S1. Synthesis of internal and external standards

2-[(4-Fluorobenzoyl)amino] acetic acid (4-FHA)

![Chemical structure of 2-[(4-Fluorobenzoyl)amino] acetic acid (4-FHA)]

A solution of glycine (0.03 mol, 2.25 g) in distilled water (20 mL) was combined with 10 mL of a 6 M aqueous solution of NaOH. Dropwise addition of 3.54 ml of 4-fluorobenzoyl chloride (0.03 mol, 4.76 g) while stirring resulted in a clear solution. Diluted HCl (1 M) was added to acidify the solution. The forming precipitate was filtered off and dried under reduced pressure over night. Three iterations of re-crystallization from water yielded the product of analytical purity.

Yield: colorless crystals (30%).

Melting point: 184 °C.

\[^1\text{H-NMR:}\] (400 MHz, DMSO-d\textsubscript{6}): \(\delta = 3.93 (d, J = 6 \text{ Hz}, C (2') H_2), 7.32 (m, J = 3.2 \text{ Hz}, C (3) + C (5) H_2), 7.95 (m, 2H, \(J = 3.20 \text{ Hz}, C (2) + C (6) H_2), 8.88 (t, J = 8.88 \text{ Hz}, N (H_2), 12.61 (S, OH).\)

\[^{13}\text{C-NMR:}\] (100 MHz, DMSO-d\textsubscript{6}): \(\delta = 41.32 (C 2'), 115.30 (J = 88 \text{ Hz}, C (3) + C (5), 130.11 (J = 12 \text{ Hz}, C (2) + C (6), 130.11 (J = 12 \text{ Hz}, C (1), 162.75 (C4), 165.33 (J = 88 \text{ Hz}, C1'), 171.28 (C3').

\[^{\text{MIR:}}\] \(\bar{\nu} = 3327 (w, \nu_{\text{N-H, amine}}), 3047 (w, \nu_{\text{O-H, carboxylic acid}}), 2874 (w, \delta_{\text{C=O, carboxylic acid}}), 1717 (s, \nu_{\text{C=O, carboxylic acid}}), 1602 (m, \nu_{\text{aromatic ring}}), 1500 (s, \nu_{\text{aromatic ring}}), 1222 (s, \nu_{\text{C=O}}).\)
2-[(3,4-Difluorobenzoyl)amino] acetic acid (3,4-FHA)

A solution of glycine (0.03 mol, 2.25 g) in distilled water (20 mL) was combined with 10 mL of a 6 M aqueous solution of NaOH. Dropwise addition of 3.78 ml of 3,4-difluorobenzoyl chloride (0.03 mol, 5.30 g) while stirring resulted in a clear solution. Diluted HCl (1 M) was added to acidify the solution. The forming precipitate was filtered off and dried under reduced pressure over night. Three iterations of re-crystallization from water yielded the product of analytical purity.

Yield: colorless crystals (35%).

Melting point: 138-139 °C.

$^1$H-NMR: (400 MHz, DMSO-d$_6$): $\delta$ = 3.94 (d, $J$ = 6 Hz, C (2') H$_2$), 7.60 (m, $J$ = 2 Hz, C (5) H), 7.80 (m, $J$ = 2 Hz, C (2) H), 7.90 (t, $J$ = 2 Hz, C (6) H), 8.98 (t, $J$ = 6 Hz, N (H$_2$)), 12.65 (s, OH).

$^{13}$C-NMR: (100 MHz, DMSO-d$_6$): $\delta$ = 41.29 (C9), 116.66 ($J$ = 76 Hz, C1), 117.66 ($J$ = 72 Hz, C6), 124.72 ($J$ = 28 Hz, C5), 131.25 ($J$ = 20 Hz, C2), 147.96 ($J$ = 52 Hz, C4), 150.31 ($J$ = 52 Hz, C3), 164.35, ($J$ = 4 Hz, C1'), 171.90 (C3').

MIR: $\tilde{\nu}$ = 3320 (m, $\nu$N-H, amine), 3061 (w, $\nu$O-H, carboxylic acid), 2942 (w, $\delta$C=H$_2$), 1720 (s, $\nu$C=O, carboxylic-acid), 1592 (s, $\nu$aromatic ring), 1505 (s, $\nu$aromatic ring), 1242 (s, $\nu$C-CH$_3$).
2-Chloro-5-methyl-3-nitropyridine-1-oxide (precursor 1)

![Chemical Structure](image)

After dissolution of urea-hydrogen peroxide (42 mmol, 3.95 g) and 2-chloro-5-methyl-3-nitropyridine (20 mmol, 3.452 g) in dichloromethane (80 mL), ice-cold water was used to cool the mixture. Trifluoroacetic anhydride (42 mmol, 6 mL) was added slowly and the mixture was stirred for 30 min. Then, the temperature was raised to room temperature and stirred for 36 hours. After completion of the reaction, it was ascertained by use of thin layer chromatography, the mixture was filtered and poured into water (50 mL). The product was extracted using dichloromethane (2 x 25 mL). The organic phase was then washed with brine and adsorbed onto diatomaceous earth. Flash chromatography on silica gel and a mixture of ethyl acetate/n-hexane in a gradient ranging from 40-60% was used to separate the product.

Yield: pale yellow crystals (82%).

Melting point: 137-138 °C (solid from n-hexane/ethyl acetate).

\(^1\)H NMR: (400 MHz, DMSO-d\(_6\)): \(\delta = 8.71 (q, \; ^4J = 0.9 \text{ Hz}, 1\text{H}, \text{C (6) H}), 8.00 (q, \; ^4J = 0.8 \text{ Hz}, 1\text{H}, \text{C (4) H}), 2.33 (m, 3\text{H, CH}_3)\).

\(^1^3\)C NMR: (100 MHz, DMSO-d\(_6\)): \(\delta = 146.6 (\text{C2}), 142.8 (\text{C6}), 134.9 (\text{C3}), 132.9 (\text{C5}), 122.3 (\text{C4}), 17.2 (\text{CH}_3)\).

MIR: \(\tilde{\nu} = 3038 (w, \nu_{\text{C-H}}), 1694 (w, \nu_{\text{C-N}}), 1598 (w, \nu_{\text{aromatic ring}}), 1532 (s, \nu_{\text{aromatic ring}}), 1385 (w, \delta_{\text{CH}_3}), 1223 (m, \nu_{\text{NO}_2, \text{nitrite}}), 747 (s, \nu_{\text{C-Cl}})\).
2-Chloro-5-methyl-3-nitropyridine-1-oxide (32.3 mmol, 6.091 g) was suspended in phosphorous oxychloride (30 mL) and refluxed for 5 hours. The mixture was carefully added to pre-warmed water, then cooled and extracted with ethyl acetate (3 x 20 mL). The ethyl acetate phase was washed with sodium bicarbonate (aq.) and adsorbed onto diatomaceous earth. Flash chromatography on silica gel and a mixture of ethyl acetate/n-hexane in a gradient ranging from 20-60% was used to separate the product.

Yield: off-white crystals (74%).

Melting point: 68-69 °C (solid from n-hexane/ethyl acetate).

1H NMR: (400 MHz, DMSO-d6): δ = 8.69 (d, 4J = 0.6 Hz, 1H, C (4) H), 2.42 (d, 4J = 0.6 Hz, 3H, CH3).

13C NMR: (100 MHz, DMSO-d6): δ = 152.0 (C2), 143.2 (C6), 138.3 (C3), 138.1 (C4), 134.1 (C5), 18.2 (CH3).

MIR: $\tilde{\nu} = 3065$ (w, $\nu_{C-H}$), 1580 (m, $\nu_{NH}$, amine), 1551 (m, $\nu_{aromatic\ ring}$), 1512 (s, $\nu_{aromatic\ ring}$), 1258 (w, $\nu_{NO2, nitrite}$), 722 (s, $\nu_{C-Cl}$).
6-Chloro-5-methyl-3-nitropyridin-2-amine (precursor-3)

![Structure of 6-Chloro-5-methyl-3-nitropyridin-2-amine](image)

After suspension of 2,6-dichloro-3-methyl-5-nitropyridine (11.85 mmol, 2.453 g) in 2-propanol (100 mL), aqueous ammonia was added in excess (10 mL, 25%). The reaction mixture was then warmed to 35 °C and stirred for 5 days. Filtration yielded the crude product, which was purified using silica gel and a mixture of ethyl acetate/n-hexane in a gradient ranging from 40-60% to obtain the product.

Yield: pale yellow crystals (50.38 %).

Melting point: 196-197 °C (solid from n-hexane/ethyl acetate).

\(^1\)H NMR: (400 MHz, DMSO-d\(_6\)): \(\delta = 8.38\) (d, \(^4J = 0.4\) Hz, 1H, C(4) H), 8.03 (s, 2H, NH\(_2\)), 2.23 (d, \(^4J = 0.4\) Hz, 3H, CH\(_3\)).

\(^{13}\)C NMR: (100 MHz, DMSO-d\(_6\)): \(\delta = 155.4\) (C6), 151.8 (C2), 137.6 (C4), 125.9 (C3), 119.5 (C5), 17.6 (CH\(_3\)).

MIR: \(\tilde{\nu} = 3460\) (w, \(\nu_{\text{C-N-H}}\)), 3074 (w, \(\nu_{\text{C-H}}\)), 2852 (w, \(\delta_{\text{CH}_3}\)), 1630 (m, \(\nu_{\text{NH}_2, \text{amine}}\)), 1585 (w, \(\nu_{\text{aromatic ring}}\)), 1552 (m, \(\nu_{\text{NO}_2, \text{nitrite}}\)), 1500 (m, \(\nu_{\text{aromatic ring}}\)), 768 (m, \(\nu_{\text{C-Cl}}\)).
6-(4-Fluorobenzylamino)-3-methyl-5-nitropyridine-2-amine (precursor 4)

![Chemical structure of the compound](image)

Triethylamine (4.5 mmol, 628 µL), 4-fluorobenzyl amine (2.7 mmol, 309 µL) and 6-chloro-5-methyl-3-nitropyridin-2-amine (1.5 mmol, 282 mg) were suspended in dimethyl sulfoxide (10 mL) and heated in a microwave reactor to 100 °C for 2.5 hours. The reaction mixture was then poured into water (150 mL) and extracted with ethyl acetate (2 x 50 mL). Evaporation yielded the product in sufficient purity for further modification.

Yield: yellow solid crystals (93%).

Melting point: 176-177 °C (solid from ethyl acetate).

\[
\begin{align*}
\text{\textsuperscript{1}H NMR:} & \quad (400 \text{ MHz, DMSO-d}_6): \delta = 7.90 (t, J = 5.98 \text{ Hz}, 1\text{H, N\textsuperscript{6} H}), 7.83 (d, J = 0.8 \text{ Hz}, 1\text{H, C (4) H}), 7.44 (m, 2\text{H, C (2') + C (6') H}_2), 7.15 (m, 2\text{H, C (3') + C (5') H}_2), 4.62 (d, J = 6.04 \text{ Hz}, 2\text{H, C (a') H}_2), 2.03 (s, 3\text{H, CH}_3). \\
\text{\textsuperscript{13}C NMR:} & \quad (100 \text{ MHz, DMSO-d}_6): \delta = 162.4 (d, J_{C,F} = 242 \text{ Hz, C4'}), 159.4 (C6), 154.5 (C2), 135.8 (d, J_{C,F} = 3 \text{ Hz, C1'}), 133.0 (C4), 129.7 (d, J_{C,F} = 8 \text{ Hz, C (2') + C (6')}), 117.3 (C3), 115.0 (d, J_{C,F} = 21 \text{ Hz, C (3') + C (5')}), 109.9 (C5), 43.3 (C\alpha'), 15.8 (CH\textsubscript{3}). \\
\text{MIR:} & \quad \tilde{\nu} = 3493 (w, \nu_{\text{N-H, amine}}), 3350 (m, \nu_{\text{C-N-H}}), 3044 (w, \nu_{\text{C-H}}), 2934 (w, \delta_{\text{CH}_2}), 1580 (s, \nu_{\text{aromatic ring}}), 1437 (w, \delta_{\text{CH}_3}), 1246 (s, \nu_{\text{NO}_2, \text{nitrite}}), 1230 (s, \nu_{\text{C-F}}).
\end{align*}
\]
**Ethyl[2-amino-6-[(4-fluorobenzyl)amino]-5-methylpyridin-3-yl]carbamate (AS-77)**

![Chemical structure of AS-77](image)

6-(4-Fluorobenzyl)-3-methyl-5-nitropyridine-2-amine (2.5 mmol, 690 mg) and Pd/C (250 mg, 10% Pd) were suspended in 2-propanol (15 mL), carefully set under hydrogen atmosphere and stirred for 24 hours at 40 °C. The reaction mixture was cooled using ice-cool water before adding triethylamine (3.75 mmol, 523 µL) and then ethyl chloroformate (3.125 mmol, 297 µL). The mixture was stirred at room temperature until the reaction was complete, as indicated by thin-layer chromatography. Water was added to precipitate the crude product, which was further purified by three iterations of re-crystallization from ethanol.

**Yield:** pale yellow crystals (38%).

**Mp:** 156-157 °C (solid from ethanol).

**1H NMR:** (400 MHz, DMSO-d$_6$): δ = 8.17 (s, 1H, N$_3$ H), 7.38 (m, 2H, C (3’) + C (5’) H$_2$), 7.11 (m, 2H, C (2’) + C (6’) H$_2$), 6.92 (s, 1H, C (4) H), 6.08 (t, $^3$J = 6.12 Hz, 1H, N$_6$ H), 4.93 (s, 2H, N$_2$ H$_2$), 4.49 (d, $^3$J = 6.04 Hz, 2H, C($\alpha’$) H$_2$), 4.06 (q, $^3$J = 7.06 Hz, 2H, OCH$_2$), 1.93 (s, 3H, CH$_3$), 1.21 (t, $^3$J = 6.34 Hz, 3H, OCH$_2$CH$_3$).

**13C NMR:** (100 MHz, DMSO-d$_6$): δ = 162.0 (d, $^1$J$_{C,F}$ = 241 Hz, C4’), 155.1 (C=O), 153.0 (C6), 150.8 (C2), 138.0 (d, $^4$J$_{C,F}$ = 3 Hz, C1’), 136.1 (C4), 129.2 (d, $^3$J$_{C,F}$ = 8 Hz, C (2’) + C (6’), 114.6 (d, $^2$J$_{C,F}$ = 21 Hz, C (3’) + C (5’), 106.1 (C3), 103.6 (C5), 59.9 (OCH$_2$), 43.2 (C$\alpha$’), 15.8 (CH$_3$), 14.6 (OCH$_2$CH$_3$).

**MIR:** $\tilde{\nu}$ = 3355 (w, $\nu_{N-H,\text{amine}}$), 3046 (w, $\nu_{C=C-H}$), 2865 (w, $\delta_{CH3}$), 1569 (m, $\nu_{NH2,\text{amine}}$), 1687 (m, $\nu_{CO2}$), 1497 (s, $\nu_{\text{aromatic ring}}$), 1218 (s, $\nu_C$-F).

**HRMS:** (ESI, m/z [M-H$^-$]) calculated: 319.1570212; found: 319.1570.
Method

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PDA End Time: 7.50 min
PDA Time Constant: 0.8400 sec

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Pump C: LC-20ADXR
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B Conc.: 5.0 %
B Curve:
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PressMin: 0 bar
Pump C PressMax: 400 bar
Pump C PressMin: 0 bar
Solenoid Valve B Name: LPGE Unit
Solenoid Valve B: C
Compressibility Setting: Off

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Enable Autosampler: Use
Rinse Type: External only
Sample Rack: Rack 1.5 mL 105 vials
Rinsing Volume: 500.0 ul
Needle Stroke: 52 mm
Control Vial Needle Stroke: 52 mm
Rinsing Speed: 35 ul/sec
Sampling Speed: 5.0 ul/sec
Sample Discharge Speed: 1.0 ul/sec
Measuring Line Purge Time: 10.0 min
Rinse Port R0 Purge Time: 10.0 min
Rinse Mode: Before and after aspiration
Rinse Dip Time: 0 sec
Cooler Temperature: 15 C
Measuring Line Purge Volume: 100 ul
Air Gap Volume: 0.1 ul
Loop Injection Type: Partial Loop
Rinse Method: Rinse port only
Rinse Time: 2 sec

<<Oven>>
Oven Model: CTO-20AC
Enable Oven: Use
Oven Temperature: 40 C
Maximum Temperature: 90 C
Ready Check: On

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BPR Temperature: 50 C

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SFC: SFC-30A
BPR Pressure: 400.0 bar
BPR Temperature: 50 C

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Cell Temp.: 40 C
Slit Width: 1.2 nm
Reference Correction: Not Used

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Initial Valve Position: -

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Event Time: 0.10 sec
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Threshold: 0
Start m/z: 100.00
End m/z: 500.00
Scan Speed: 5000 u/sec
Interface Volt.: Use the Data in the Tuning File
Qarray DC Voltage: Use the Data in the Tuning File

--Segment 1 Event 2--
Start Time: 0.00 min
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Acquisition Mode: SIM
Polarity: Positive
Event Time: 0.02 sec
Micro Scan Width: 0.00 u
Detector Voltage: +1.20 kV
CH1 (m/z) 305.00 (DL Vol.) ---- (Qarray DC) ---- (Qarray RF) ----
CH2 (m/z) 275.00 (DL Vol.) ---- (Qarray DC) ---- (Qarray RF) ----
CH3 (m/z) 319.00 (DL Vol.) ---- (Qarray DC) ---- (Qarray RF) ----
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Qarray DC Voltage: Use the Data in the Tuning File

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Detector Voltage: +1.30 kV
CH1 (m/z) 196.00 (DL Vol.) ---- (Qarray DC) ---- (Qarray RF) ----
CH2 (m/z) 214.00 (DL Vol.) ---- (Qarray DC) ---- (Qarray RF) ----
CH3 (m/z) 129.00 (DL Vol.) ---- (Qarray DC) ---- (Qarray RF) ----
Interface Volt.: Use the Data in the Tuning File
DL Volt: Use the Data in the Tuning File
Qarray DC Voltage: Use the Data in the Tuning File

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DL Temperature: 250 C
Nebulizing Gas Flow: 1.50 L/min
Heat Block: 200 C
Drying Gas: On
5.00 L/min
Analysis Report

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Date Acquired : 22.08.2018 10:22:07
Acquired by : System Administrator

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Acquired by : System Administrator

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MS Chromatogram

(x1,000,000)

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MS Chromatogram

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Analysis Report

Sample Name: Rekons1_LUX_H2O
Method Filename: Reconstitution.icm
Batch Filename: Reconstitution.icb
Injection Volume: 5 uL
Date Acquired: 18.05.2018 11:05:25

MS Chromatogram

(x100,000)

Method

Total Flow: 4.0000 mL/min
Pump C Flow: 0.1000 mL/min
B Conc.: 5.0 %
B Curve: 0

LC Time Program

Time Module Command Value Comment
6.00 Pumps Pump B Conc. 25
6.50 Pumps Pump B Conc. 25
7.00 Pumps Pump B Conc. 5
7.50 Controller Stop

Oven

Temperature: 40 C

Interface

DL Temperature: 250 C
Nebulizing Gas Flow: 1.50 L/min
Heat Block: 200 C
Drying Gas: On

5.00 L/min

C:\LabSolutions\Data\Flupirtine\Reports\Reconstitution\Rekons1_LUX_H2O_007.lcd
<table>
<thead>
<tr>
<th>Compound</th>
<th>m/z</th>
<th>Rt</th>
<th>Tailing factor</th>
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<th>Noise</th>
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<td>1.26</td>
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<td>1.23</td>
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Analysis Report

Sample Name: Rekons1_LUX_ACN
Method Filename: Reconstitution.icm
Batch Filename: Reconstitution.lcb
Injection Volume: 5 uL
Date Acquired: 18.05.2018 10:48:31

MS Chromatogram

305.00 (+)@2/275.00 (+)@2/319.00 (+)@2/196.00 (-)@4/214.00 (-)@4

(x100,000)

Method

<<Pump>>
Total Flow: 4.0000 mL/min
Pump C Flow: 0.1000 mL/min
B Conc.: 5.0 %
B Curve: 0

<<LC Time Program>>

<table>
<thead>
<tr>
<th>Time</th>
<th>Module</th>
<th>Command</th>
<th>Value</th>
<th>Comment</th>
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<td>Stop</td>
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<<Oven>>

Oven Temperature: 40 C

<<SFC A>>

BPR Pressure: 150.0 bar

<<SFC B>>

BPR Pressure: 400.0 bar

<<Interface>>

Interface: ESI
DL Temperature: 250 C
Nebulizing Gas Flow: 1.50 L/min
Heat Block: 200 C
Drying Gas: On
Drying Gas Flow: 5.00 L/min
<table>
<thead>
<tr>
<th>Compound</th>
<th>m/z</th>
<th>Rt</th>
<th>Tailing factor</th>
<th>S/N</th>
<th>Noise</th>
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<tbody>
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Analysis Report

Sample Name: Rekons1_LUX_IPA
Method Filename: Reconstitution.icm
Batch Filename: Reconstitution.icb
Injection Volume: 5 µL
Date Acquired: 18.05.2018 10:14:38

MS Chromatogram

(x100,000)

Method

<<Pump>>
Total Flow: 4.0000 mL/min
Pump C Flow: 0.1000 mL/min
B Conc.: 5.0 %
B Curve: 0

<<LC Time Program>>

<table>
<thead>
<tr>
<th>Time</th>
<th>Module</th>
<th>Command</th>
<th>Value</th>
<th>Comment</th>
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<td>Stop</td>
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<<Oven>>

Oven Temperature: 40 C

<<SFC A>>
BPR Pressure: 150.0 bar

<<SFC B>>
BPR Pressure: 400.0 bar

<<Interface>>

Interface: ESI
DL Temperature: 250 C
Nebulizing Gas Flow: 1.50 L/min
Heat Block: 200 C
Drying Gas: On

5.00 L/min
### Compound Analysis

<table>
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<th>Compound</th>
<th>m/z</th>
<th>Rt</th>
<th>Tailing factor</th>
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<th>Noise</th>
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Analysis Report

Sample Name: Rekons1_LUX_MeOH
Method Filename: Reconstitution.icm
Batch Filename: Reconstitution.icb
Injection Volume: 5 uL
Date Acquired: 18.05.2018 10:31:35

MS Chromatogram

Chromatogram:
- 305.00 (+)@2
- 275.00 (+)@2
- 319.00 (+)@2
- 196.00 (-)@4
- 214.00 (-)@4

Method:
- Pump:
  - Total Flow: 4.0000 mL/min
  - Pump C Flow: 0.1000 mL/min
  - B Conc.: 5.0%
  - B Curve: 0

-LC Time Program:
<table>
<thead>
<tr>
<th>Time</th>
<th>Module</th>
<th>Command</th>
<th>Value</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
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<td>6.00</td>
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<td>7.50</td>
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</table>

-Oven:
- Temperature: 40 C

-SFC A:
- BPR Pressure: 150.0 bar

-SFC B:
- BPR Pressure: 400.0 bar

<<Interface>>
- Interface: ESI
- DL Temperature: 250 C
- Nebulizing Gas Flow: 1.50 L/min
- Heat Block: 200 C
- Drying Gas: On
- 5.00 L/min

C:\LabSolutions\Data\Flupirtine\Reports\Reconstitution\Rekons1_LUX_MeOH_003.lcd
<table>
<thead>
<tr>
<th>Compound</th>
<th>m/z</th>
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