Analysis of Mitragynine in Blood Samples using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)
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INTRODUCTION

A method using solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry was performed to detect and quantify for mitragynine in 15 human serum samples. These samples were submitted to USAINS Biomics Laboratory Testing Services Sdn Bhd (BIOMICS) by the Centre for Drug Research, Universiti Sains Malaysia (USM). The sample extraction procedure was modified from a published and validated method by Lee, M.J. (2016) and Lee et al. (2018). Mitragynine was identified with multiple reaction monitoring (MRM) in the positive electrospray ionization (ESI) mode using nalorphine as the internal standard (IS).

A batch of 9 human serum samples were submitted for analysis and quantification of mitragynine. These samples were subjected to solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis modified from a method validated by Lee, M.J. (2018).

| No. | Laboratory Code | Sample ID   |
|-----|----------------|-------------|
| 1   | 107553         | 10039288    |
| 2   | 107554         | 10039286    |
| 3   | 107559         | 10039287    |
| 4   | 107561         | 20038840    |
| 5   | 107563         | 10040642    |
| 6   | 107564         | 10040935    |
| 7   | 107565         | 10040906    |
| 8   | 107566         | 10040890    |
| 9   | 107567         | 10040891    |

Table 1: Sample identification and coding for the 9 human serum samples received for mitragynine analysis.
MATERIALS AND METHODS

Chemicals and Standards

| Chemicals                                | Company / Source                                      |
|------------------------------------------|-------------------------------------------------------|
| Mitragynine                              | Chemtron Biotechnology Sdn Bhd, Malaysia.             |
| Nalorphine                               | Cerilliant Corporation, Round Rock, Texas            |
| Methanol (HPLC grade)                    | Fischer Scientific, UK.                              |
| Acetonitrile (ACN) HPLC grade            | Merck KGaA, Darmstadt, Germany                        |
| Ammonium formate                         | Fluka, Switzerland                                    |
| Formic acid, OPTIMA LC/MS                | Fischer Scientific, Fair Lawn, NJ07410               |
| Ammonium hydroxide                       | J.T. Baker®, USA                                      |
| Hydrochloric acid                        | Fischer Chemicals, USA                                |
| Formic acid                              | Fluka Analytical, Germany, Sigma-Aldrich Corporation  |
| Puriss p.a. eluent additive for LC-MS ~ 98% (T) |                                                       |
| Methanol (HPLC grade)                    | Fisher Scientific UK                                  |

Materials

| Materials Description                  | Company / Source                                      |
|----------------------------------------|-------------------------------------------------------|
| Bond-Elut Certify SPE (100 mg, 1 mL)   | Agilent Technologies Inc., USA                        |
| SB-C18 Guard Cartridge Dimension: 4.6 mm x 12.5 mm, 5 µm | Agilent Technologies Inc., USA |
| ZORBAX Eclipse XDB-C18 HPLC Column Dimension: 4.6 mm x 150 mm, 5 µm | Agilent Technologies Inc., USA |

Instrumentation

| Instrument Description                  | Company / Source                                      |
|-----------------------------------------|-------------------------------------------------------|
| Agilent Technologies 1290 Infinity II High-Performance Liquid Chromatograph (HPLC) with quaternary pump | Agilent Technologies Inc., USA |
| Liquid Chromatography-Mass Spectrometry (LC-MS) System Model: 6470 Triple Quad LC/MS with 1290 Infinity II HPLC using Agilent Infinity II 1290 quaternary pump | Agilent Technologies Inc., USA |
**Instrument Parameters**

The following table showed the instrument parameters used for the Agilent HPLC and LC-MS System for the analysis of mitragynine.

| Instrument Parameter       | Parameter Description                  |
|---------------------------|---------------------------------------|
| Sample injection volume:  | 5 µL                                  |
| Column compartment temperature: | 35°C                                |
| Mobile phases:             | **A**: 0.1% formic acid in 10 mM ammonium formate  |
|                           | **B**: 0.1% formic acid in acetonitrile |
| Solvent gradient programme: |                                      |
|                           | **Time** (minute) | **Mobile Phase** % A | % B | **Flow rate** (mL/min) |
|                           | 0              | 90              | 10  | 1.0                     |
|                           | 9              | 0               | 100 | 1.0                     |
|                           | 12             | 0               | 100 | 1.0                     |
|                           | 12.2           | 90              | 10  | 1.5                     |
|                           | 20             | 90              | 10  | 1.5                     |
| Post-run time:            | 2 minutes     |                  |     |                         |
| MS/MS conditions:         |                         |                  |     |                         |
|                           | Gas temperature: 300°C           |                  |     |                         |
|                           | Gas flow: 5 L/min                |                  |     |                         |
|                           | Nebulizer pressure: 45 psi        |                  |     |                         |
|                           | Sheath gas temperature: 250°C     |                  |     |                         |
|                           | Sheath gas flow: 11 L/min         |                  |     |                         |
|                           | Capillary voltage: 3500 V         |                  |     |                         |
|                           | Nozzle voltage: 500 V             |                  |     |                         |
|                           | MS1 temperature: 100°C            |                  |     |                         |
|                           | MS2 temperature: 100°C            |                  |     |                         |

Table 2: The Agilent HPLC and 6470 Triple Quad LC-MS instrument parameters for the analysis of mitragynine in human serum sample.
### Analyte MS/MS Fragmentation Parameters

| Analyte  | Precursor ion | Product ion | Collision energy (CE) | Fragmentor voltage (V) |
|----------|---------------|-------------|-----------------------|------------------------|
| Mitragynine | 399.2         | 174.1       | 21                    | 176                    |
|          | 238.1         | 50          | 29                    | 176                    |
|          | 226.1         | 29          | 176                    |
|          | 159.1         | 50          | 176                    |
| Nalorphine | 312.2         | 152.1       | 74                    | 62                     |
|          | 77.1          | 110         | 62                     |

The following figure is a proposed fragmentation and structural rearrangements pathway of mitragynine.

![Proposed fragmentation and structural rearrangements pathway of mitragynine (Avula et al., 2015).](image)

Figure 1: Proposed fragmentation and structural rearrangements pathway of mitragynine (Avula et al., 2015).
Mobile Phase A: 0.1% Formic Acid in 10 mM Ammonium Formate

Weigh 0.63 g ammonium formate into a 1 L volumetric flask and dissolve in approximately 500 mL of deionised water. Then, add 1 mL of formic acid into the solution and make up volume to 1 L with deionised water.

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Dispense 1 mL of formic acid into a 1 L volumetric flask and make up volume to 1 L with acetonitrile.

Sample Preparation and Solid-Phase Extraction

In this experiment, nalorphine was used as the internal standard to correct for extraction efficiency and helped to improve the method accuracy and precision. The mitragynine 1 mg/mL stock solution was prepared in duplicates (stock solution A and stock solution B). One stock was used for calibration standards preparation and another stock used for quality control purposes.

The 1 mg/mL mitragynine stock solution A was diluted to 1 µg/mL, 10 µg/mL, and 100 µg/mL working solutions with methanol to prepare the series of calibrators.

| Calibrator | Calibrator Concentration (ng/mL) | Concentration of Working Solution A (µg/mL) | Volume of Working Solution Required (µL) | Volume of Blank Serum Sample (µL) |
|------------|----------------------------------|---------------------------------------------|-----------------------------------------|----------------------------------|
| C0         | 0                                | -                                           | -                                       | 1,000                            |
| C1         | 10                               | 1                                           | 10                                      | 990                              |
| C2         | 100                              | 10                                          | 10                                      | 990                              |
| C3         | 250                              | 10                                          | 25                                      | 975                              |
| C4         | 500                              | 100                                         | 5                                       | 995                              |
| C5         | 750                              | 100                                         | 7.5                                     | 993                              |
| C6         | 1,000                            | 100                                         | 10                                      | 990                              |

Table 3: Table summarizing the preparation of the series of calibrators in blank serum matrix.

The 1 mg/mL mitragynine stock solution B was diluted to 10 µg/mL and 100 µg/mL working solutions with methanol to be used for spiking into blank serum sample.

| QC Sample | Calibrator Concentration (ng/mL) | Concentration of Working Solution B (µg/mL) | Volume of Working Solution Required (µL) | Volume of Blank Serum Sample (µL) |
|-----------|----------------------------------|---------------------------------------------|-----------------------------------------|----------------------------------|
| QC-L      | 55                               | 10                                          | 5.5                                     | 995                              |
| QC-M      | 375                              | 10                                          | 37.5                                    | 963                              |
| QC-H      | 875                              | 100                                         | 8.75                                    | 992                              |

Table 4: Table summarizing the preparation of the 3 levels of QC in blank serum matrix.
All the coded serum samples were mixed well and centrifuged at 3,000 rpm at room temperature for 5 minutes. The same was applied to the series of calibrators and QC samples.

A 100 µL of each calibrator and QC sample was dispensed into the respective microcentrifuge tube and added with 2.5 µL of 10 µg/mL nalorphine (internal standard). They were mixed thoroughly ready to be loaded onto the solid-phase extraction (SPE) cartridges.

A 100 µL of the centrifuged serum samples were aliquoted into the respective microcentrifuge tubes and diluted with 900 µL of blank serum. The diluted serum samples were mixed thoroughly and 100 µL of each diluted sample was transferred to a new microcentrifuge. Each sample was then added with 2.5 µL of 10 µg/mL nalorphine and vortexed to mix well ready to be loaded onto the SPE cartridges.

The Bond-Elut Certify II Solid-Phase Extraction cartridges were conditioned sequentially with 1 mL of methanol and distilled water. Then, 100 µL of the respective calibrators, QC samples, and subject test samples were loaded onto the assigned SPE cartridges. The SPE cartridges were then washed with 1 mL of distilled water followed by 10 mM hydrochloric acid, and finally methanol. The cartridges were then vacuum-dried before eluting with freshly prepared 2% (v/v) ammonium hydroxide in methanol (elution solvent). The eluent was dried at 60°C under a gentle stream of nitrogen. The dried sample was reconstituted with 100 µL of the starting mobile phase (mobile phase A : mobile phase B, 90:10, v/v) and filtered prior to analysis with the Agilent Technologies 6470 Triple Quad LC/MS System coupled with the Agilent Technologies Infinity II 1290 High Performance Liquid Chromatography System (LC-MS/MS).
RESULTS

The following table summarized the concentration (µg/mL) of mitragynine detected in the 9 human plasma samples using the extraction method mentioned in the previous section. Each sample was subjected to a 100 times dilution. Therefore, the final concentrations reported in table 5 were after taking the dilution factor into consideration.

| No. | Laboratory Code | Sample ID | Mitragynine Concentration in serum (µg/mL) |
|-----|-----------------|-----------|------------------------------------------|
| 1   | 107553          | 10039288  | 11.3                                     |
| 2   | 107554          | 10039286  | 9.6                                      |
| 3   | 107559          | 10039287  | 20.4                                     |
| 4   | 107561          | 20038840  | 22.4                                     |
| 5   | 107563          | 10040642  | 3.6                                      |
| 6   | 107564          | 10040935  | 8.5                                      |
| 7   | 107565          | 10040906  | 6.8                                      |
| 8   | 107566          | 10040890  | 2.5                                      |
| 9   | 107567          | 10040891  | 8.0                                      |

Table 5: Summary of analysis for the detection of mitragynine in 9 human serum samples. *ND – Not detected.

A typical MRM chromatogram for mitragynine reference standard was depicted in figure 2.

A typical MRM chromatogram for mitragynine in human serum sample was shown in figure 3. Mitragynine isomers (i.e. speciogynine, mitracilliatine, speciocilliatine, paynantheine, and isopaynantheine) might contribute to the chromatographic peaks after the mitragynine chromatographic peak. However, the data was non conclusive since the reference standards for the isomers were not available.
Figure 2: Typical MRM chromatogram of mitragynine reference standard using the ZORBAX Eclipse XDB-C18 HPLC column (4.6 mm x 150 mm; 5 μm).
Figure 3: Typical MRM chromatogram of mitragynine in human serum sample using the ZORBAX Eclipse XDB-C18 HPLC column (4.6 mm x 150 mm; 5 μm).
The calibration curve for calculating the 15 human serum samples were as shown in figure 4 below.

![Graph of the calibration curve](image)

*Figure 4: Typical MRM chromatogram of mitragynine reference standard using the ZORBAX Eclipse XDB-C18 HPLC column (4.6 mm x 150 mm; 5 μm).*

The raw data for the calibration curve, QC levels, and 15 human serum samples were as shown in the table below.

| Calibrator | Concentration (ng/mL) | AUC | Ratio | Average Ratio |
|------------|-----------------------|-----|-------|---------------|
|            |                       | Mitragynine | Nalorphine (IS) |               |
| C0         | 0                     | 0   | 599,030 | 0.00          | 0.00          |
| C1         | 10                    | 58,356 | 590,624 | 0.10          | 0.08          |
| C2         | 100                   | 448,965 | 639,946 | 0.70          | 0.64          |
| C3         | 250                   | 1,395,946 | 724,915 | 1.93          | 1.91          |
| C4         | 500                   | 2,223,959 | 735,630 | 3.02          | 3.09          |
| C5         | 750                   | 2,484,201 | 495,904 | 5.01          | 5.94          |
| C6         | 1,000                 | 6,200,765 | 706,166 | 8.78          | 7.92          |

|        |                       | 4,496,430 | 636,970 | 7.06          |

Table 6: Chromatographic area under the curve (AUC) data for the mitragynine calibration curve shown in figure 4.
### Table 6: Chromatographic area under the curve (AUC) data for the mitragynine calibration curve shown in figure 4.

| Sample ID | Conc. (ng/mL) | AUC | Ratio | Calculated Conc. (ng/mL) | Final Conc. (ng/mL) | % Recovery |
|-----------|---------------|-----|-------|--------------------------|---------------------|------------|
| QC-L      | 0             | 199,606 | 650,844 | 0.31                     | 55.25               | 56.90      | 103.5    |
|           |               | 173,033 | 520,096 | 0.33                     | 58.54               |            |          |
| QC-M      | 10            | 1,483,402 | 575,035 | 2.58                     | 342.97              | 330.26     | 88.1     |
|           |               | 1,557,071 | 654,538 | 2.38                     | 317.56              |            |          |
| QC-H      | 100           | 3,387,103 | 604,964 | 5.60                     | 725.15              | 778.85     | 89.0     |
|           |               | 3,452,392 | 535,468 | 6.45                     | 832.56              |            |          |

### Table 7: Chromatographic area under the curve (AUC) data for mitragynine in the 15 human serum samples.

| Sample ID | AUC | Ratio | Calculated Conc. (ng/mL) | Final Conc. (ng/mL) |
|-----------|-----|-------|--------------------------|---------------------|
| 107553    | 462,534 | 606,406 | 0.76                     | 112.98              | 11,298.06 |
| 107554    | 397,323 | 629,384 | 0.63                     | 96.34               | 9,634.03  |
| 107555    | -      | 776,545 | -                        | -                   | -         |
| 107556    | 261,734 | 561,029 | 0.47                     | 75.48               | 7,548.42  |
| 107557    | 279,886 | 522,271 | 0.54                     | 84.27               | 8,426.61  |
| 107558    | 466,885 | 610,617 | 0.76                     | 113.22              | 11,321.67 |
| 107559    | 768,454 | 517,878 | 1.48                     | 204.26              | 20,425.97 |
| 107560    | 1,884,687 | 538,746 | 3.50                     | 459.25              | 45,925.13 |
| 107561    | 908,487 | 552,172 | 1.65                     | 224.70              | 22,469.58 |
| 107562    | 640,183 | 575,793 | 1.11                     | 157.17              | 15,716.81 |
| 107563    | 82,000  | 522,649 | 0.16                     | 36.29               | 3,629.03  |
| 107564    | 358,446 | 666,446 | 0.54                     | 84.51               | 8,451.23  |
| 107565    | 242,210 | 596,805 | 0.41                     | 67.80               | 6,780.31  |
| 107566    | 39,247  | 575,859 | 0.07                     | 25.06               | 2,505.74  |
| 107567    | 372,319 | 736,799 | 0.51                     | 80.39               | 8,039.49  |
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APPENDIX

Appendix 1: MRM Mitragynine Reference Standard

### Qualitative Analysis Report

| Data File      | PS1.d                        |
|----------------|------------------------------|
| Sample Type    | Calibration                  |
| Instrument Name| Instrument 1                 |
| Acq Method     | Mitragynine_LRU_Flex_2.m     |
| IRM Calibration| Not Applicable               |
| Comment        |                              |
| Sample Group   | LC 1                         |
| Stream Name    |                              |
| Acquisition SW | 6400 Series Triple Quadrupole B.08.02 (B8260.0) |

### Chromatograms

| Fragmentor Voltage | 17   |
|--------------------|------|
| Collision Energy   | 5    |
| Ionization Mode    | 1    |

**Mitragynine: +ESI MRM Frag=176.0V CID@50.0 (399.2 -> 159.1) PS1.d**

### Integration Peak List

| Peak | Start | RT  | Height | Area  | Area % |
|------|-------|-----|--------|-------|--------|
| 1    | 6.77  | 6.89| 7.634  | 83621 | 100    |

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Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End  | Height | Area  | Area % |
|------|-------|-----|------|--------|-------|--------|
| 1    | 6.771 | 6.89| 7.690| 70571  | 345910| 100    |

Fragmentor | 6 | Collision | 11 | Ionization | ES
Voltage     | 2 | Energy    | 0  | Mode       | 1

Integration Peak List

| Peak | Start | RT  | End  | Height | Area  | Area % |
|------|-------|-----|------|--------|-------|--------|
| 1    | 3.5   | 3.619| 4.172| 11514  | 46983 | 100    |
| 2    | 7.28  | 7.423| 7.621| 25     | 136   | 0.29   |
| 3    | 10.577| 10.697| 10.864| 110    | 557   | 1.18   |

Fragmentor | 6 | Collision | 7 | Ionization | ES
Voltage     | 2 | Energy    | 4 | Mode       | 1
Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT    | End | Height | Area   | Area % |
|------|-------|-------|-----|--------|--------|--------|
| 1    | 3.5   | 3.619 | 4.156 | 16599  | 67499  | 100    |
| 2    | 10.733| 10.877| 10.975| 55     | 296    | 0.44   |

--- End Of Report ---
Appendix 2: MRM Sample 107553

Qualitative Analysis Report

Data File: Sample 107553b.d
Sample Type: Sample
Instrument Name: Instrument 1
Acq Method: Mitragynine_LM_Flex_2.m
IRM Calibration Status: Not Applicable
Comment: Sample 107553b
User Name: P2:C2
Acquired Time: 31/10/2020 3:56:17 AM (UTC+08:00)
DA Method: APCI Chemstation.m
Info.
Acquisition Time (Local): 31/10/2020 3:56:17 AM (UTC+08:00)

Chromatograms

| Fragmentor Voltage | Collision Energy | Ionization Mode | ES |
|--------------------|------------------|-----------------|----|
| 1                  | 7.044            | 7.163           | 7.260 |
| 2                  | 7.358            | 7.451           | 7.984 |

Mitragynine: +ESI MRM Frag=176.0V CID@50.0 (399.2 -> 159.1) Sample 107553b.d

Integration Peak List

| Peak | Start | RT | End | Height | Area | Area % |
|------|-------|----|-----|--------|------|--------|
| 1    | 7.044 | 7.163 | 7.260 | 37977  | 132098 | 100    |
| 2    | 7.358 | 7.451 | 7.984 | 12999  | 87693  | 45.65  |

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Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End  | Height | Area | Area % |
|------|-------|-----|------|--------|------|--------|
| 1    | 4.794 | 4.913| 5.075| 34     | 129  | 0.08   |
| 2    | 5.26  | 5.403| 5.679| 186    | 1381 | 0.9    |
| 3    | 7.072 | 7.167| 7.268| 30841  | 153967| 100    |
| 4    | 7.266 | 7.296| 7.328| 3370   | 0    | 0      |
| 5    | 7.274 | 7.302| 7.354| 3139   | 11678| 7.58   |
| 6    | 7.355 | 7.45 | 8.015| 9445   | 62890| 40.84  |

Fragmentor Voltage 17
Collision Energy 2
Ionization Mode 9
ES 1

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## Qualitative Analysis Report

### Integration Peak List

| Peak | Start | RT  | End  | Height | Area  | Area % |
|------|-------|-----|------|--------|-------|--------|
| 1    | 7.066 | 7.167 | 7.266 | 30347  | 155950 | 100    |
| 2    | 7.311 | 7.311 | 7.358 | 3777   | 8029  | 5.15   |
| 3    | 7.359 | 7.449 | 8.047 | 10159  | 68718 | 44.06  |

### Fragmentation
- **Emission**: 6
- **Collision**: 2
- **Energy**: 11
- **Ionization**: 1
- **Mode**: 1

### Nalorphine

**ESI MRM Frag**: 52.0V CID@110.0 (312.2 → 77.1) Sample 107553b.d

### Integration Peak List

| Peak | Start | RT  | End  | Height | Area  | Area % |
|------|-------|-----|------|--------|-------|--------|
| 1    | 3.751 | 3.871 | 4.585 | 92023  | 426995 | 100    |

### Fragmentation
- **Emission**: 6
- **Collision**: 7
- **Energy**: 4
- **Mode**: 1

---

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Qualitative Analysis Report

![Graph showing counts vs. acquisition time with peak at 3.871 at 4.5 min]

**Integration Peak List**

| Peak | Start | RT  | End  | Height | Area | Area % |
|------|-------|-----|------|--------|------|--------|
| 1    | 3.752 | 3.871| 4.617| 130682 | 605466 | 100    |

--- End Of Report ---
Appendix 3: MRM Sample 107554

Qualitative Analysis Report

| Data File          | Sample 107554d.d                      | Sample Name       | Sample 107554b                      |
|--------------------|--------------------------------------|-------------------|------------------------------------|
| Sample Type        | Sample                               | Position          | P2-C4                              |
| Instrument Name    | Instrument 1                         | User Name         |                                    |
| Acq Method         | Mitragynine_U4_Flex_2.m              | Acquired Time     | 31/10/2020 4:40:34 AM (UTC+08:00)  |
| IRM Calibration    | Not Applicable                       | DA Method         | APCI Chemstation.m                 |
| Status             |                                      | Info.             | 31/10/2020 4:40:34 AM (UTC+08:00)  |
| Comment            |                                      | Acquisition Time  |                                    |
| Sample Group       |                                      | (Local)           |                                    |
| Stream Name        | LC 1                                 |                  |                                    |
| Acquisition SW     | 6400 Series Triple Quadrupole B.08.02 (B8260.0) |                  |                                    |

Chromatograms

Fragmentor 17 6 Collision Energy 0 Ionization Mode ES

Integration Peak List

| Peak | Start  | RT   | End   | Height | Area   | Area % |
|------|--------|------|-------|--------|--------|--------|
| 1    | 7.127  | 7.243| 7.338 | 31689  | 165513 | 100    |
| 2    | 7.426  | 7.532| 8.254 | 20853  | 157511 | 98.93  |

Fragmentor Voltage 6 Collision Energy 2 Ionization Mode ES
Qualitative Analysis Report

Integration Peak List

| Peak | Start   | RT   | End   | Height | Area   | Area % |
|------|---------|------|-------|--------|--------|--------|
| 1    | 7.048   | 7.048| 7.243 | 7.338  | 75425  | 397323 | 100    |
| 2    | 7.427   | 7.552| 8.572 | 51058  | 387023 | 97.41  |

Fragmentor Voltage: 17
Collision Energy: 2
Ionization Mode: I

Integration Peak List

| Peak | Start   | RT   | End   | Height | Area   | Area % |
|------|---------|------|-------|--------|--------|--------|
| 1    | 5.339   | 5.468| 5.742 | 5.742  | 200    | 1168   | 0.89   |
| 2    | 7.076   | 7.247| 7.413 | 7.413  | 24925  | 131035 | 100    |
| 3    | 7.413   | 7.413| 7.421 | 2758   | 1090   | 0.83   |
| 4    | 7.424   | 7.531| 8.143 | 15039  | 111645 | 85.2   |

Fragmentor Voltage: 17
Collision Energy: 2
Ionization Mode: I

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Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End   | Height | Area  | Area % |
|------|-------|-----|-------|--------|-------|--------|
| 1    | 7.119 | 7.247 | 7.342 | 25820 | 13333 | 100    |
| 2    | 7.429 | 7.469 | 7.469 | 2954   | 3159  | 3.23   |

Fragmentor Voltage 2
Collision Energy 0
Ionization Mode I
ES

Integration Peak List

| Peak | Start | RT  | End   | Height | Area  | Area % |
|------|-------|-----|-------|--------|-------|--------|
| 1    | 3.827 | 3.948 | 4.833 | 93333  | 448480| 100    |
| 2    | 10.161| 10.971| 10.992| 33     | 722   | 0.16   |

Fragmentor Voltage 2
Collision Energy 0
Ionization Mode I
ES
Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End | Height | Area | Area % |
|------|-------|-----|-----|--------|------|--------|
| 1    | 3.827 | 3.946 | 4.681 | 131164 | 629384 | 100    |

--- End Of Report ---
Appendix 4: MRM Sample 107559

Qualitative Analysis Report

| Data File     | Sample 107559b.d                     | Sample Name | Sample 107559b |
|---------------|--------------------------------------|-------------|----------------|
| Sample Type   | Sample                               | Position    | F2-D5          |
| Instrument Name | Instrument 1                        | User Name   |                |
| Acq Method    | Mitragynine_LMU_Rexi_2.m             | Acquired Time | 31/10/2020 8:22:00 AM (UTC+08:00) |
| IRM Calibration Status | Not Applicable            | DA Method   | APCI Chemstation.m |
| Comment       |                                      | Info.       |                |
| Stream Name   | LC 1                                 | Acquisition Time | 31/10/2020 8:22:00 AM (UTC+08:00) |
| Acquisition SW | 6400 Series Triple Quadrupole B.03.02 (B8260.0) | (Local)           |                |

Chromatograms

Fragmentor Voltage 17 Collision Energy 5 Ionization Mode 0 E5 1

Mitragynine: +ESI MRM Frag=175.0V CID@50.0 (399.2 -> 159.1) Sample 107559b.d

Counts vs. Acquisition Time (min)

Integration Peak List

| Peak | Start | RT  | End  | Height | Area | Area % |
|------|-------|-----|------|--------|------|--------|
| 1    | 6.313 | 6.893 | 6.992 | 63058 | 311960 | 95.76  |
| 2    | 7.086 | 7.184 | 7.484 | 481101 | 325721 | 100    |

Fragmentor Voltage 17 Collision Energy 5 Ionization Mode 0 E5 1
## Qualitative Analysis Report

### Integration Peak List

| Peak | Start  | RT     | End   | Height | Area  | Area % |
|------|--------|--------|-------|--------|-------|--------|
| 1    | 3.772  | 4.021  | 4.343 | 39     | 326   | 0.13   |
| 2    | 4.808  | 4.677  | 4.918 | 34     | 171   | 0.07   |
| 3    | 5.021  | 5.163  | 5.408 | 204    | 1239  | 0.48   |
| 4    | 6.774  | 6.897  | 6.996 | 53187  | 25763 | 100    |
| 5    | 7.081  | 7.184  | 7.872 | 37579  | 237844| 92.31  |

### Fragmentor

| Voltage | 6 |
|---------|---|
| Energy  | 1 |

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Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End  | Height | Area  | Area % |
|------|-------|-----|------|--------|-------|--------|
| 1    | 6.774 | 6.897| 6.992| 51559  | 250556| 100    |
| 2    | 7.088 | 7.184| 7.968| 39938  | 250375| 99.80  |

Fragmentor Voltage: 6
Collision Energy: 11
Ionization Mode: ES

Integration Peak List

| Peak | Start | RT  | End  | Height | Area  | Area % |
|------|-------|-----|------|--------|-------|--------|
| 1    | 3.501 | 3.521| 4.538| 83842  | 356729| 100    |

Fragmentor Voltage: 6
Collision Energy: 11
Ionization Mode: ES
Qualitative Analysis Report

Integratioon Peak List

| Peak | Start  | RT   | End  | Height | Area  | Area % |
|------|--------|------|------|--------|-------|--------|
| 1    | 3.502  | 3.621| 4.871| 118818 | 517878| 100    |

--- End Of Report ---
Appendix 5: MRM Sample 107561

Qualitative Analysis Report

Data File: Sample 107561b.d
Sample Type: Sample
Instrument Name: Instrument 1
Acq Method: Mitragynine_LMU_Field_2.m
IRM Calibration Status: Not Applicable
Comment: 
Sample Group: 
Stream Name: LC 1
Acquisition SW Version: 6400 Series Triple Quad: pole B.08.02 (B0260.0)

Sample Name: Sample 107561b
Position: P2-D9
User Name: 
Acquired Time: 31/10/2020 9:50:35 AM (UTC+08:00)
DA Method: APCI Chemstation.m
Info.
Acquisition Time (Local): 31/10/2020 9:50:35 AM (UTC+08:00)

Chromatograms

Fragmentor Voltage: 17
Collision Energy: 6
Ionization Mode: 5
ES: 0

Integration Peak List

| Peak | Start | End  | Height | Area  | Area % |
|------|-------|------|--------|-------|--------|
| 1    | 6.685 | 7.024| 7.115  | 74019 | 363512 | 100    |
| 2    | 7.215 | 7.313| 8.095  | 49831 | 334445 | 91.98  |

Fragmentor Voltage: 17
Collision Energy: 6
Ionization Mode: 5
ES: 0
Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT   | End  | Height | Area | Area % |
|------|-------|------|------|--------|------|--------|
| 1    | 5.325 | 5.368| 5.603| 190    | 1099 | 0.12   |
| 2    | 6.754 | 7.024| 7.123| 24253  | 98487| 1.00   |
| 3    | 7.210 | 7.313| 8.238| 212195 | 805803| 86.7  |
| 4    | 9.490 | 9.601| 9.891| 25     | 276  | 0.03   |

Fragmentor: 17, Collision: 2, Ionization: ES, Voltage: 6, Energy: 9, Mode: 1

Integration Peak List

| Peak | Start | RT   | End  | Height | Area   | Area % |
|------|-------|------|------|--------|--------|--------|
| 1    | 5.000 | 5.246| 5.599| 221    | 1892   | 0.63   |
| 2    | 6.905 | 7.024| 7.123| 81901  | 302595 | 100    |
| 3    | 7.213 | 7.312| 8.063| 37620  | 244159 | 80.69  |

Fragmentor: 17, Collision: 2, Ionization: ES, Voltage: 6, Energy: 1, Mode: 1

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Qualitative Analysis Report

Intetration Peak List

| Peak | Start | RT  | End  | Height | Area | Area % |
|------|-------|-----|------|--------|------|--------|
| 1    | 3.582 | 3.701 | 4.506 | 125409 | 552172 | 100 |

--- End Of Report ---
Appendix 6: MRM Sample 107563

Qualitative Analysis Report

Data File: Sample 107563a.d
Sample Type: Sample
Instrument Name: Instrument 1
Acq Method: Mitragynine_LIQ_Hexv_2.m
IBM Calibration Status: Not Applicable
Comment:
Sample Group:
Stream Name: LC 1
Acquisition SW Version: 6400 Series Triple Quadrupole B.03.02 (B0260.0)
Sample Name: Sample 107563a
Position: P2-E3
User Name:
Acquired Time: 31/10/2020 10:57:02 AM (UTC+08:00)
DA Method: APCI Chemstation.m
Info.
Acquisition Time (Local): 31/10/2020 10:57:02 AM (UTC+08:00)

Chromatograms

Fragmentor Voltage: 6
Collision Energy: 5
Ionization Mode: E5

Mitragynine: +ESI MRM Frag=179.0V CID@50.0 (399.2 -> 159.1) Sample 107563a.d

Integration Peak List

| Peak | Start | RT   | End  | Height | Area  | Area % |
|------|-------|------|------|--------|-------|--------|
| 1    | 6.944 | 7.06 | 7.165| 6685   | 32997 | 100    |
| 2    | 7.255 | 7.349| 7.682| 3475   | 25663 | 77.76  |

Fragmentor Voltage: 17
Collision Energy: 2
Ionization Mode: E5
Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End  | Height | Area  | Area % |
|------|-------|-----|------|--------|-------|--------|
| 1    | 6.94  | 7.056 | 7.151 | 17104  | 82000 | 100    |
| 2    | 7.256 | 7.349 | 7.867 | 9099   | 63422 | 77.34  |

Fragmentor 17  
Voltage 6  
Collision Energy 2  
Ionization ES 9  
Mode 1

Integration Peak List

| Peak | Start | RT | End | Height | Area | Area % |
|------|-------|----|-----|--------|------|--------|
| 1    | 3.827 | 4.045 | 4.359 | 35     | 393  | 1.47   |
| 2    | 5.18  | 5.279 | 5.456 | 223    | 1123 | 4.2    |
| 3    | 6.929 | 7.056 | 7.159 | 5516   | 26056| 100    |
| 4    | 7.253 | 7.349 | 7.818 | 2094   | 18282| 68.7   |

Fragmentor 17  
Voltage 6  
Collision Energy 2  
Ionization ES 1  
Mode 1

Agilent Technologies
Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End  | Height | Area    | Area % |
|------|-------|-----|------|--------|---------|--------|
| 1    | 6.844 | 7.050 | 7.155 | 5589   | 26832  | 100    |
| 2    | 7.208 | 7.349 | 7.655 | 2916   | 19707  | 73.31  |

Fragmentor 6  
Voltage 2
Collision 11  
Energy 0
Ionization ESI
Mode I

Integration Peak List

| Peak | Start | RT  | End  | Height | Area    | Area % |
|------|-------|-----|------|--------|---------|--------|
| 1    | 3.605 | 3.724 | 4.553 | 80338  | 357756 | 100    |
| 2    | 7.108 | 7.298 | 7.445 | 18     | 159    | 0.04   |
| 3    | 7.446 | 7.599 | 7.798 | 18     | 108    | 0.03   |
| 4    | 10.862 | 10.973 | 10.992 | 89     | 382    | 0.1    |

Fragmentor 6  
Voltage 2
Collision 7  
Energy 4
Ionization ESI
Mode I
Qualitative Analysis Report

nalorphine: +ESI MRM Frag=62.0 V Cl(D)@74.0 (312.2 -> 152.1) Sample 107563a.d

Counts vs. Acquisition Time (min)

Integration Peak List

| Peak | Start | RT  | End  | Height | Area  | Area % |
|------|-------|-----|------|--------|-------|--------|
| 1    | 3.605 | 3.724 | 4.776 | 114617 | 522649 | 100    |

--- End Of Report ---
Appendix 7: MRM Sample 107564

Qualitative Analysis Report

Data File: Sample 107564a.d
Sample Type: Sample
Instrument Name: Instrument 1
Acq Method: Mitragynine_LRU_Flexi_3.m
IBRM Calibration Status: Not Applicable
Comment: Info.
Sample Group: LC 1
Stream Name: LC 1
Acquisition SW Version: 6400 Series Triple Quadrupole 8.08.02 (85280.0)

Sample Name: Sample 107564a
Position: P2-ES
User Name: Info.
Acquired Time: 31/10/2020 11:41:20 AM (UTC+08:00)
DA Method: APCE Chemstation.m
Acquisition Time (Local): 31/10/2020 11:41:20 AM (UTC+08:00)

Chromatograms

Fragmentor Voltage: 17
Collision Energy: 6
Ionization Mode: 0
ES: 1

Integration Peak List

| Peak | Start | RT | End | Height | Area | Area % |
|------|-------|----|-----|--------|------|--------|
| 1    | 6.897 | 7.000 | 7.107 | 30241 | 140719 | 100    |
| 2    | 7.202 | 7.258 | 7.902 | 20530 | 139731 | 94.8   |

Fragmentor Voltage: 17
Collision Energy: 6
Ionization Mode: 0
ES: 1

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Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT   | End  | Height | Area  | Area % |
|------|-------|------|------|--------|-------|--------|
| 1    | 6.296 | 7.008| 7.103| 71866  | 358946| 100    |
| 2    | 7.202 | 7.298| 8.015| 51228  | 343612| 95.86  |

Fragmentor 17    Collision 2    Ionization E5    Voltage 6    Energy 9    Mode I

Integration Peak List

| Peak | Start | RT   | End  | Height | Area  | Area % |
|------|-------|------|------|--------|-------|--------|
| 1    | 3.724 | 4.113| 4.63 | 36     | 593   | 0.49   |
| 2    | 4.647 | 4.771| 4.963| 39     | 190   | 0.16   |
| 3    | 5.118 | 5.261| 5.504| 190    | 1206  | 1      |
| 4    | 6.897 | 7.008| 7.111| 24684  | 119905| 100    |
| 5    | 7.199 | 7.299| 7.857| 15673  | 100546| 83.85  |

Fragmentor 17    Collision 2    Ionization E5    Voltage 6    Energy 9    Mode I
Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT | End | Height | Area | Area % |
|------|-------|----|-----|--------|------|--------|
| 1    | 6.895 | 7.008 | 7.103 | 24787  | 118853 | 100    |
| 2    | 7.204 | 7.298 | 7.857 | 16505  | 105708 | 88.94  |

Fragmentor Voltage: 6
Collision Voltage: 11
Ionization Mode: II

Integration Peak List

| Peak | Start | RT | End | Height | Area | Area % |
|------|-------|----|-----|--------|------|--------|
| 1    | 3.586 | 3.705 | 4.522 | 99857  | 473313 | 100    |

Fragmentor Voltage: 5
Collision Voltage: 7
Ionization Mode: I

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Qualitative Analysis Report

![Graph showing counts vs. acquisition time with peak at 3.705 minutes.]

Integration Peak List

| Peak | Start | RT  | End  | Height | Area  | Area % |
|------|-------|-----|------|--------|-------|--------|
| 1    | 3.585 | 3.705 | 4.363 | 140546 | 665446 | 100    |

--- End Of Report ---
Appendix 8: MRM Sample 107565

Qualitative Analysis Report

Data File Sample 107565a.d
Sample Type Sample
Instrument Name Instrument 1
Acq Method Mitragynine_LU_Relix_Z.m
IRM Calibration Status Not Applicable
Comment
Sample Group LC 1
Stream Name LC 1
Acquisition SW 6400 Series Triple Quadrupole 8.08.02 (B8260.0)
Version

Sample Name Sample 107565a
Position P2-E7
User Name
Acquired Time 31/10/2020 12:25:35 PM (UTC+08:00)
DA Method APCI Chemstation.m
Info.
Acquisition Time (Local) 31/10/2020 12:25:35 PM (UTC+08:00)

Chromatograms

Fragmentor Voltage 17
Collision Energy 6
Ionization Mode 5
ES

Counts vs. Acquisition Time (min)

Mitragynine: +ESI MRM Frag=176.0V CID@50.0 (399.2 -> 159.1) Sample 107565a.d

Integration Peak List

| Peak | Start | RT  | End  | Height | Area   | Area % |
|------|-------|-----|------|--------|--------|--------|
| 1    | 6.669 | 6.994| 7.084| 20342  | 97318  | 76.83  |
| 2    | 7.109 | 7.209| 7.004| 21815  | 137418 | 100    |

Fragmentor Voltage 17
Collision Energy 2
Ionization Mode 9
ES

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Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT | End | Height | Area | Area % |
|------|-------|----|-----|--------|------|--------|
| 1    | 5.311 | 5.581 | 5.901 | 47    | 594  | 0.17   |
| 2    | 6.779 | 6.984 | 7.084 | 59283 | 242210 | 71.3   |
| 3    | 7.166 | 7.209 | 8.238 | 53460 | 339695 | 100    |

Fragmentor Voltage 17  
Collision Energy 2  
Ionization ES 9  
Mode I

Integration Peak List

| Peak | Start | RT | End | Height | Area | Area % |
|------|-------|----|-----|--------|------|--------|
| 1    | 3.803 | 4.114 | 4.566 | 31    | 412  | 0.42   |
| 2    | 4.366 | 4.750 | 5.043 | 24    | 175  | 0.18   |
| 3    | 5.109 | 5.24  | 5.552 | 188   | 1265 | 1.29   |
| 4    | 6.859 | 6.988 | 7.08  | 16259 | 70782 | 78.45  |
| 5    | 7.163 | 7.268 | 7.763 | 15890 | 98258 | 100    |

Fragmentor Voltage 6  
Collision Energy 1  
Ionization ES Mode I

Agilent Technologies
Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End | Height | Area | Area % |
|------|-------|-----|-----|--------|------|--------|
| 1    | 6.859 | 6.984 | 7.076 | 16612 | 78193 | 74.56  |
| 2    | 7.165 | 7.268 | 7.809 | 17058 | 104730| 100    |

Fragmentor 6
Collision 11
Ionization ES
Voltage 2
Energy 0
Mode I

Integration Peak List

| Peak | Start | RT  | End | Height | Area | Area % |
|------|-------|-----|-----|--------|------|--------|
| 1    | 3.559 | 3.678 | 4.49 | 92736  | 421905| 100    |
| 2    | 7.335 | 7.529 | 8.098| 22     | 346   | 0.08   |

Fragmentor 6
Collision 7
Ionization ES
Voltage 2
Energy 4
Mode I
Qualitative Analysis Report

nalorphine: +ESI MRM Frag=62.0V CID@74.0 (312.2 -> 152.1) Sample 107565a.d

| Peak | Start  | RT   | End  | Height | Area     | Area % |
|------|--------|------|------|--------|----------|--------|
| 1    | 3.559  | 3.678| 4.776| 132236 | 595808   | 100    |

--- End Of Report ---
Appendix 9: MRM Sample 107566

Qualitative Analysis Report

| Data File         | Sample 107566b.d                        | Sample Name       | Sample 107566b                        |
|-------------------|-----------------------------------------|-------------------|--------------------------------------|
| Sample Type       | Sample                                  | Position          | P2:F1                                |
| Instrument Name   | Instrument 1                            | User Name         |                                      |
| Acq Method        | Mitragynine_LHU_Flex_2.m                 | Acquired Time     | 31/10/2020 1:32:01 PM (UTC+08:00)    |
| IBM Calibration   | Not Applicable                          | DA Method         | APCI Chemstation.m                   |
| Status            |                                        |                   |                                      |
| Comment           |                                        |                   |                                      |
| Sample Group      |                                        |                   |                                      |
| Stream Name       | LC 1                                    | Info.             |                                      |
| Acquisition SW    | 0400 Series Triple Quadrupole B.08.02  | Acquisition Time  | 31/10/2020 1:32:01 PM (UTC+08:00)    |
| Version           |                                        | (Local)           |                                      |

Chromatograms

Fragmentor Voltage 17 6 5 0 1
Collision Energy
Ionization Mode
ES

Mitragynine: +ESI MRM Frag=175.0V CID@50.0 (399.2 -> 159.1) Sample 107566b.d

Integration Peak List

| Peak | Start | End  | Height | Area | Area % |
|------|-------|------|--------|------|--------|
| 1    | 6.231 | 6.25 | 7.045  | 3334 | 16064  | 46.54 |
| 2    | 7.045 | 7.23 | 7.602  | 4427 | 34512  | 100  |

Fragmentor Voltage 17 6 9 1
Collision Energy
Ionization Mode
ES

Agilent Technologies
Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End  | Height | Area | Area % |
|------|-------|-----|------|--------|------|--------|
| 1    | 6.623 | 6.95| 7.044| 7769   | 39247| 44.35  |
| 2    | 7.044 | 7.229| 7.713| 11292  | 87516| 100    |

Fragmentsor Voltage: 17 Collision Energy: 6 Ionization ES Rate: 3

Integration Peak List

| Peak | Start | RT  | End  | Height | Area | Area % |
|------|-------|-----|------|--------|------|--------|
| 1    | 4.636 | 4.732| 4.863| 94     | 142  | 0.6    |
| 2    | 5.068 | 5.211| 5.424| 204    | 1156 | 4.84   |
| 3    | 6.83  | 6.949| 7.05 | 2772   | 13542| 56.73  |
| 4    | 7.05  | 7.23 | 7.65 | 3202   | 23870| 100    |

Fragmentsor Voltage: 17 Collision Energy: 6 Ionization ES Rate: 3

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Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End  | Height | Area  | Area % |
|------|-------|-----|------|--------|-------|--------|
| 1    | 6.633 | 6.952| 7.043| 2657   | 13113 | 47.14  |
| 2    | 7.043 | 7.229| 7.87 | 4009   | 27819 | 100    |

Fragmentor Voltage 2
Collision Energy 0
Ionization Mode 1

Integration Peak List

| Peak | Start | RT  | End  | Height | Area  | Area % |
|------|-------|-----|------|--------|-------|--------|
| 1    | 3.54  | 3.659| 4.363| 88718  | 405414| 100    |
| 2    | 7.37  | 7.499| 7.653| 26    | 196   | 0.05   |
| 3    | 9.911 | 10.79| 10.927| 33    | 327   | 0.08   |

Fragmentor Voltage 6
Collision Energy 4
Ionization Mode 1

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Qualitative Analysis Report

![Graph showing counts vs. acquisition time](image)

**Integration Peak List**

| Peak | Start | RT  | End  | Height | Area    | Area % |
|------|-------|-----|------|--------|---------|--------|
| 1    | 3.541 | 3.66| 4.697| 125451 | 575859  | 100    |
| 2    | 10.921| 10.950| 10.952| 47     | 143     | 0.02   |

--- End Of Report ---
Appendix 10: MRM Sample 107567

Qualitative Analysis Report

Data File: Sample 107567a.d
Sample Type: Sample
Instrument Name: Instrument 1
Acq Method: Mitragynine_LH1_Flexi_2.m
IRM Calibration Status: Not Applicable
Comment:
Sample Group:
Stream Name: LC 1
Acquisition SW Version: 6400 Series Triple Quadrupole B.08.02 (B8260.0)

Sample Name: Sample 107567a
Position: P2-F2
User Name:
Acquired Time: 31/10/2020 1:54:10 PM (UTC+08:00)
DA Method: APCI Chemstation.m
Info.
Acquisition Time (Local): 31/10/2020 1:54:10 PM (UTC+08:00)

Chromatograms

Fragmentor Voltage: 17
Collision Energy: 6
Ionization Mode: 5
ES: 0

Mitragynine: ESI MRM Frag=176.0V CID@50.0 (399.2 -> 159.1) Sample 107567a.d

Integration Peak List

| Peak | Start | End | Height | Area | Area % |
|------|-------|-----|--------|------|--------|
| 1    | 6.793 | 6.933 | 7.02   | 32356 | 151585 | 100   |
| 2    | 7.119 | 7.213 | 7.872  | 20483 | 135530 | 89.41 |

Fragmentor Voltage: 17
Collision Energy: 6
Ionization Mode: 5
ES: 1
Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT   | End   | Height | Area | Area % |
|------|-------|------|-------|--------|------|--------|
| 1    | 5.059 | 5.315| 5.725 | 25     | 398  | 8.11   |
| 2    | 6.313 | 6.933| 7.028 | 77849  | 372319| 100    |
| 3    | 7.119 | 7.215| 7.92  | 51916  | 335545| 90.07  |

Fragmentor Voltage 17
Energy 6
Ionization Mode ES 1

Integration Peak List

| Peak | Start | RT   | End   | Height | Area   | Area % |
|------|-------|------|-------|--------|--------|--------|
| 1    | 4.581 | 4.7  | 4.861 | 34     | 131   | 0.13   |
| 2    | 5.066 | 5.211| 5.455 | 210    | 1355  | 1.11   |
| 3    | 6.817 | 6.937| 7.032 | 25561  | 122006| 100    |
| 4    | 7.116 | 7.214| 7.745 | 15285  | 96939 | 79.45  |

Fragmentor Voltage 17
Energy 6
Ionization Mode ES 1

Agilent Technologies
Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End  | Height | Area   | Area % |
|------|-------|-----|------|--------|--------|--------|
| 1    | 6.317 | 6.933 | 7.024 | 6577   | 12371  | 100    |
| 2    | 7.121 | 7.213 | 7.761 | 10515  | 104331 | 84.61  |

Fragmentor 6 Collision 11 Ionization ES
Voltage 2 Energy 0 Mode I

Integration Peak List

| Peak | Start | RT  | End  | Height | Area   | Area % |
|------|-------|-----|------|--------|--------|--------|
| 1    | 3.532 | 3.651 | 4.299 | 51594  | 514930 | 100    |
| 2    | 6.652 | 6.811 | 6.967 | 18     | 152    | 0.03   |
| 3    | 6.977 | 7.123 | 7.558 | 29     | 318    | 0.06   |
| 4    | 8.975 | 9.353 | 9.708 | 31     | 568    | 0.11   |
| 5    | 9.708 | 10.767 | 10.93 | 104    | 1667   | 0.32   |
| 6    | 10.9  | 10.974 | 10.992 | 59     | 222    | 0.04   |

Fragmentor 6 Collision 7 Ionization ES
Voltage 2 Energy 4 Mode I

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Qualitative Analysis Report

Integration Peak List

| Peak | Start | RT  | End | Height  | Area   | Area % |
|------|-------|-----|-----|---------|--------|--------|
| 1    | 3.533 | 3.652 | 4.633 | 156673  | 736799 | 100    |
| 2    | 10.864 | 10.944 | 10.992 | 51      | 254    | 0.03   |

--- End Of Report ---