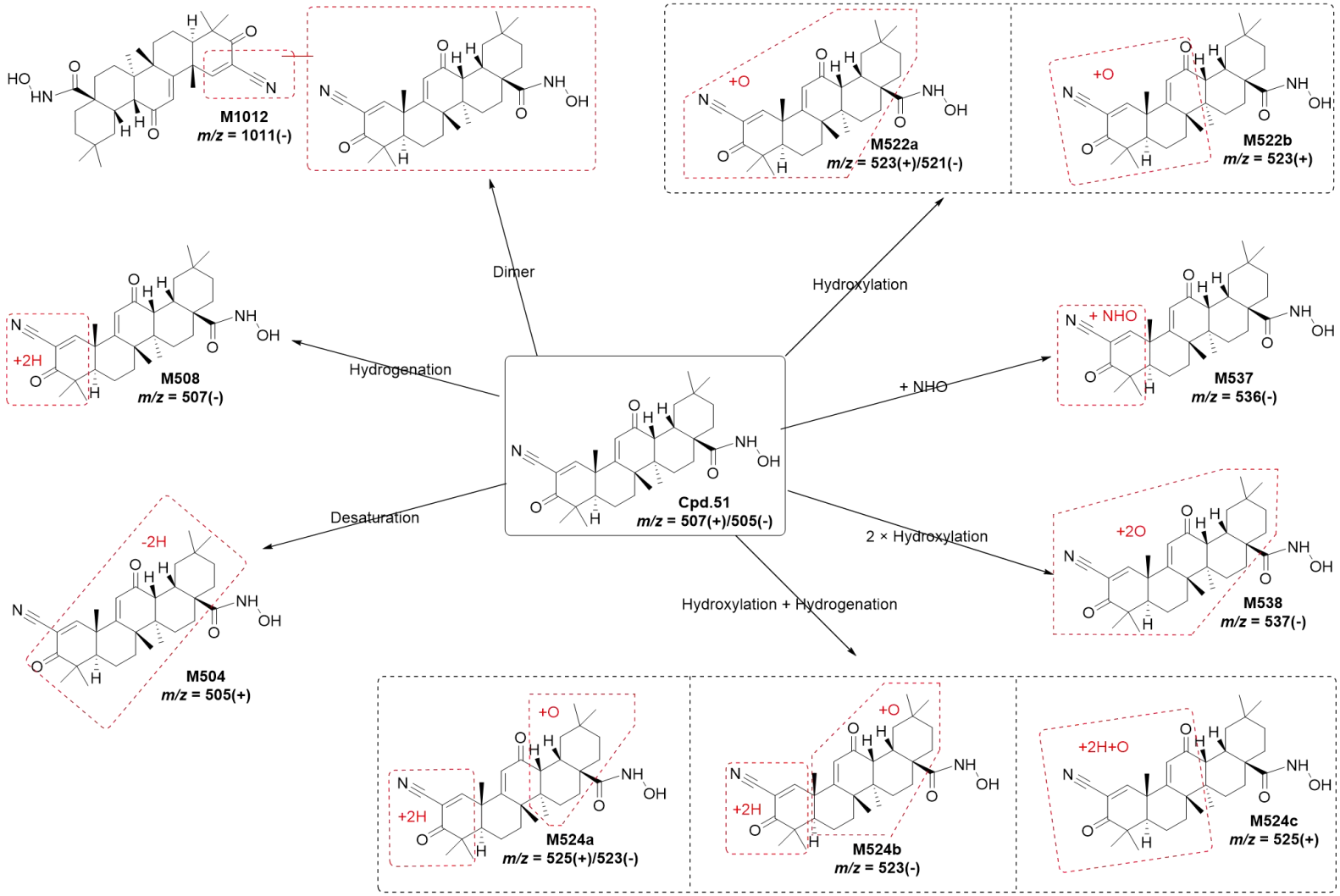


**Table 1.** Major metabolites of Cpd.51 incubated in human liver microsomes in the presence of NADPH

| Peak ID | Found *m/z* | Mass Shift | Biotransformation | R.T. (min) | Human liver microsome | |
|---|---|---|---|---|---|---|
| | | | | | Relative UV* | MS peak area |
| Parent ($T_0$) | 507.3223 $[M+H]^+$ | n/a | n/a | 5.60 | 100.00% | 1.19E+04 |
| | 505.3066 $[M-H]^-$ | | | | | 1.59E+04 |
| Parent ($T_5$) | 507.3223 $[M+H]^+$ | n/a | n/a | 5.60 | 54.06% | 6.69E+03 |
| | 505.3066 $[M-H]^-$ | | | | | 8.97E+03 |
| M504 | 505.3064 $[M+H]^+$ | -2.0157 | Desaturation | 5.91 | 1.09% | 1.11E+02 |
| | 503.2909 $[M-H]^-$ | | | | | ND |
| M508 | 509.3382 $[M+H]^+$ | 2.0156 | Hydrogenation | 5.32 | 11.61% | 2.40E+03 |
| | 507.3222 $[M-H]^-$ | | | | | 2.59E+03 |
| M522a | 523.3130 $[M+H]^+$ | 15.9933 | Hydroxylation | 4.33 | + | 6.00E+01 |
| | 521.2999 $[M-H]^-$ | | | | | 1.08E+02 |
| M522b | 523.3158 $[M+H]^+$ | 15.9968 | Hydroxylation | 4.90 | 8.13% | 7.86E+02 |
| | 521.3034 $[M-H]^-$ | | | | | 9.78E+02 |
| M524a | 525.3320 $[M+H]^+$ | 18.0100 | Hydroxylation + Hydrogenation | 3.96 | + | 3.90E+01 |
| | 523.3166 $[M-H]^-$ | | | | | 5.00E+01 |
| M524b | 525.3320 $[M+H]^+$ | 18.0064 | Hydroxylation + Hydrogenation | 4.29 | + | 1.00E+01 |
| | 523.3130 $[M-H]^-$ | | | | | 1.20E+01 |
| M524c | 525.3319 $[M+H]^+$ | 18.0089 | Hydroxylation + Hydrogenation | 5.09 | + | 5.90E+01 |
| | 523.3155 $[M-H]^-$ | | | | | 1.00E+02 |
| M537 | 538.3284 $[M+H]^+$ | 31.0069 | + NHO | 4.85 | + | 5.50E+01 |
| | 536.3135 $[M-H]^-$ | | | | | 4.30E+01 |
| M538 | 539.3121 $[M+H]^+$ | 31.9907 | 2 × Hydroxylation | 3.49 | 0.69% | 2.50E+01 |
| | 537.2973 $[M-H]^-$ | | | | | 4.10E+01 |
| M1012 | 1013.6331 $[M+H]^+$ | 506.3124 | Dimer | 7.83 | 5.16% | 7.87E+02 |
| | 1011.6190 $[M-H]^-$ | | | | | 8.54E+02 |

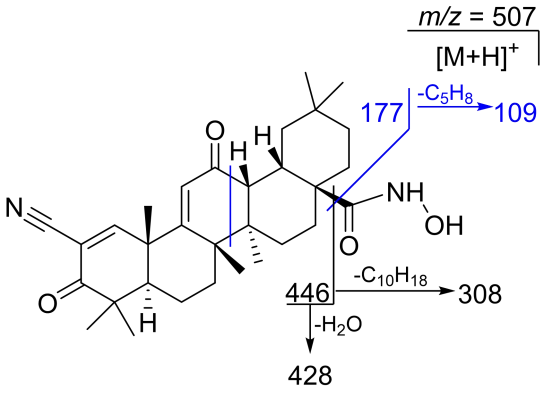
*: All percentages were calculated based on the detected UV (λ = 250-260 nm) absorption relative to that of parent in $T_0$ sample (normalized as 100%);
+: Only detected in MS;
n/a: Not applicable.

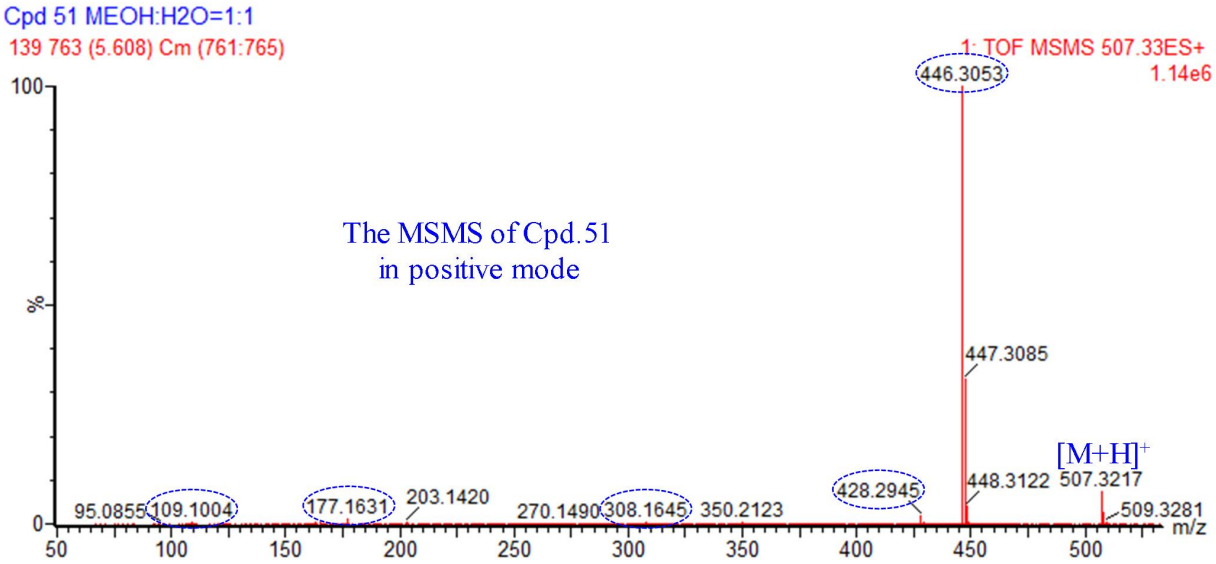
# RESULTS AND DISCUSSION

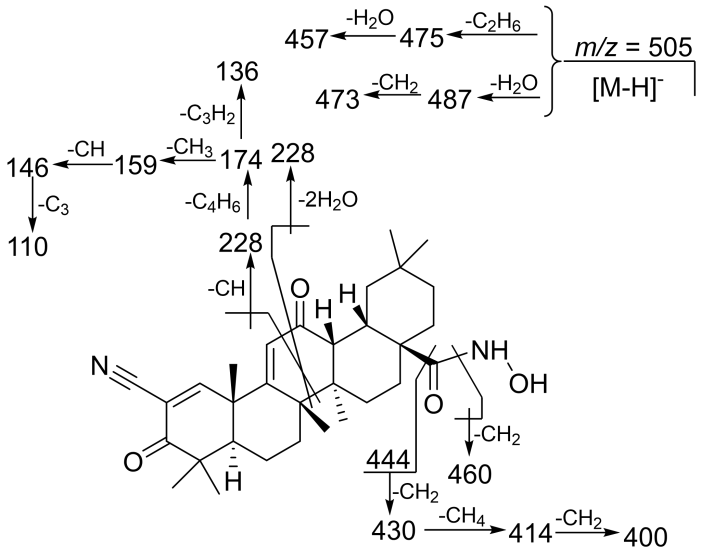
## Structure elucidation of Cpd.51 and its metabolites

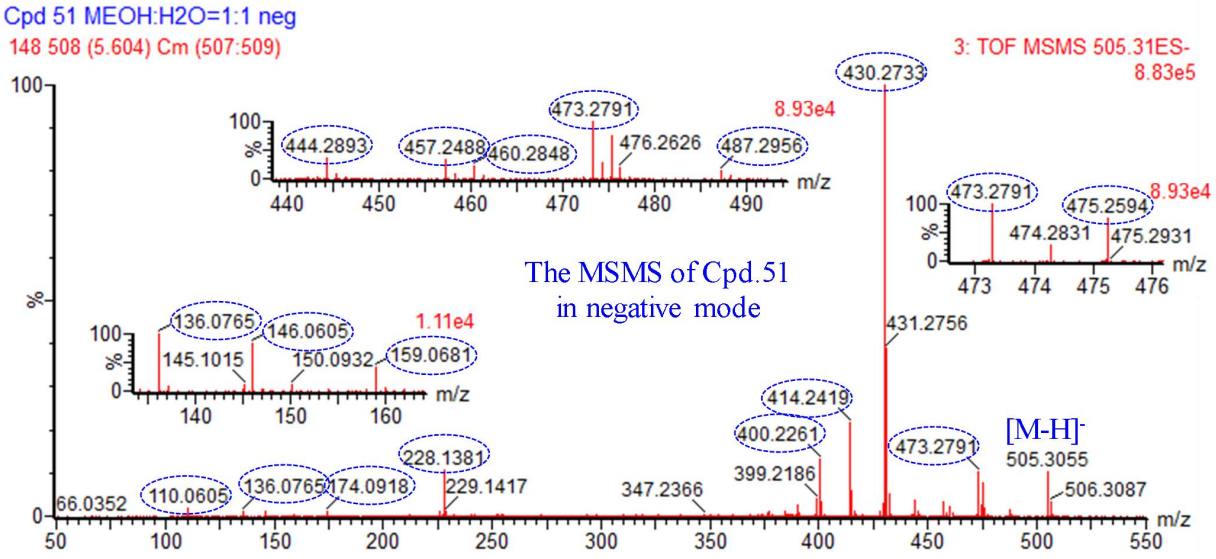
### 1) MS fragmentation assignment of Cpd.51

MSMS spectrum was obtained for Cpd.51 and the fragments were assigned to facilitate structure elucidation of Cpd.51 metabolites.

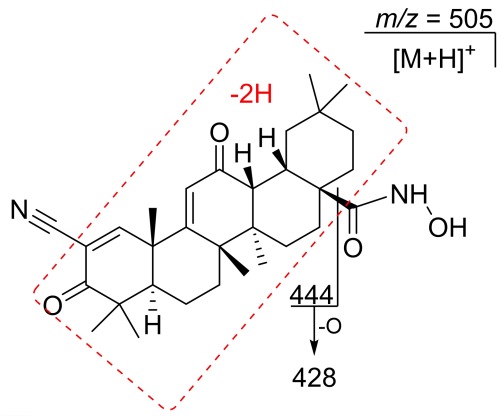


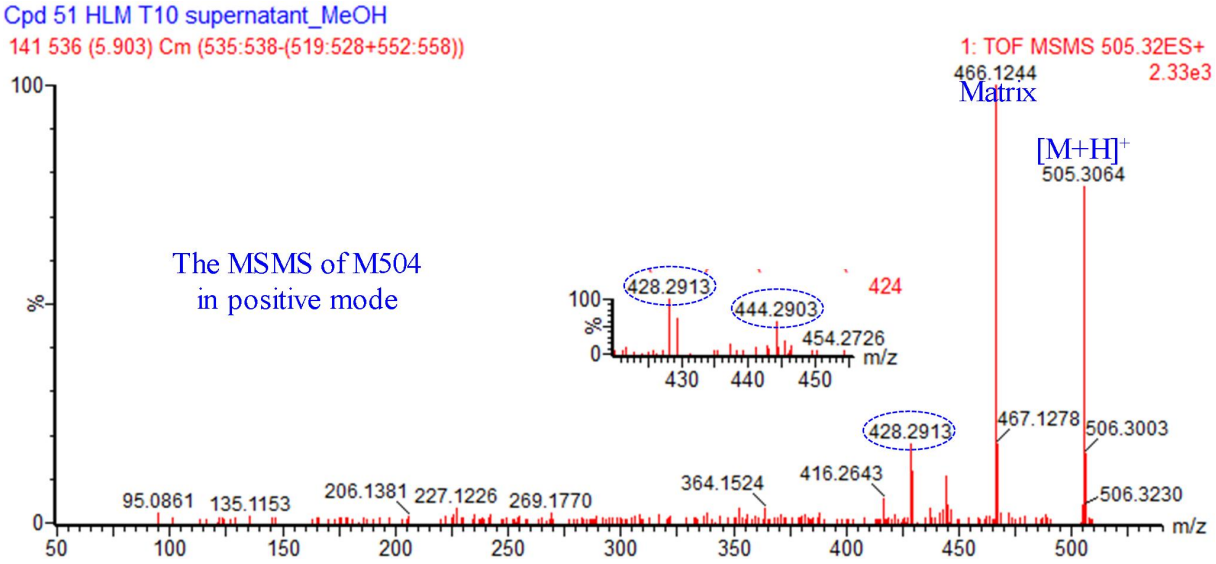




457
-H2O
475
-C2H6
m/z = 505
[M-H]-
473
-CH2
487
-H2O
136
-C3H2
146
-CH
159
-CH3
174
228
-C3
110
-C4H6
-2H2O
228
-CH
O
H
H
N
NH
OH
O
O
-CH2
444
460
H
-CH2
430
-CH4
414
-CH2
400

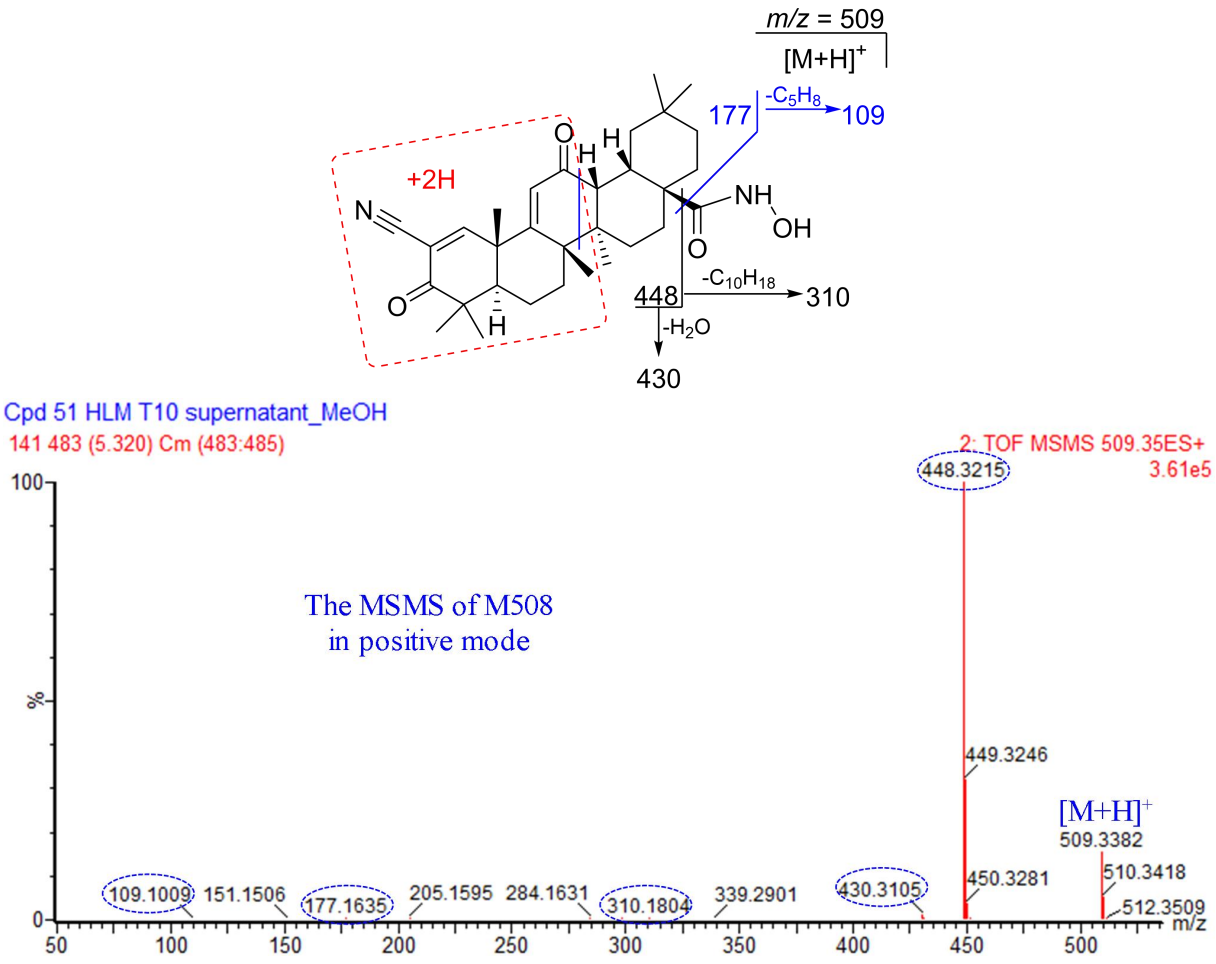

Cpd 51 MEOH:H2O=1:1 neg
148 508 (5.604) Cm (507:509)
3: TOF MSMS 505.31ES-
8.83e5
The MSMS of Cpd.51
in negative mode
[M-H]-
505.3055
430.2733
431.2756
473.2791
414.2419
400.2261
399.2186
347.2366
228.1381
229.1417
136.0765
174.0918
110.0605
66.0352
506.3087
8.93e4
444.2893
457.2488
460.2848
476.2626
487.2956
1.11e4
136.0765
146.0605
145.1015
150.0932
159.0681
475.2594
474.2831
475.2931
%
m/z

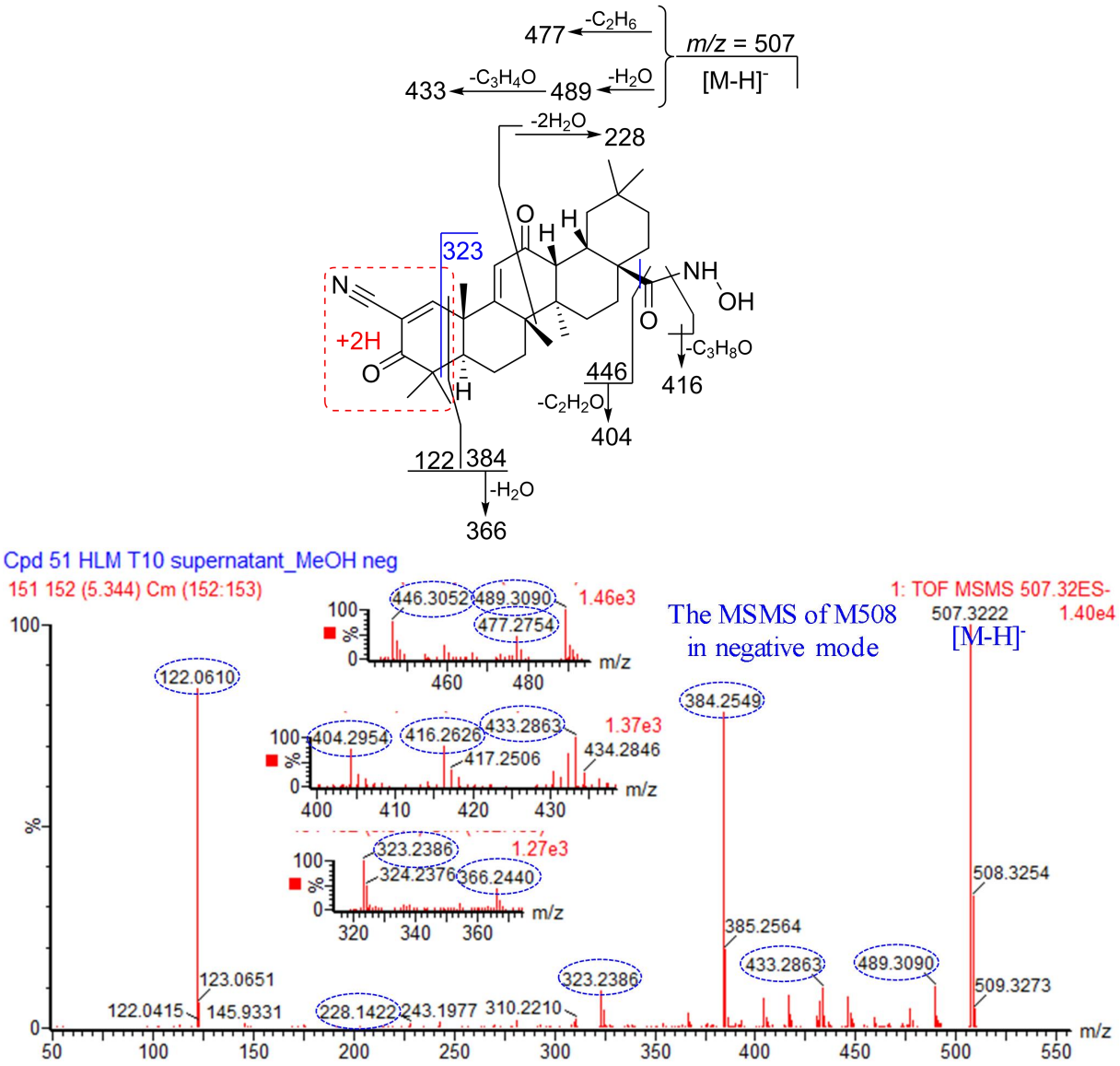
**2) M504 (Desaturation)**



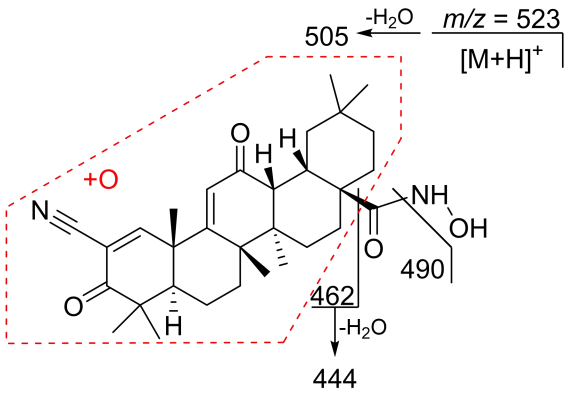



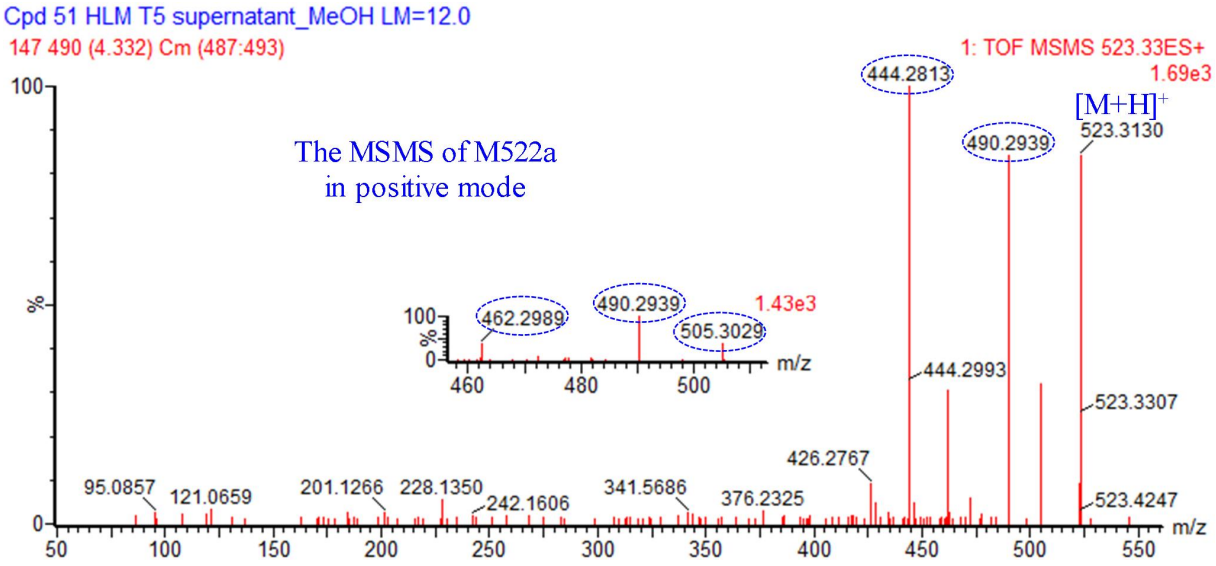
### 3) M508 (Hydrogenation)

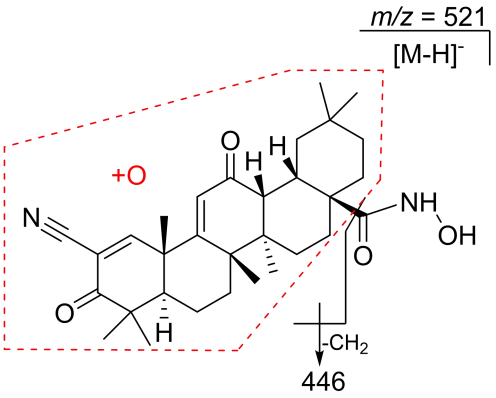


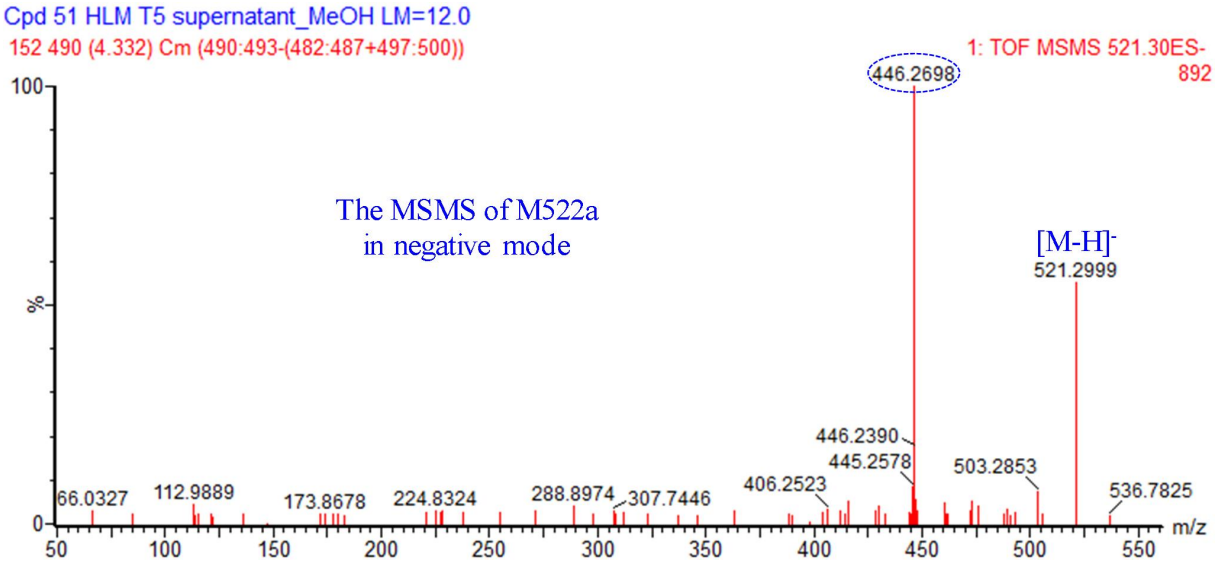

477
-C2H6
m/z = 507
[M-H]-
433
-C3H4O
489
-H2O
-2H2O
228
323
+2H
446
416
-C3H8O
-C2H2O
404
122
384
-H2O
366
Cpd 51 HLM T10 supernatant_MeOH neg
151 152 (5.344) Cm (152:153)
1: TOF MSMS 507.32ES-
The MSMS of M508
in negative mode
507.3222
1.40e4
[M-H]-
1.46e3
1.37e3
1.27e3
m/z
%

**4) M522a (Hydroxylation)**

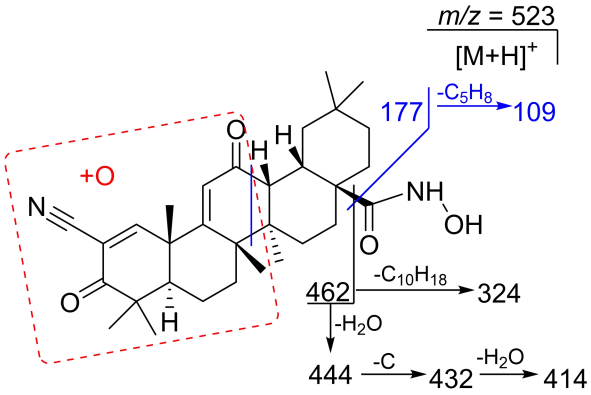


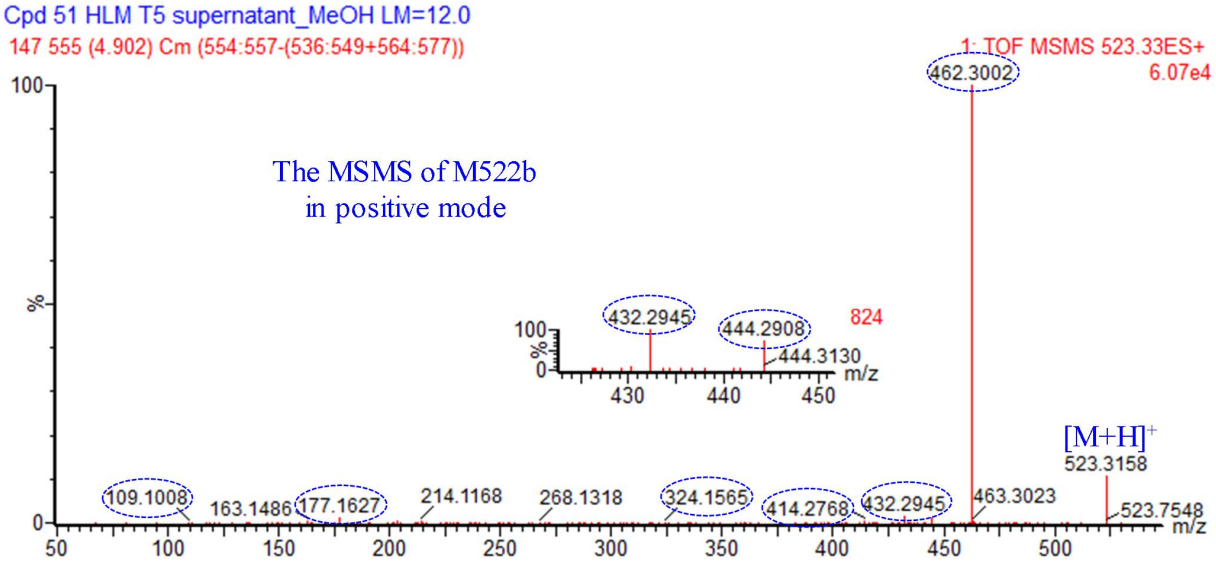



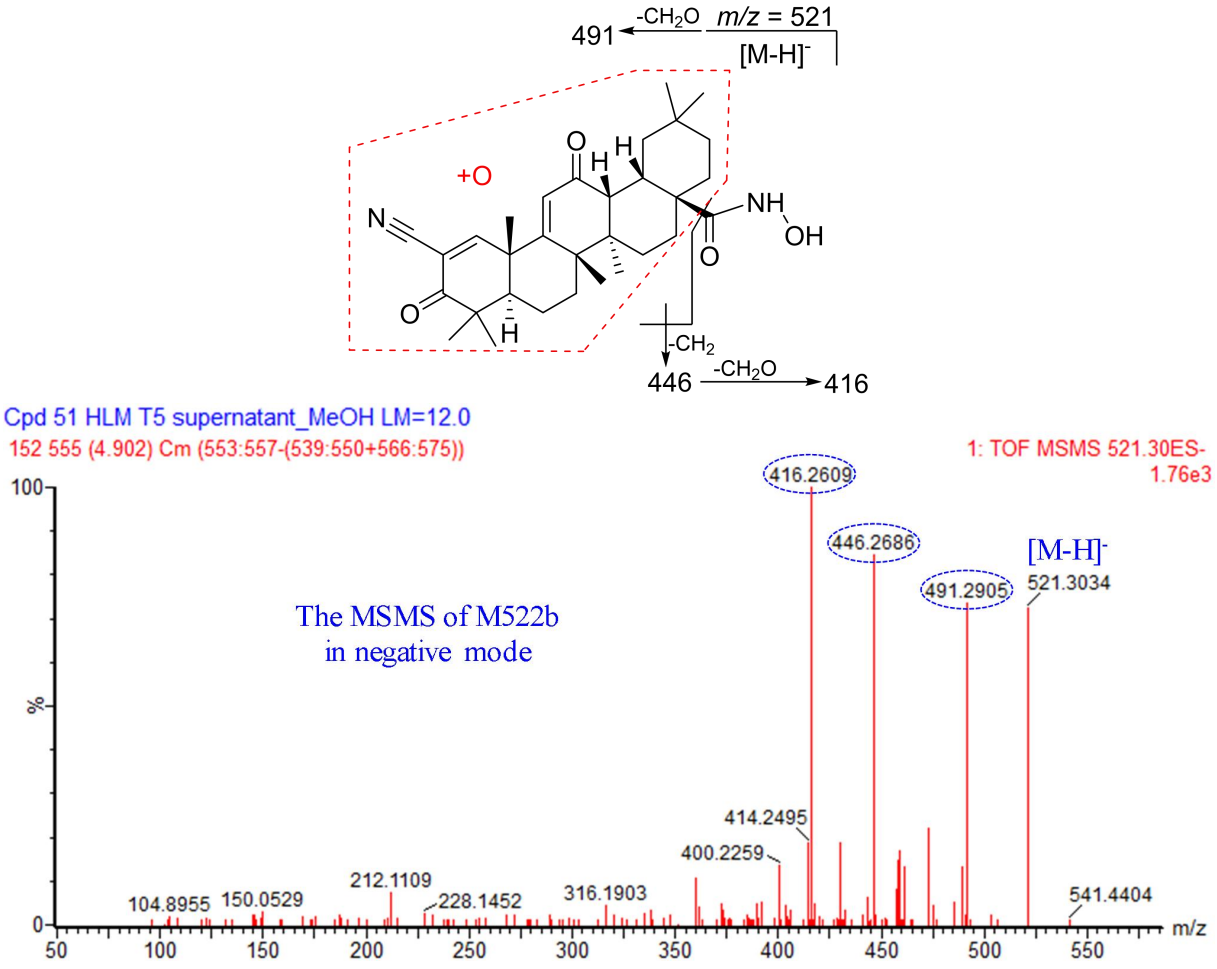

m/z = 521
[M-H]-
+O
O
H
H
N
NH
OH
O
O
H
CH2
446


Cpd 51 HLM T5 supernatant_MeOH LM=12.0
152 490 (4.332) Cm (490:493-(482:487+497:500))
1: TOF MSMS 521.30ES-892
446.2698
The MSMS of M522a
in negative mode
[M-H]-
521.2999
100
%
0
446.2390
445.2578
503.2853
66.0327
112.9889
173.8678
224.8324
288.8974
307.7446
406.2523
536.7825
m/z
50
100
150
200
250
300
350
400
450
500
550

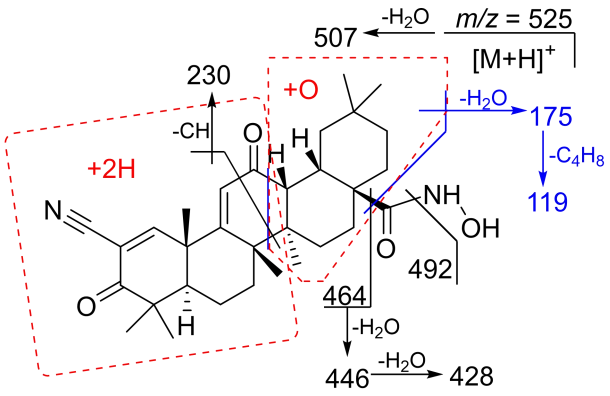
**5) M522b (Hydroxylation)**

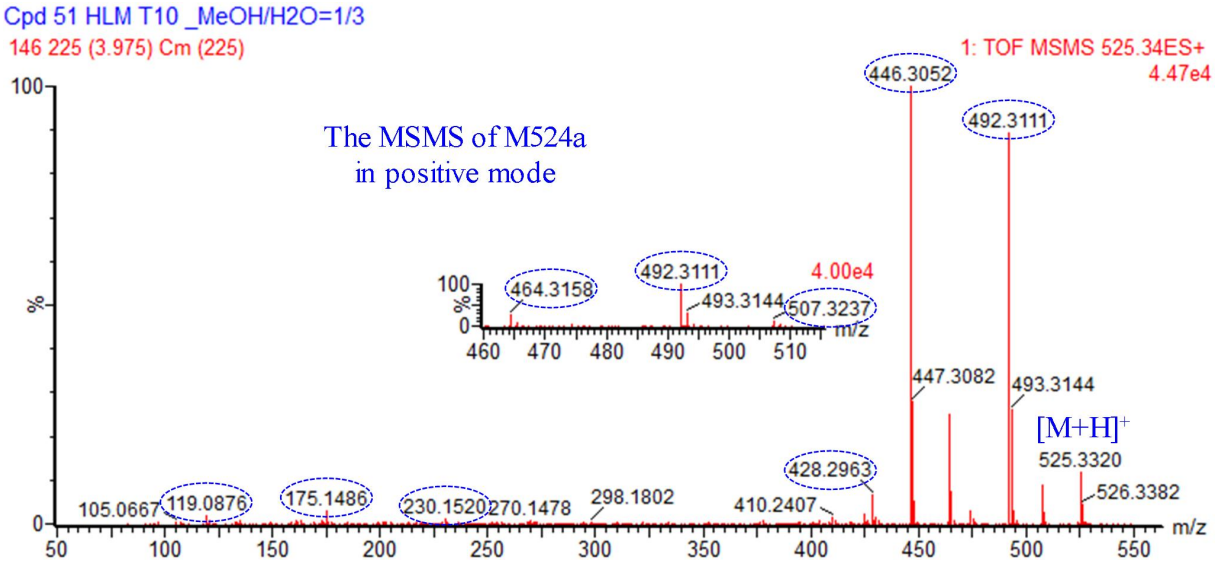


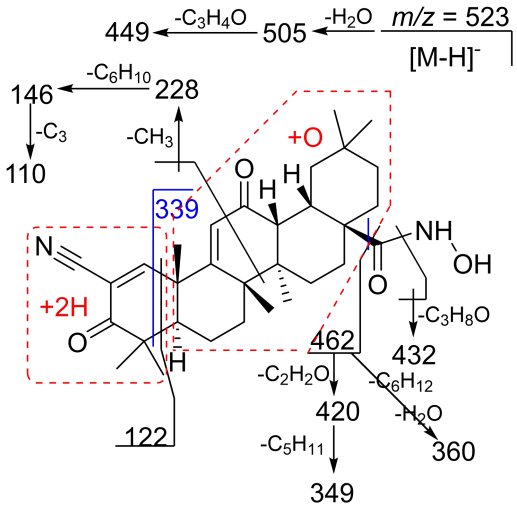



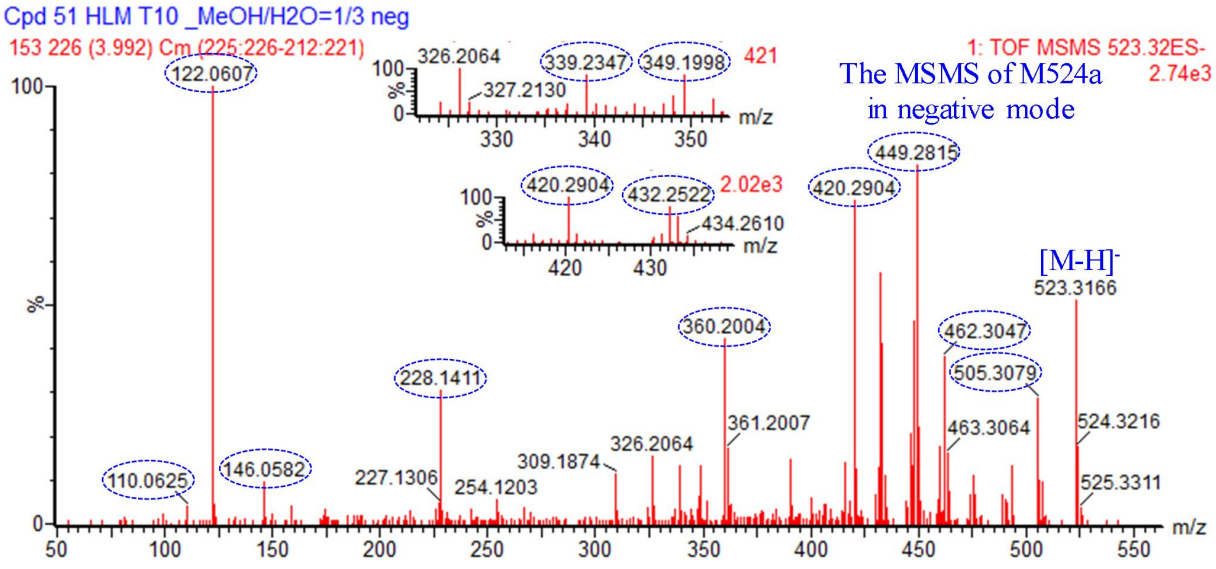

491
-CH2O
m/z = 521
[M-H]-
+O
O
H
H
N
NH
OH
O
O
H
-CH2
446
-CH2O
416
Cpd 51 HLM T5 supernatant_MeOH LM=12.0
152 555 (4.902) Cm (553:557-(539:550+566:575))
1: TOF MSMS 521.30ES-
1.76e3
The MSMS of M522b
in negative mode
[M-H]-
521.3034
416.2609
446.2686
491.2905
414.2495
400.2259
212.1109
104.8955
150.0529
228.1452
316.1903
541.4404
%
m/z
100
0
50
100
150
200
250
300
350
400
450
500
550

### 6) M524a (Hydroxylation + Hydrogenation)

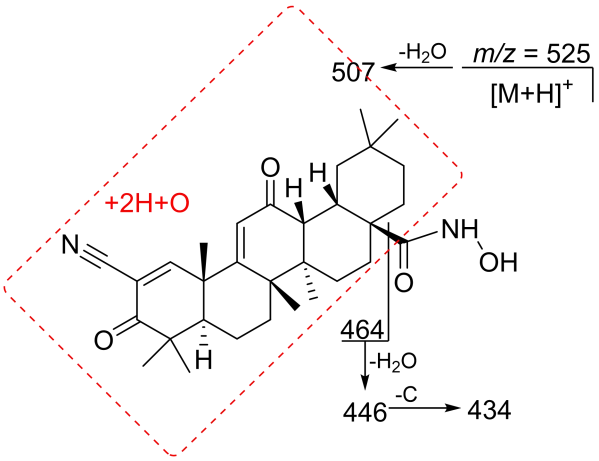


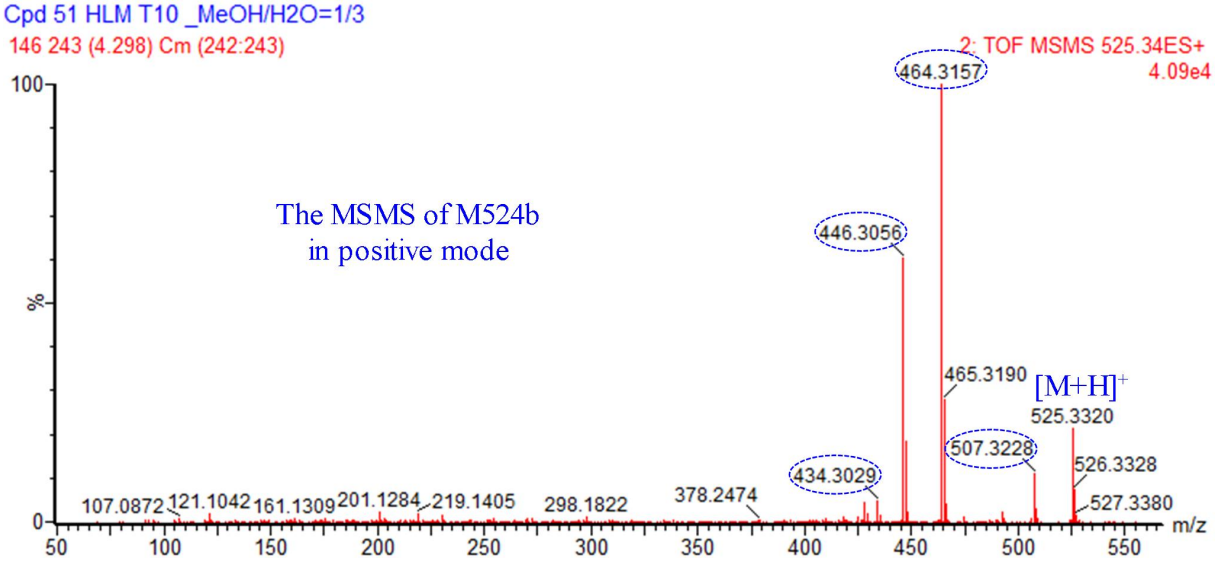



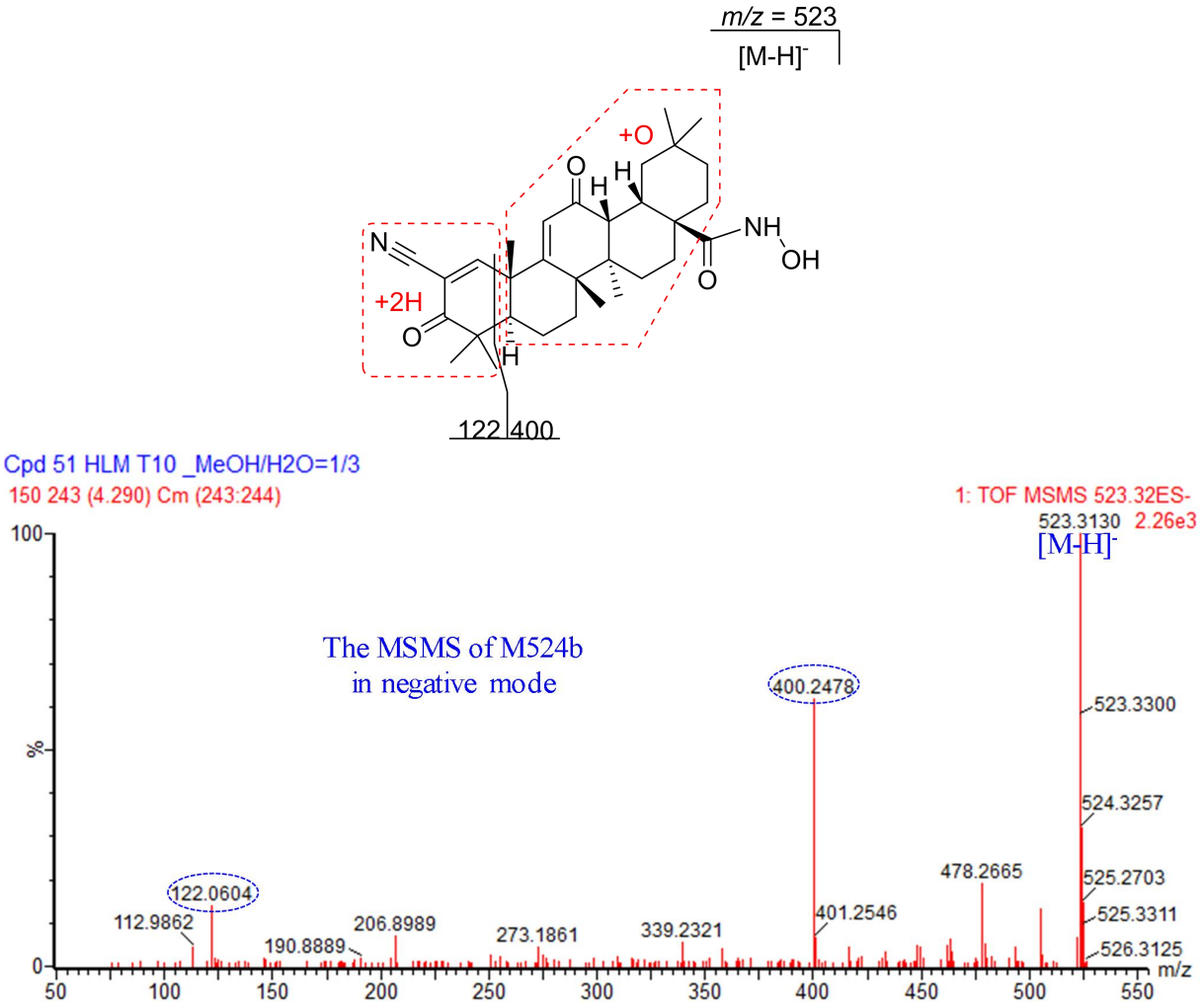

449
-C3H4O
505
-H2O
m/z = 523
[M-H]-
-C6H10
146
228
-C3
-CH3
110
+O
339
+2H
462
432
-C2H2O
-C6H12
420
-H2O
-C5H11
360
122
349


Cpd 51 HLM T10 _MeOH/H2O=1/3 neg
153 226 (3.992) Cm (225:226-212:221)
1: TOF MSMS 523.32ES-
2.74e3
The MSMS of M524a
in negative mode
[M-H]-
523.3166
122.0607
110.0625
146.0582
227.1306
228.1411
254.1203
309.1874
326.2064
360.2004
361.2007
420.2904
449.2815
462.3047
463.3064
505.3079
524.3216
525.3311
326.2064
339.2347
349.1998
421
327.2130
420.2904
432.2522
2.02e3
434.2610
%
m/z

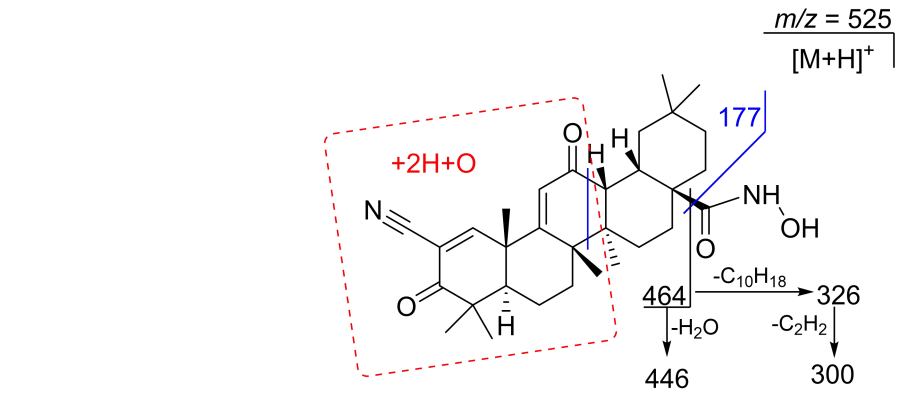
**7) M524b (Hydroxylation + Hydrogenation)**

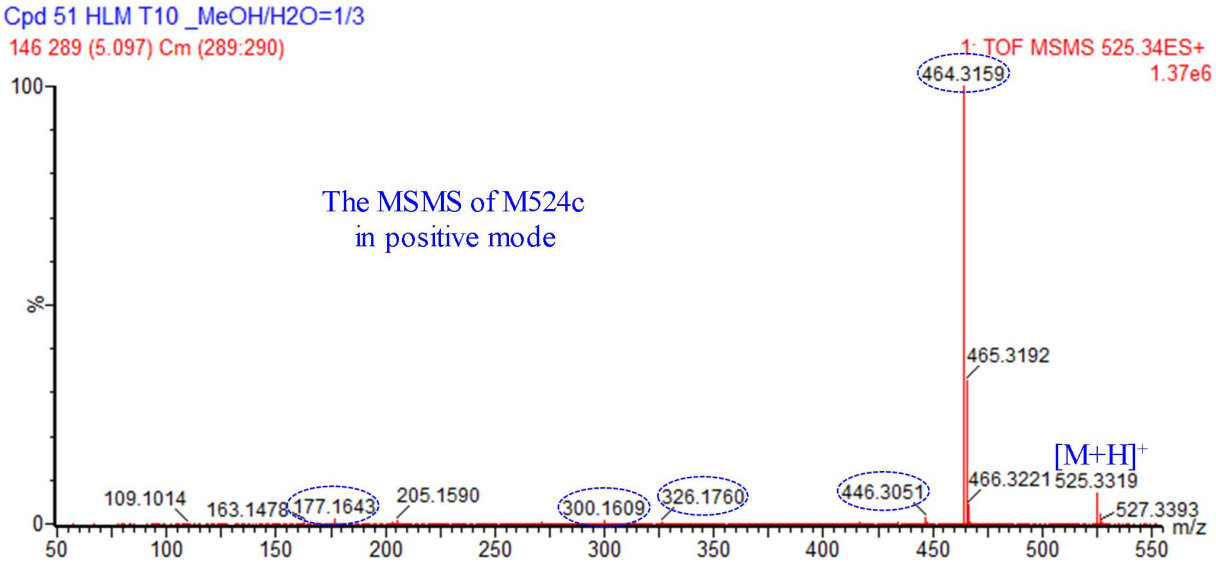


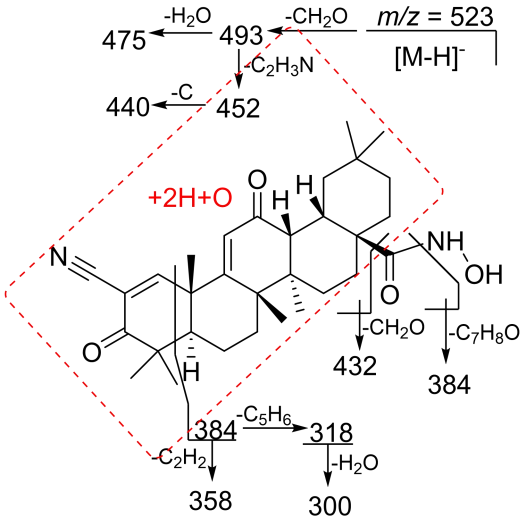



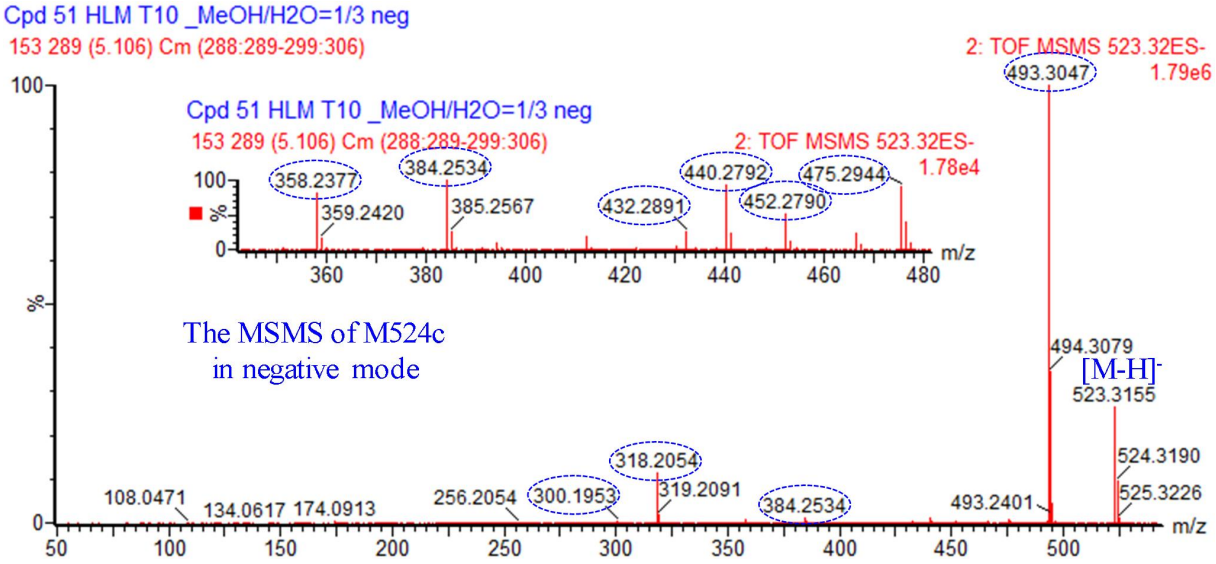

m/z = 523
[M-H]-
+O
O
H
H
NH
OH
O
N
+2H
O
H
122
400
Cpd 51 HLM T10 _MeOH/H2O=1/3
150 243 (4.290) Cm (243:244)
1: TOF MSMS 523.32ES-
523.3130 2.26e3
[M-H]-
The MSMS of M524b
in negative mode
400.2478
523.3300
524.3257
478.2665
525.2703
525.3311
526.3125
122.0604
112.9862
206.8989
190.8889
273.1861
339.2321
401.2546
%
m/z
100
0
50
100
150
200
250
300
350
400
450
500
550

**8) M524c (Hydroxylation + Hydrogenation)**

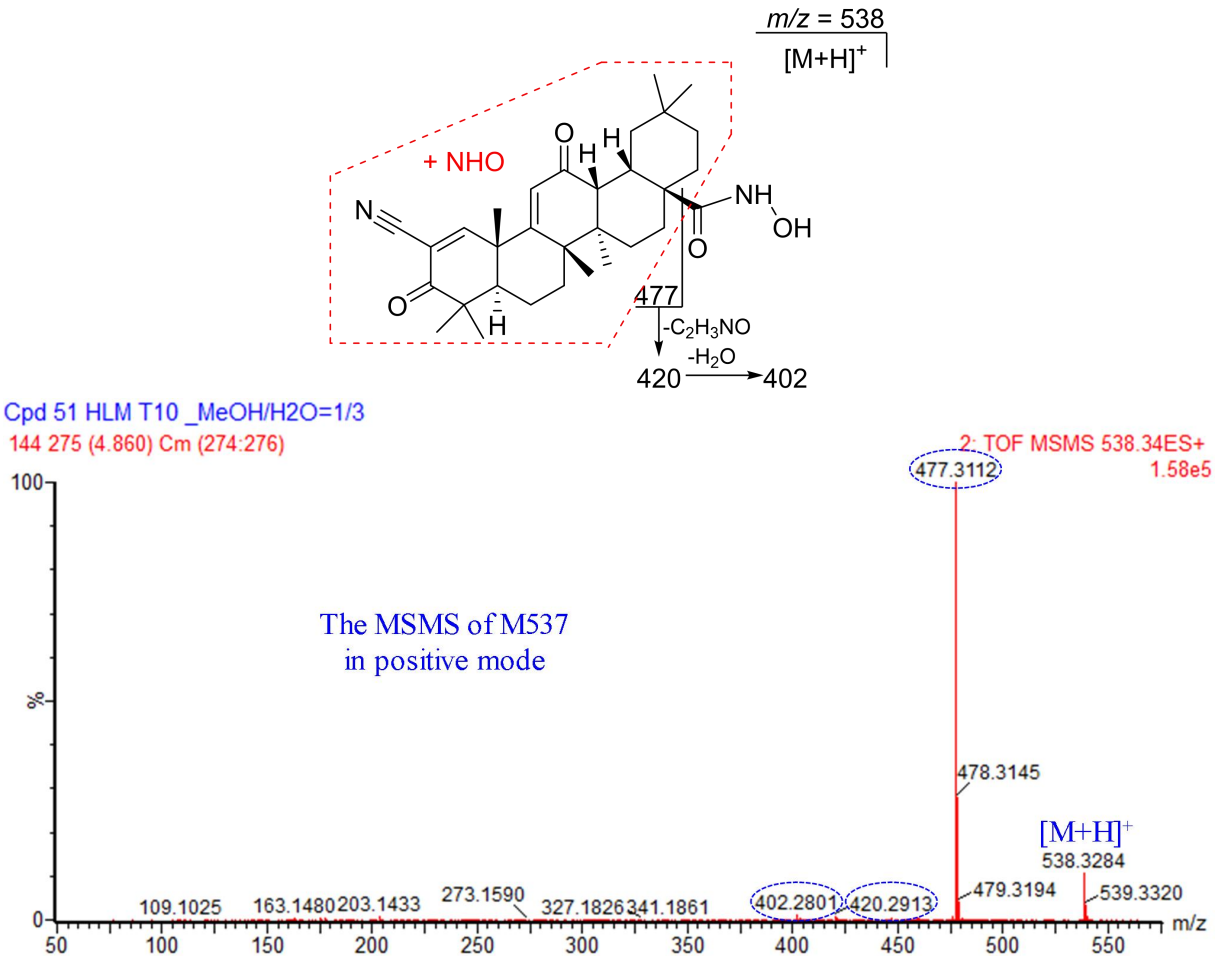


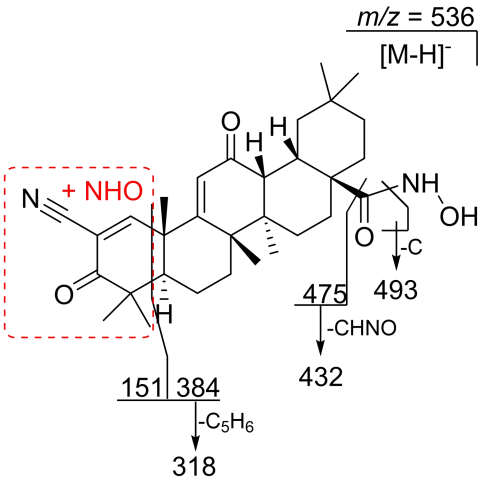



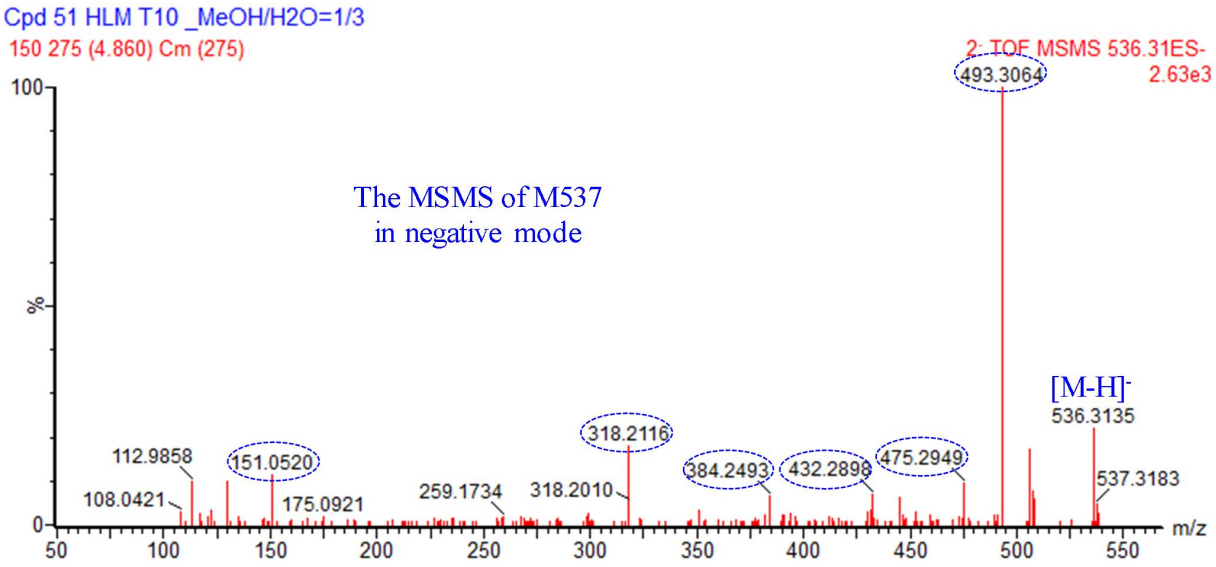

-H2O
-CH2O
m/z = 523
[M-H]-
475
493
-C2H3N
-C
440
452
+2H+O
O
H
H
N
NH
OH
O
O
H
-CH2O
-C7H8O
432
384
-C5H6
384
318
-C2H2
-H2O
358
300


Cpd 51 HLM T10 _MeOH/H2O=1/3 neg
153 289 (5.106) Cm (288:289-299:306)
2: TOF MSMS 523.32ES-
1.79e6
493.3047
Cpd 51 HLM T10 _MeOH/H2O=1/3 neg
153 289 (5.106) Cm (288:289-299:306)
2: TOF MSMS 523.32ES-
1.78e4
358.2377
384.2534
359.2420
385.2567
432.2891
440.2792
452.2790
475.2944
m/z
The MSMS of M524c
in negative mode
494.3079
[M-H]-
523.3155
524.3190
525.3226
318.2054
319.2091
108.0471
134.0617
174.0913
256.2054
300.1953
384.2534
493.2401
%
m/z

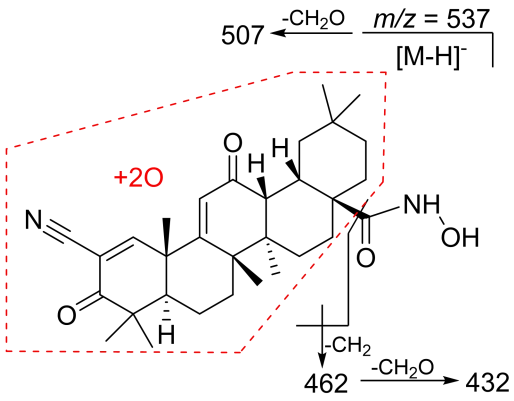
**9) M537 (+ NHO)**

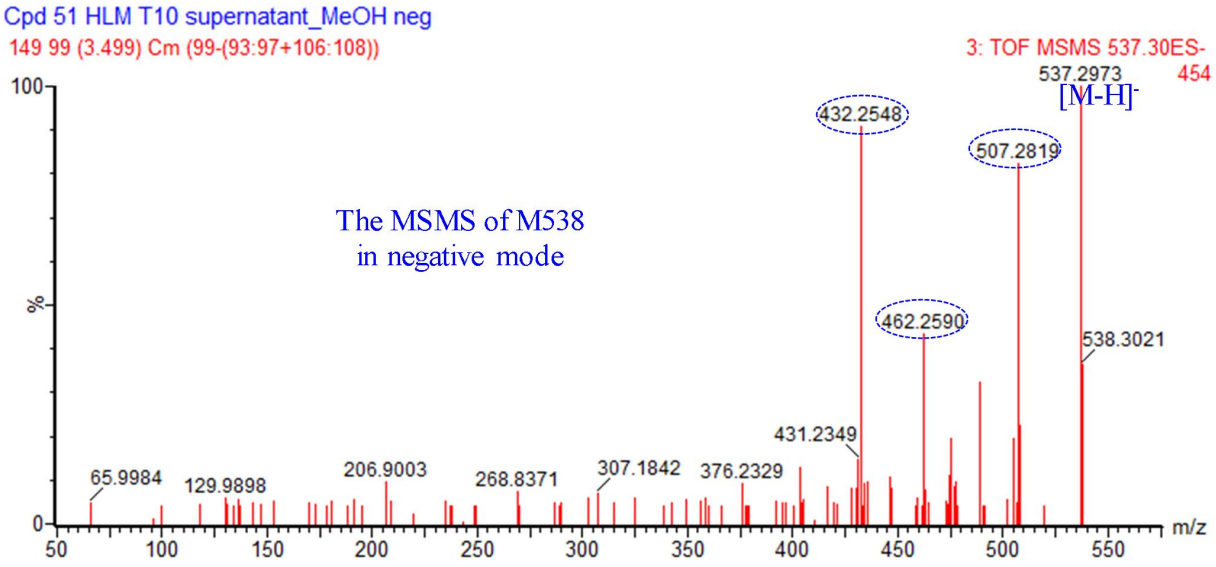

m/z = 538
[M+H]+
+ NHO
O
H
H
N
NH
OH
O
O
H
477
-C2H3NO
-H2O
420
402
Cpd 51 HLM T10 _MeOH/H2O=1/3
144 275 (4.860) Cm (274:276)
2: TOF MSMS 538.34ES+
1.58e5
477.3112
The MSMS of M537
in positive mode
478.3145
[M+H]+
538.3284
479.3194
539.3320
109.1025
163.1480
203.1433
273.1590
327.1826
341.1861
402.2801
420.2913
%
m/z


m/z = 536
[M-H]-
+ NHO
N
O
H
H
NH
OH
O
-C
475
493
-CHNO
432
151
384
-C5H6
318


Cpd 51 HLM T10 _MeOH/H2O=1/3
150 275 (4.860) Cm (275)
2: TOF MSMS 536.31ES-
2.63e3
493.3064
The MSMS of M537
in negative mode
[M-H]-
536.3135
537.3183
318.2116
384.2493
432.2898
475.2949
112.9858
151.0520
108.0421
175.0921
259.1734
318.2010
100
%
0
m/z
50
100
150
200
250
300
350
400
450
500
550

**10) M538 (2 × Hydroxylation)**



**11) M1012 (Dimer)**