

Modification of Liquid Chromatography / Mass Spectrometry Methods

Currently the Forensic Chemistry unit utilizes one instrumental method (TBI Drug Screen) on the Agilent Ultivo Triple Quadrupole LC/MS for drug identification. This method serves to analyze a vast majority of controlled substances that our laboratory receives. However, lately some combinations of compounds are not being separated with that method's liquid chromatography parameters. Developing new methods from the current method might help to improve the chromatography of these mixtures by varying the percentages of Mobile A (1 % Formic Acid in Water) and Mobile B (1 % Formic Acid in Methanol) and the time frames at which these gradients are run. These modifications are analogous to the oven temperature profiles used in gas chromatography.

The first method developed was designed to decrease the amount of time to run an instrument wash. Currently, the TBI Drug Screen method is not only used to analyze samples, but also to rinse or wash off any residual compounds that might remain in the LC system after analyzing samples. The Fast Wash method doubles the flow rate (0.700 mL/Min) and quickly goes to a high polarity mobile phase (95 % Methanol to 5 % Water) and runs for four minutes as compared to the ten minutes of the TBI Drug Screen method. While conducting this study the Fast Wash method was used after every standard mixture run, and no carryover of compounds was observed in the study.

The TBI Long Scan method is a variation of the TBI Drug Screen method. TBI Long Scan method increases the total run time from 10 minutes to 20 minutes and halves the rate at which the polarity of the mobile phase is increased. These modifications were made to improve separation between compounds having similar elution times. When Quinine and Heroin or isomers of Fluorofentanyl and Fentanyl are both present in a sample, the TBI Drug Screen method does not resolve these compounds and they typically coelute. This makes identification difficult or impossible in these cases leading to inconclusive reporting. In this study a mixture of Quinine (Merke Co Lot # 70260), Heroin (Cerilliant Lot # FE06151603), Fentanyl (USP Lot # 1275-F), and para-Fluorofentanyl (Cayman Lot # 0541563-27) was prepared with approximately a concentration of 1 mg/mL each. This mixture was diluted and run on the TBI Drug Screen method and the TBI Long Scan methods. As seen in case work, the Quinine and Heroin coeluted as did the para-Fluorofentanyl and the Fentanyl with the TBI Drug Screen method. However, with the TBI Long Scan method Quinine and Heroin were baseline resolved and Fentanyl and para-Fluorofentanyl experienced better separation although they were not baseline resolved.

Several isocratic methods were developed, potentially helping to improve separation of mixtures that might arise in future case work. The mixture described above was run with each of these isocratic methods and it was demonstrated that the retention times and peak resolution of Quinine, Heroin, Fentanyl, and para-Fluorofentanyl are influenced by the polarity of the mobile phase. In the table below the new methods are described.

New Methods

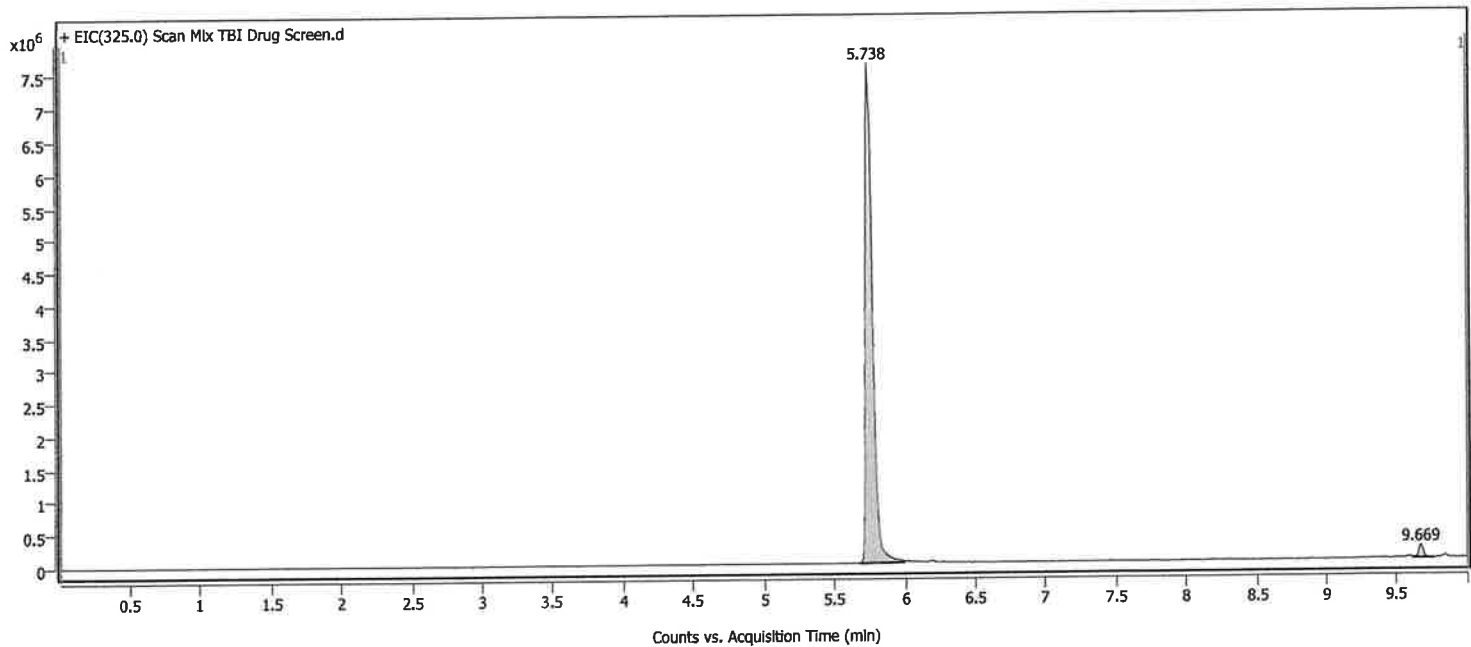
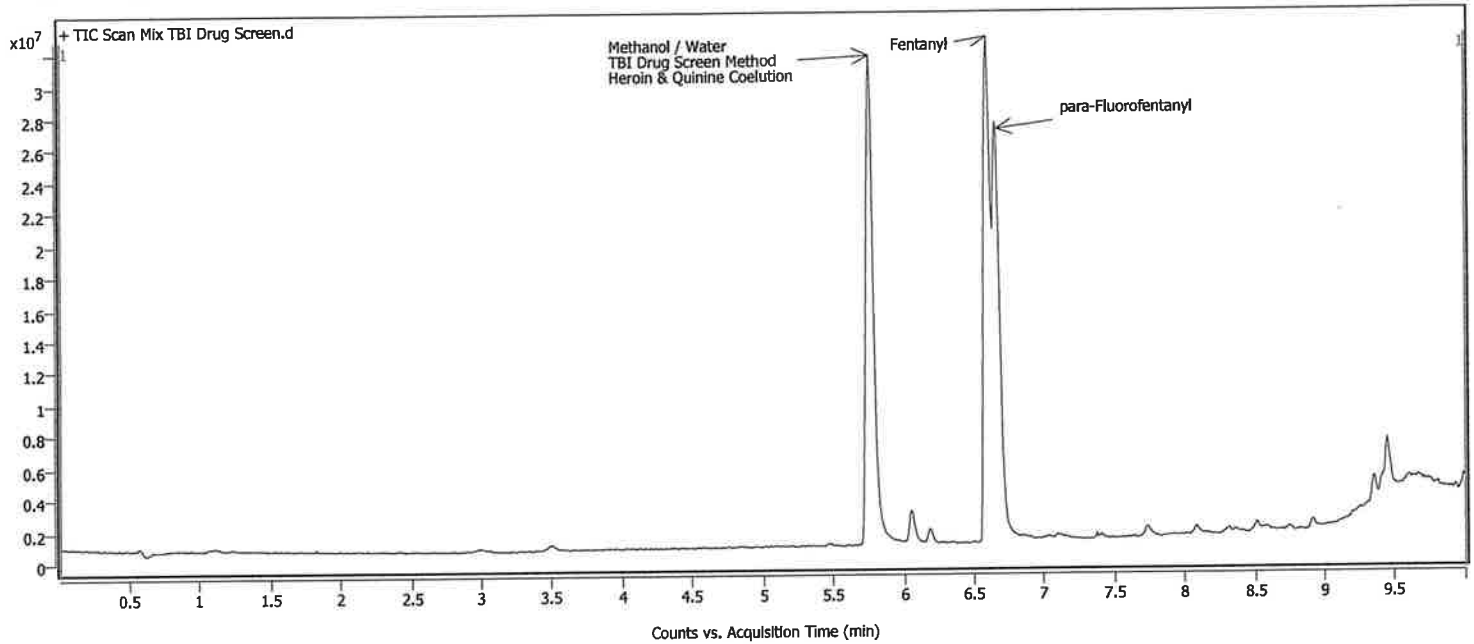
Method Name	Flow (mL/Min)	Description
Fast Wash	0.700	0 – 0.5 Minutes 99% Mobile A & 1% Mobile B 0.5 – 1 Minutes 5% Mobile A & 95% Mobile B 1 – 3.5 Minutes 5% Mobile A & 95% Mobile B 3.5 – 4 Minutes 2% Mobile A & 98% Mobile B
TBI Long Scan	0.350	0 – 1 Minutes 99% Mobile A & 1% Mobile B 1 – 19.5 Minutes 10% Mobile A & 90% Mobile B 19.5 – 20 Minutes 2% Mobile A & 98% Mobile B
Isocratic M10W90	0.350	0 – 1 Minutes 99% Mobile A & 1% Mobile B 1 – 1.5 Minutes 90% Mobile A & 10% Mobile B 1.5 – 9 Minutes 90% Mobile A & 10% Mobile B 9 – 10 Minutes 2% Mobile A & 98% Mobile B
Isocratic M20W90	0.350	0 – 1 Minutes 99% Mobile A & 1% Mobile B 1 – 1.5 Minutes 80% Mobile A & 20% Mobile B 1.5 – 9 Minutes 80% Mobile A & 20% Mobile B 9 – 10 Minutes 2% Mobile A & 98% Mobile B
Isocratic M30W90	0.350	0 – 1 Minutes 99% Mobile A & 1% Mobile B 1 – 1.5 Minutes 70% Mobile A & 30% Mobile B 1.5 – 9 Minutes 70% Mobile A & 30% Mobile B 9 – 10 Minutes 2% Mobile A & 98% Mobile B
Isocratic M40W90	0.350	0 – 1 Minutes 99% Mobile A & 1% Mobile B 1 – 1.5 Minutes 60% Mobile A & 40% Mobile B 1.5 – 9 Minutes 60% Mobile A & 40% Mobile B 9 – 10 Minutes 2% Mobile A & 98% Mobile B
Isocratic M50W90	0.350	0 – 1 Minutes 99% Mobile A & 1% Mobile B 1 – 1.5 Minutes 50% Mobile A & 50% Mobile B 1.5 – 9 Minutes 50% Mobile A & 50% Mobile B 9 – 10 Minutes 2% Mobile A & 98% Mobile B
Isocratic M60W90	0.350	0 – 1 Minutes 99% Mobile A & 1% Mobile B 1 – 1.5 Minutes 40% Mobile A & 60% Mobile B 1.5 – 9 Minutes 40% Mobile A & 60% Mobile B 9 – 10 Minutes 2% Mobile A & 98% Mobile B
Isocratic M70W90	0.350	0 – 1 Minutes 99% Mobile A & 1% Mobile B 1 – 1.5 Minutes 30% Mobile A & 70% Mobile B 1.5 – 9 Minutes 30% Mobile A & 70% Mobile B 9 – 10 Minutes 2% Mobile A & 98% Mobile B
Isocratic M80W90	0.350	0 – 1 Minutes 99% Mobile A & 1% Mobile B 1 – 1.5 Minutes 20% Mobile A & 80% Mobile B 1.5 – 9 Minutes 20% Mobile A & 80% Mobile B 9 – 10 Minutes 2% Mobile A & 98% Mobile B
Isocratic M90W90	0.350	0 – 1 Minutes 99% Mobile A & 1% Mobile B 1 – 1.5 Minutes 10% Mobile A & 90% Mobile B 1.5 – 9 Minutes 10% Mobile A & 90% Mobile B 9 – 10 Minutes 2% Mobile A & 98% Mobile B

Analysis Report

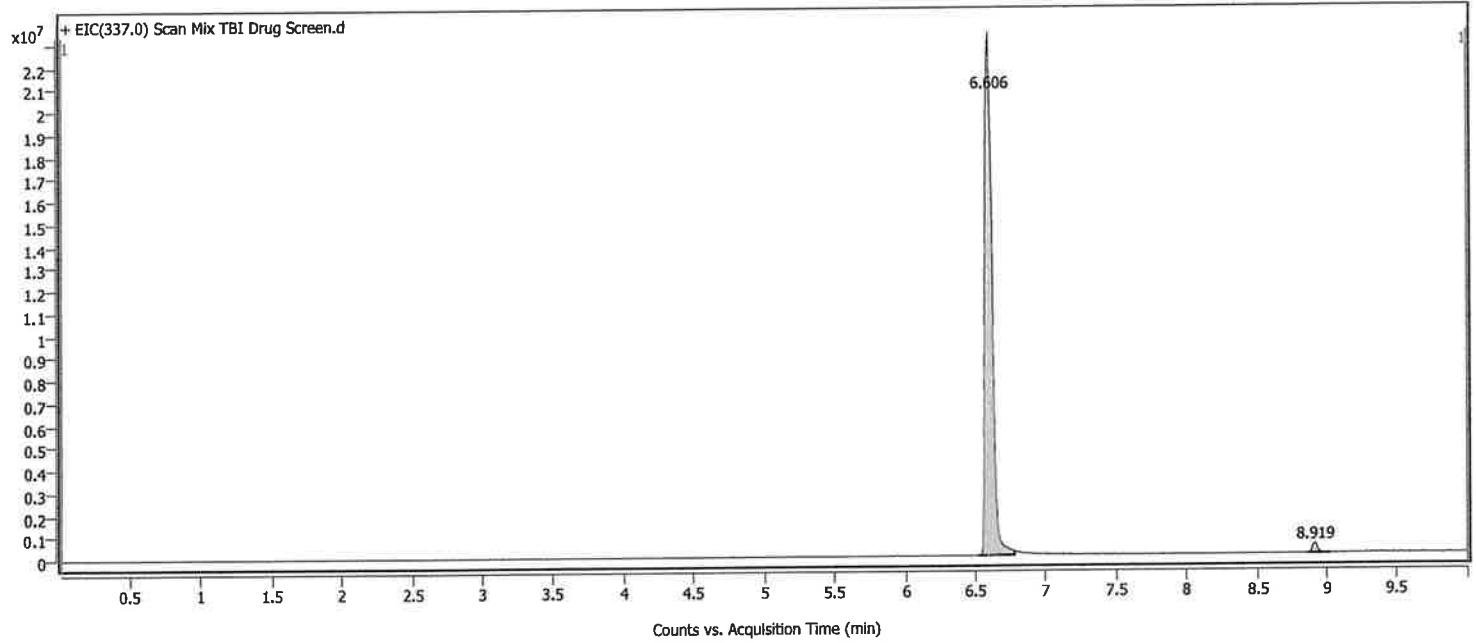
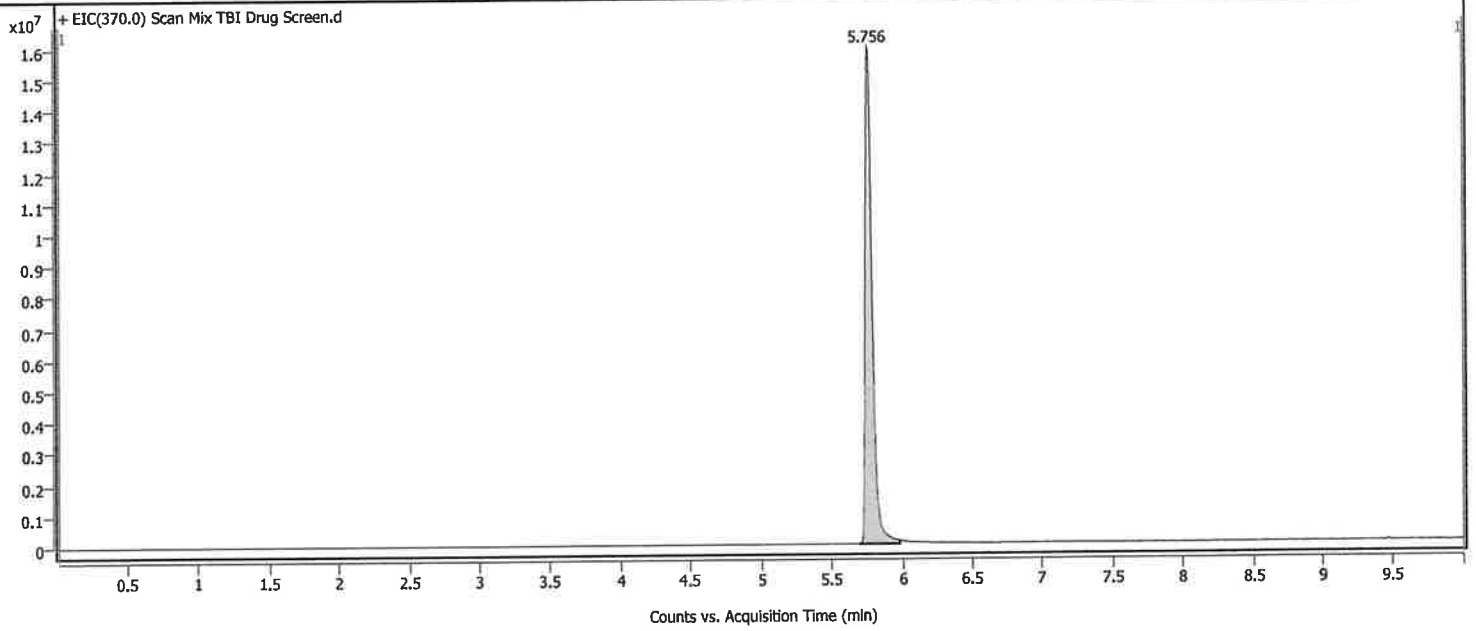
Sample Information

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Sample ID		Acq. Time (Local)	2/10/2022 10:52:55 AM (UTC-06:00)
Instrument	Ocoee	Method Path (Acq)	D:\MassHunter\Methods\TBI Drug Screen.m
MS Type	QQQ	Version (Acq SW)	Ultivo LC/TQ 1.2 (23)
Inj. Vol. (ul)	5	IRM Status	
Position	P2-F2	Method Path (DA)	
Plate Pos.		Target Source Path	
Operator		Result Summary	

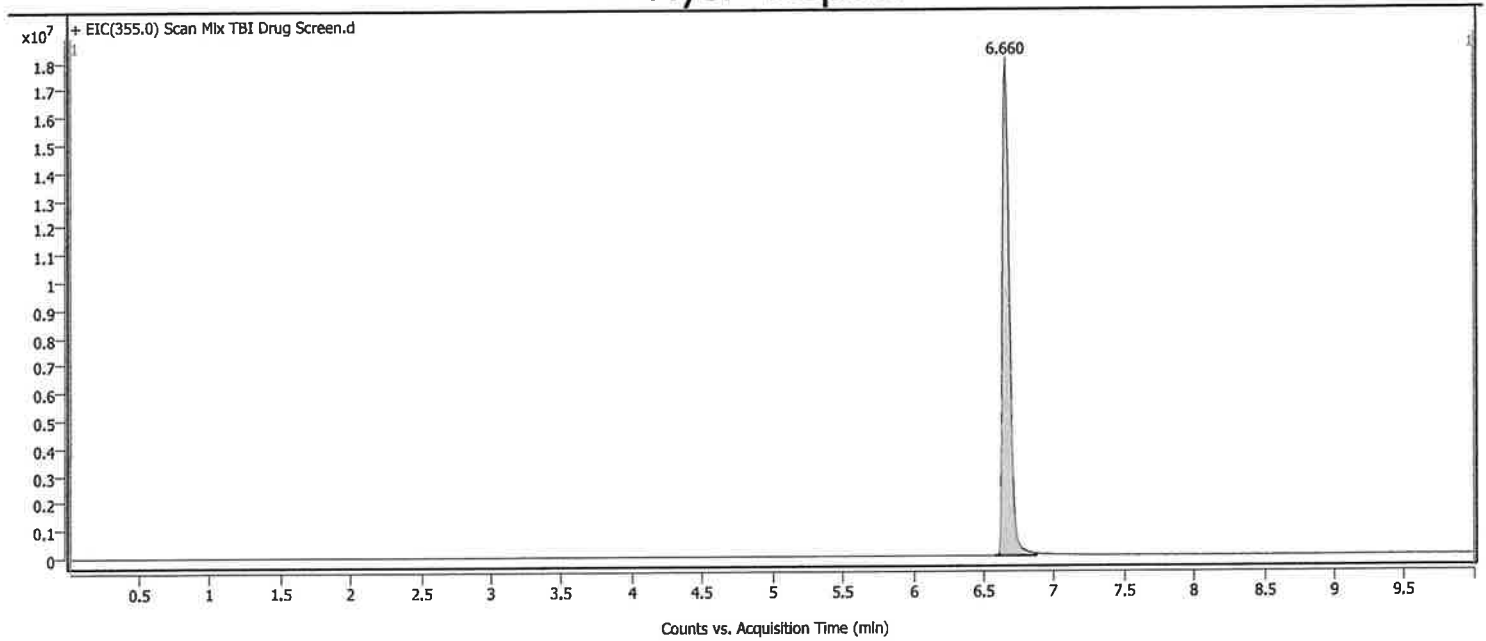
Sample Chromatograms



Analysis Report

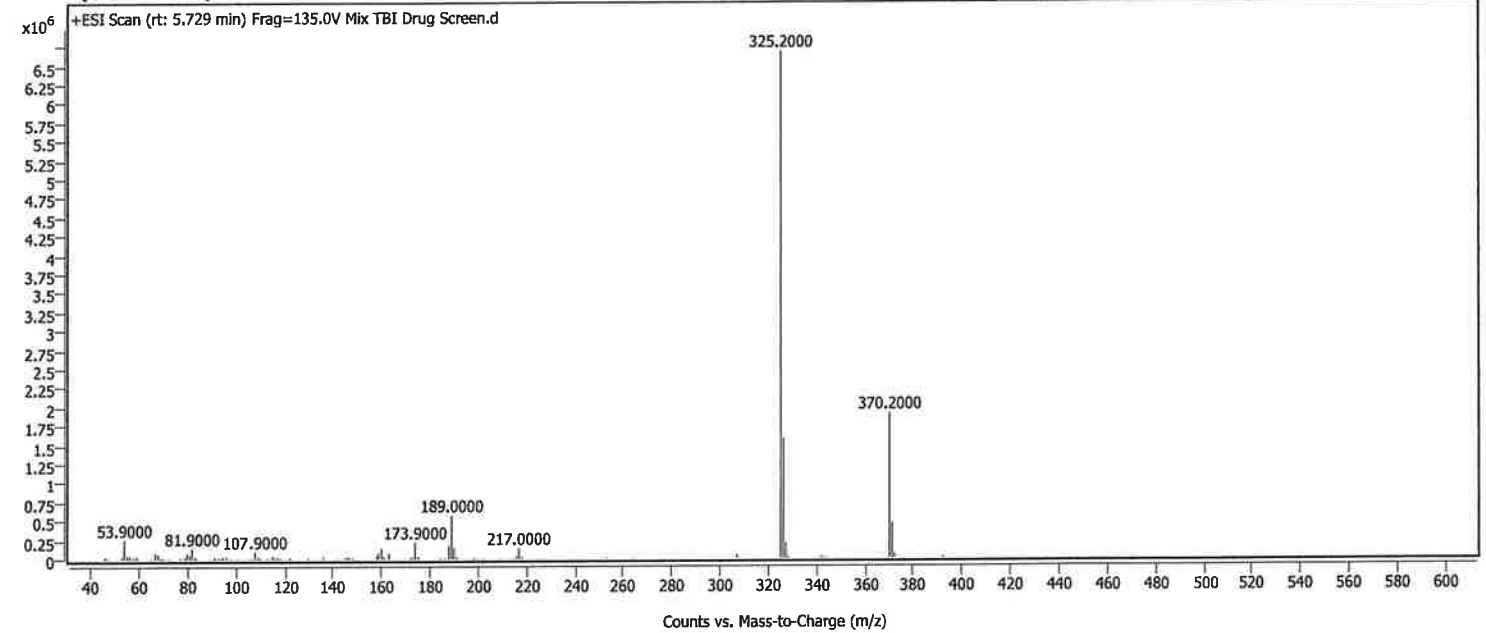


Analysis Report



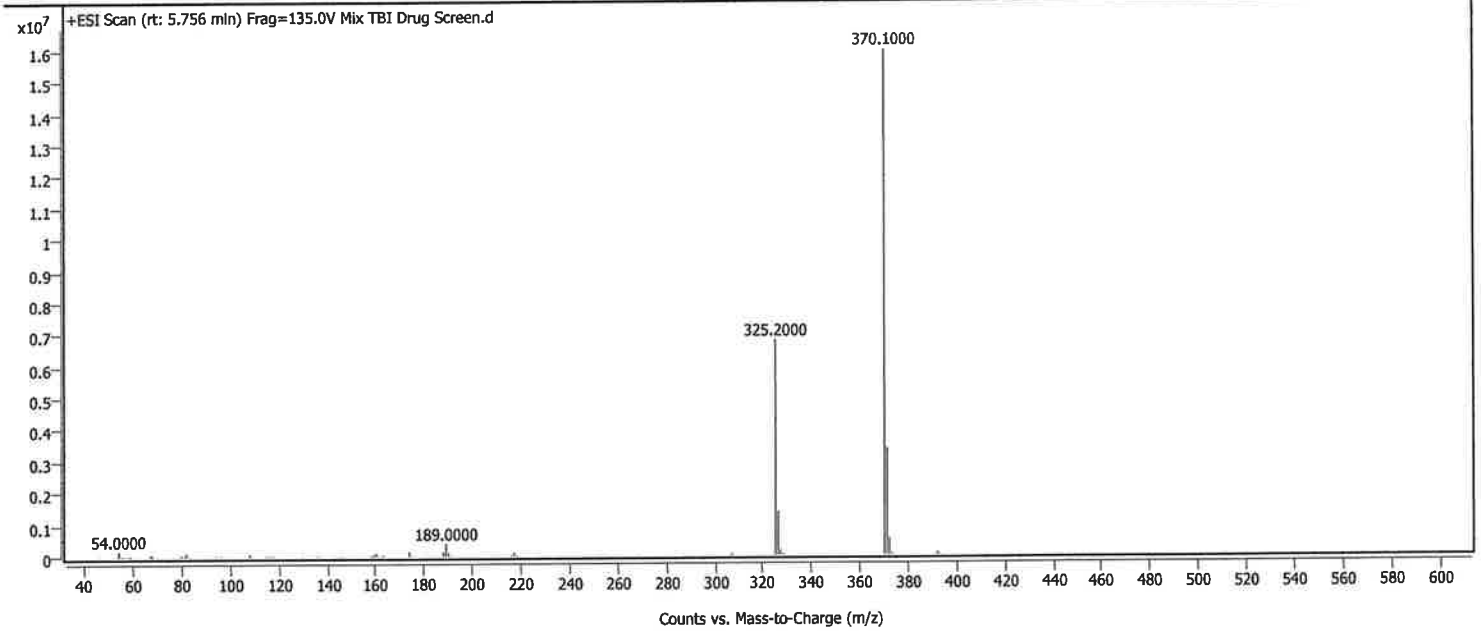
Sample Spectra

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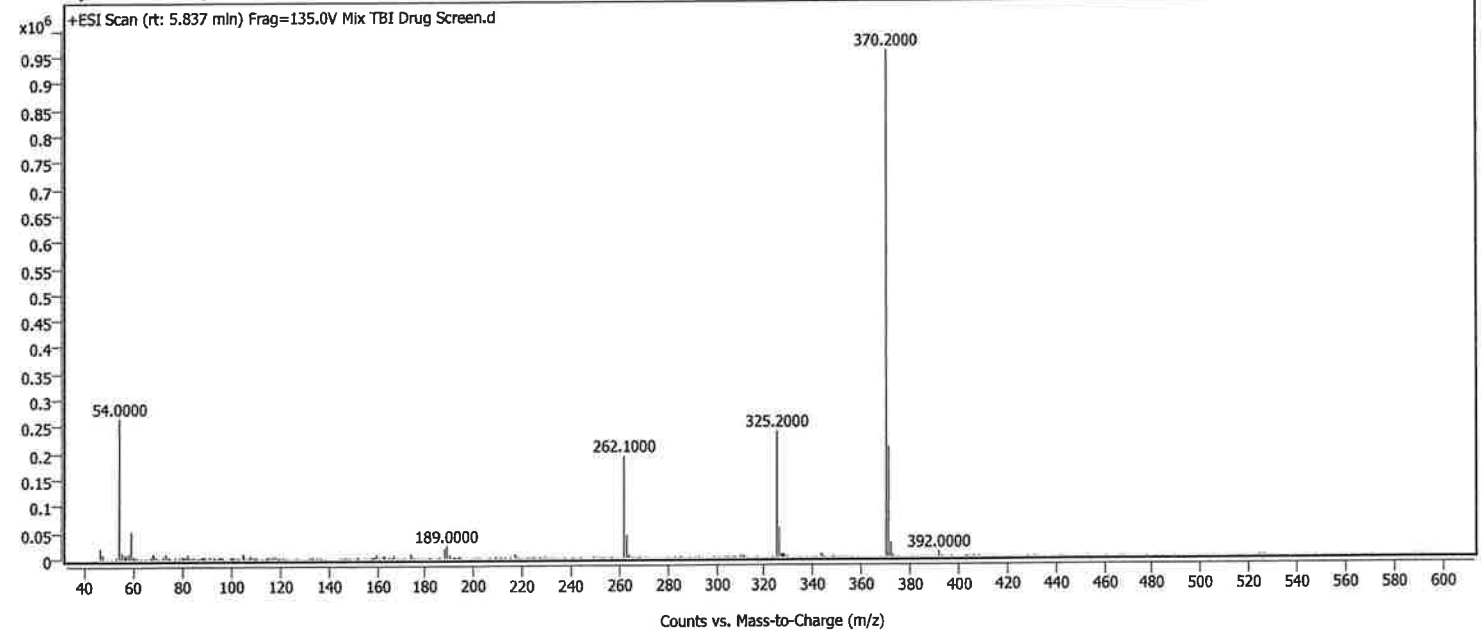


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Analysis Report

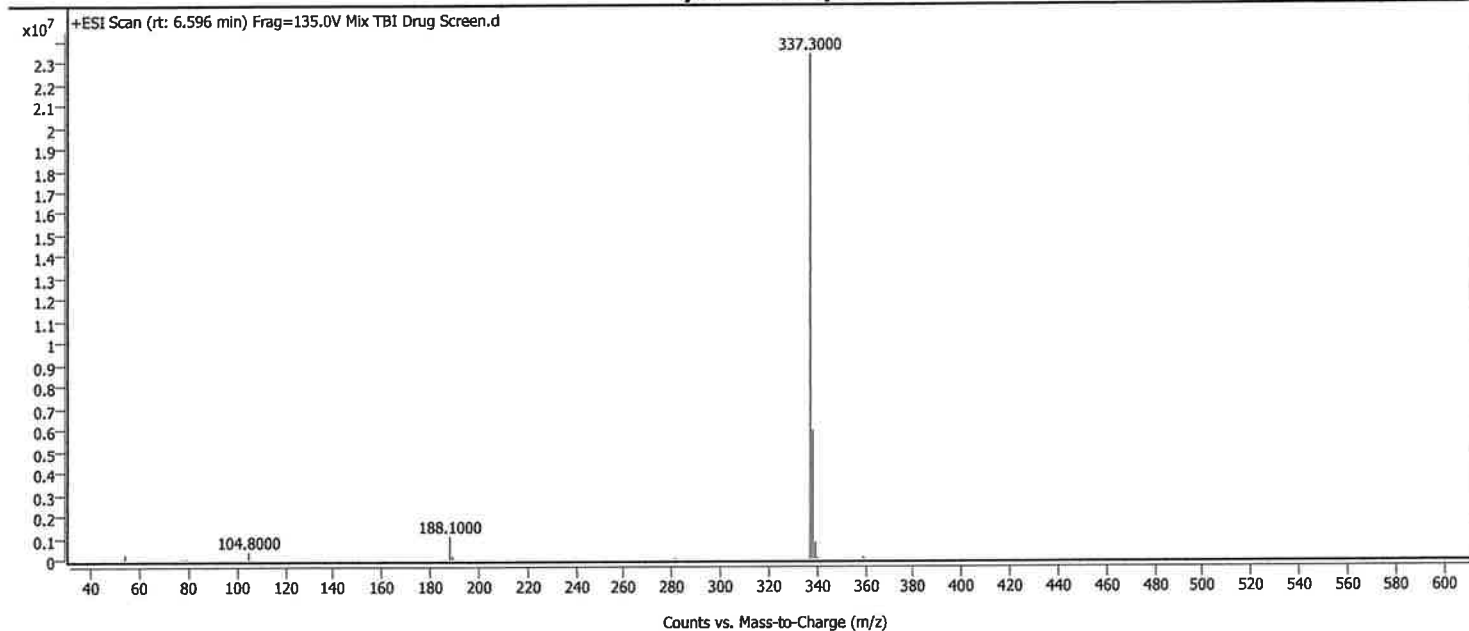


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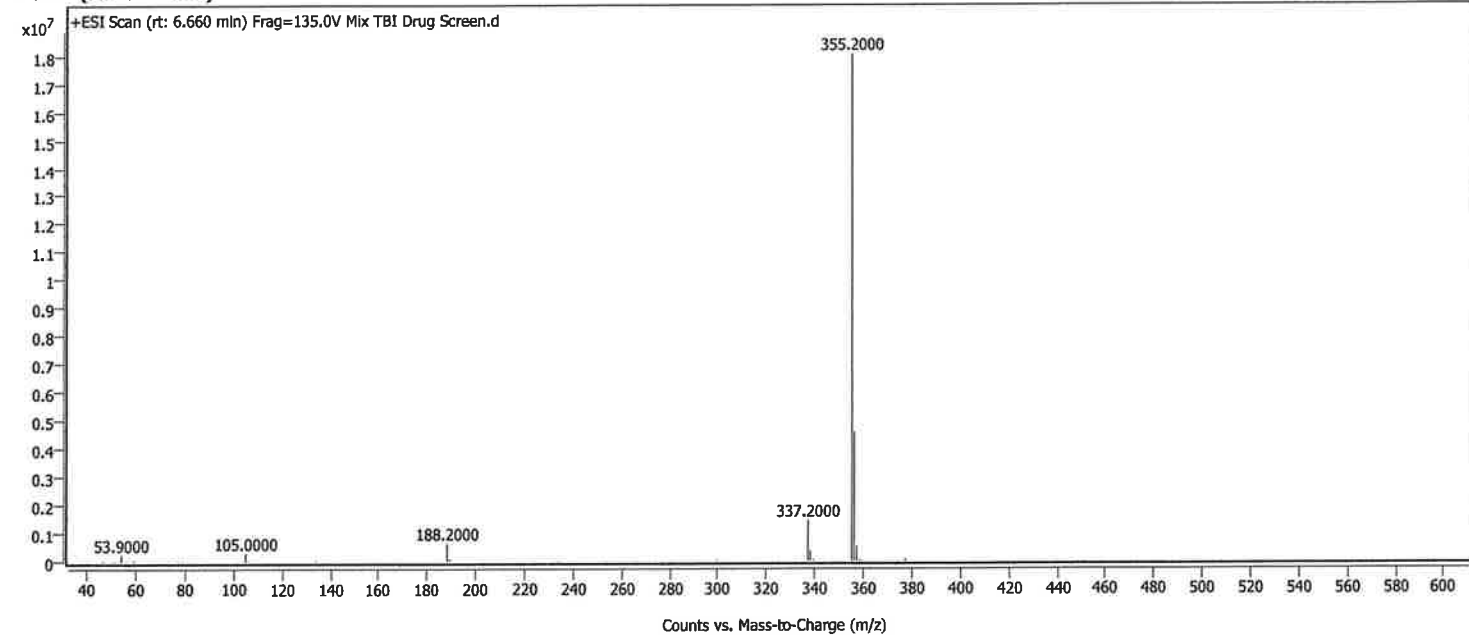


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Analysis Report



+ Scan (rt: 6.660 min)



MassHunter Qual 10.0
(End of Report)

Analysis Report

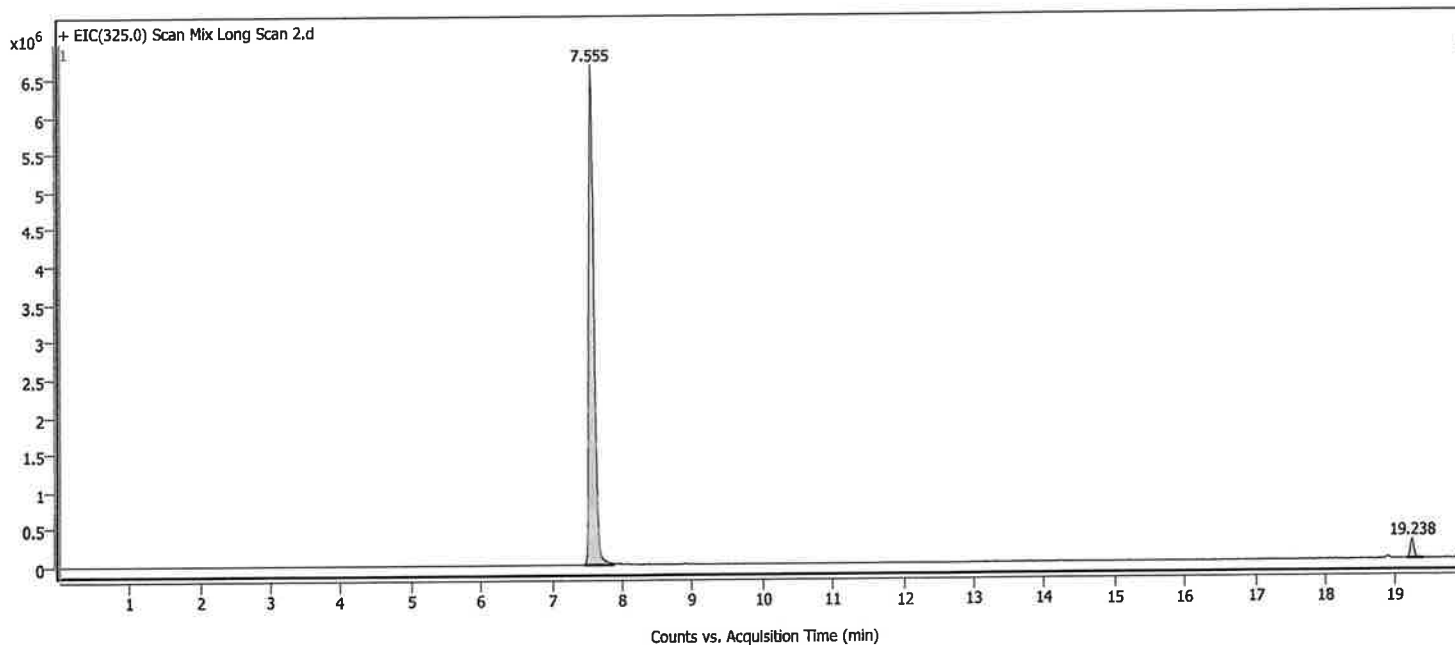
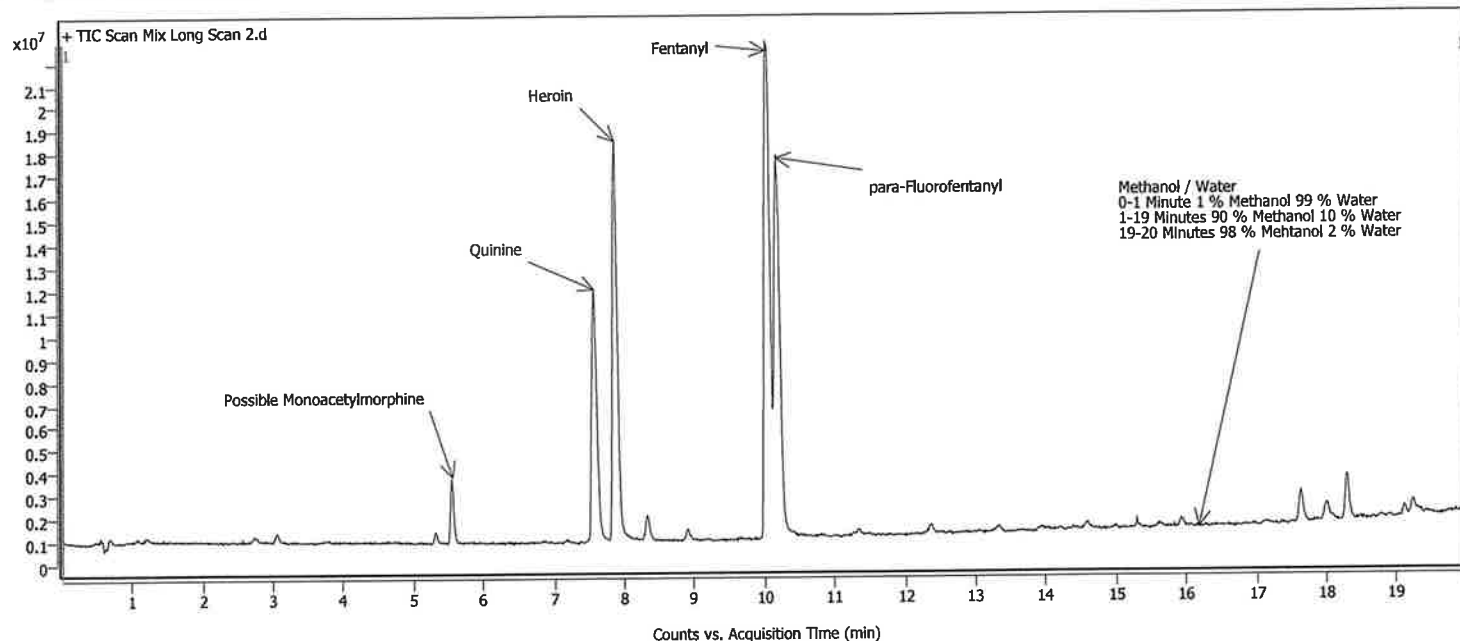
Sample Information

Name Mix Standard
Sample ID
Instrument Ocoee
MS Type QQQ
Inj. Vol. (ul) 5
Position P2-F2
Plate Pos.
Operator

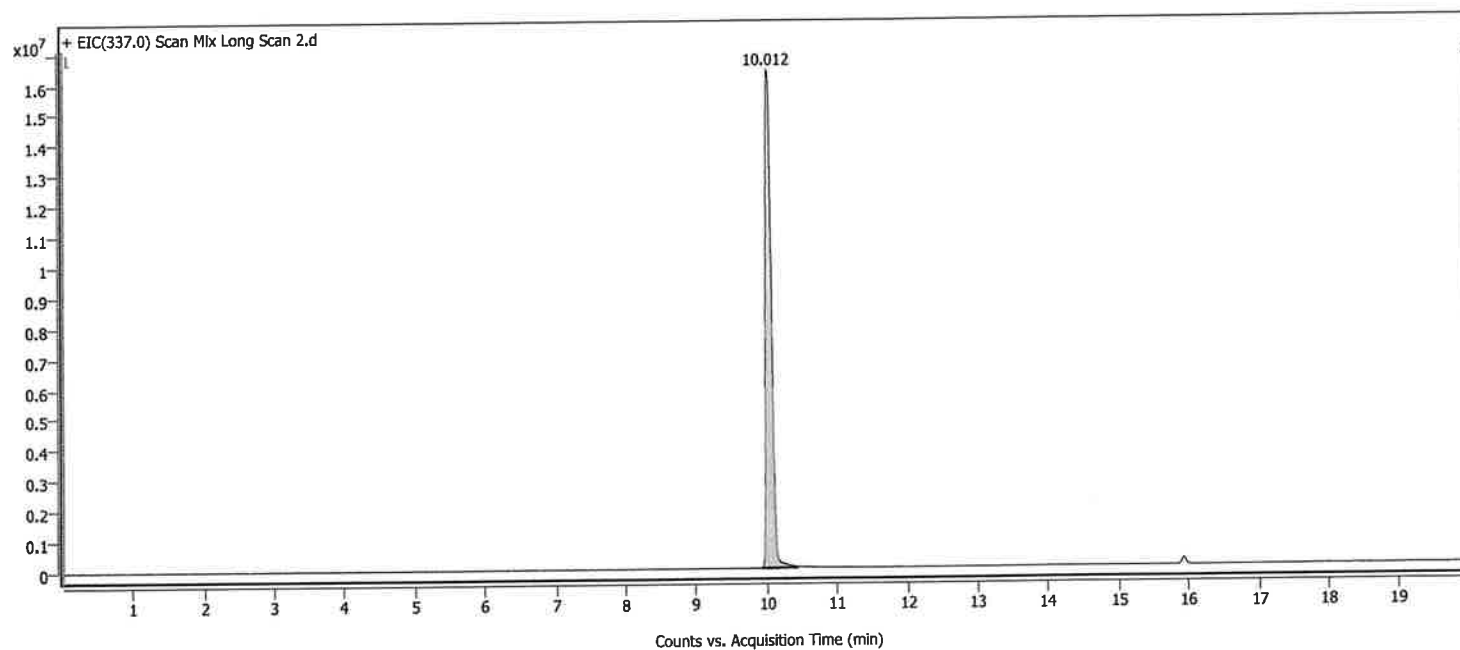
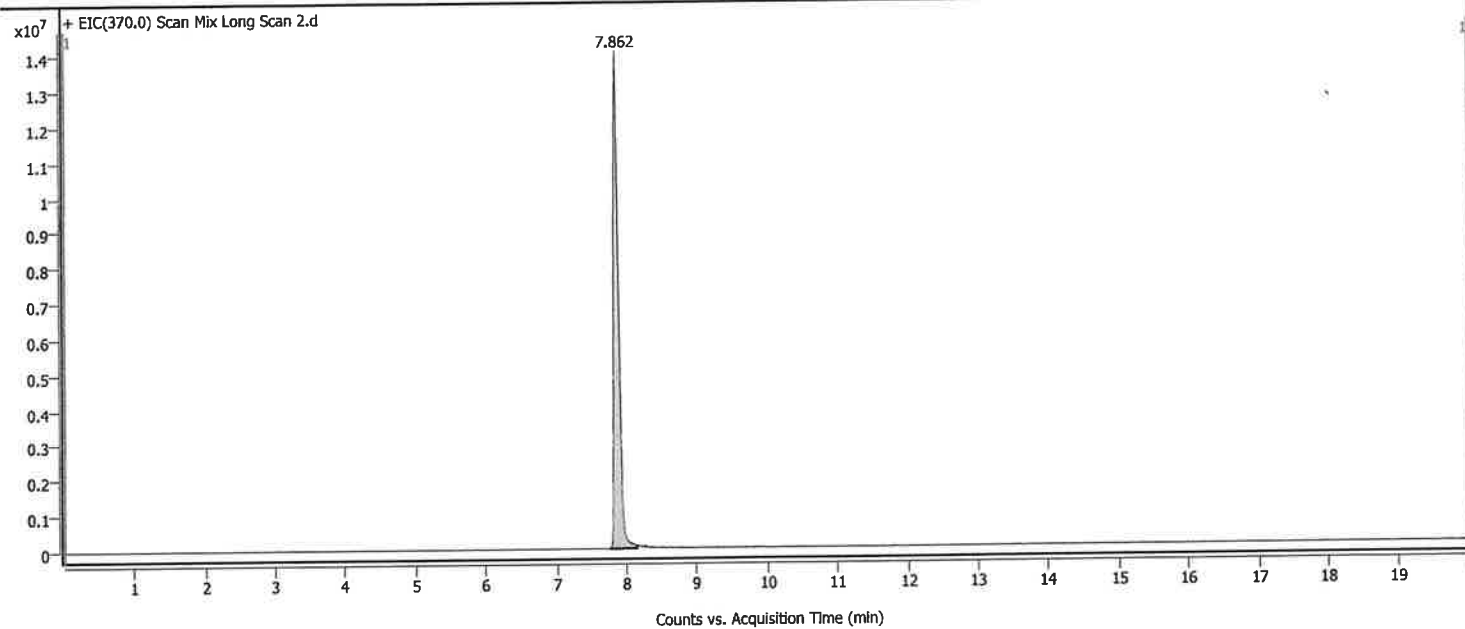
Data File Path
Acq. Time (Local)
Method Path (Acq)
Version (Acq SW)
IRM Status
Method Path (DA)
Target Source Path
Result Summary

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Ultivo LC/TQ 1.2 (23)

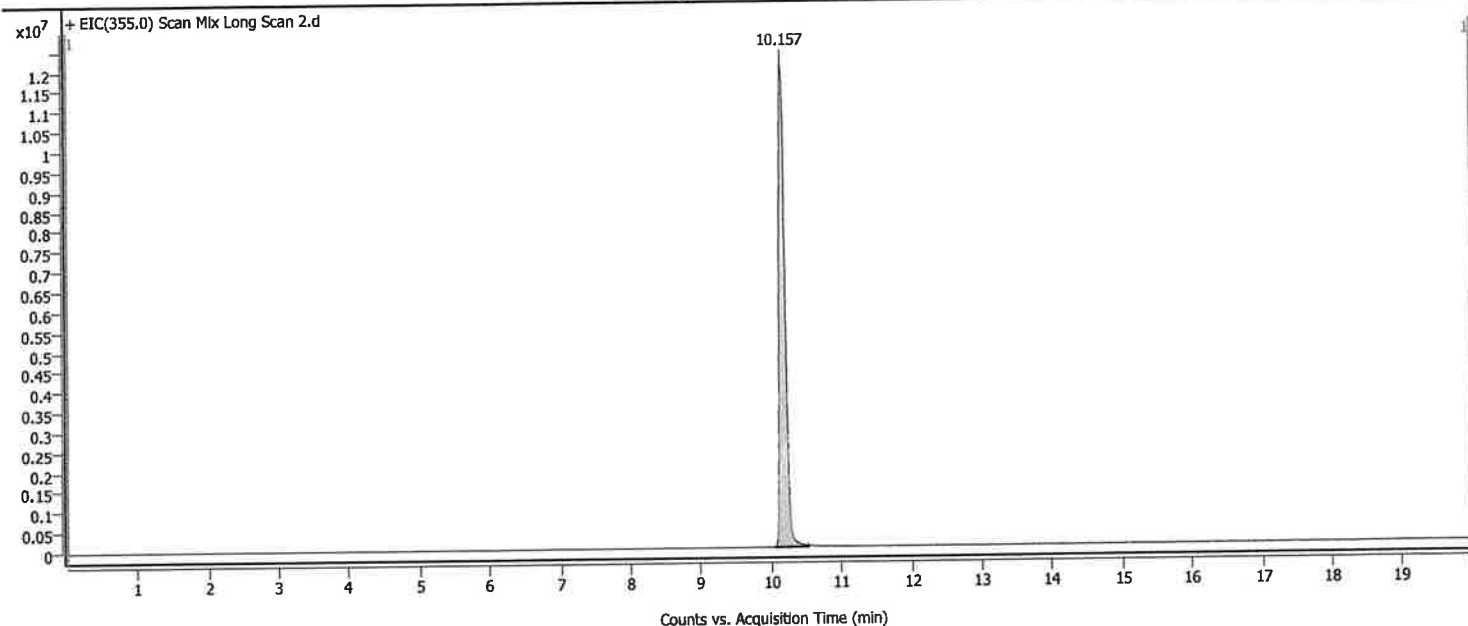
Sample Chromatograms



Analysis Report

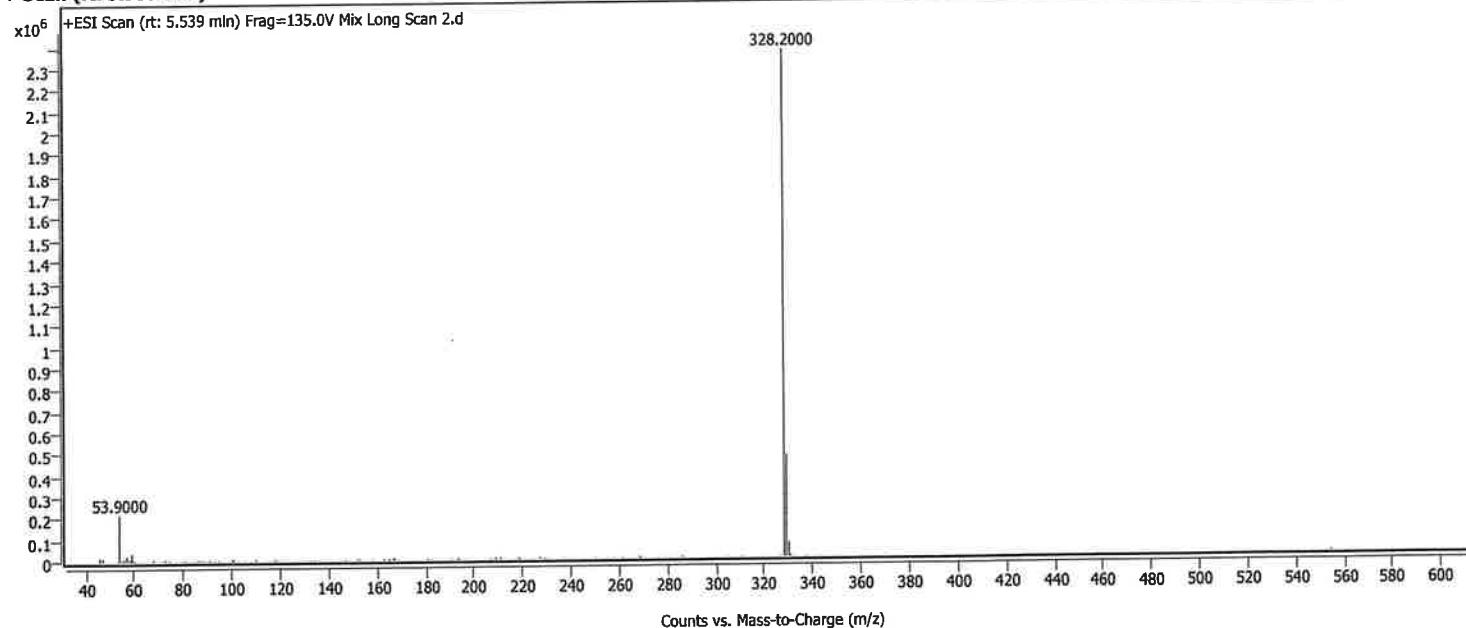


Analysis Report



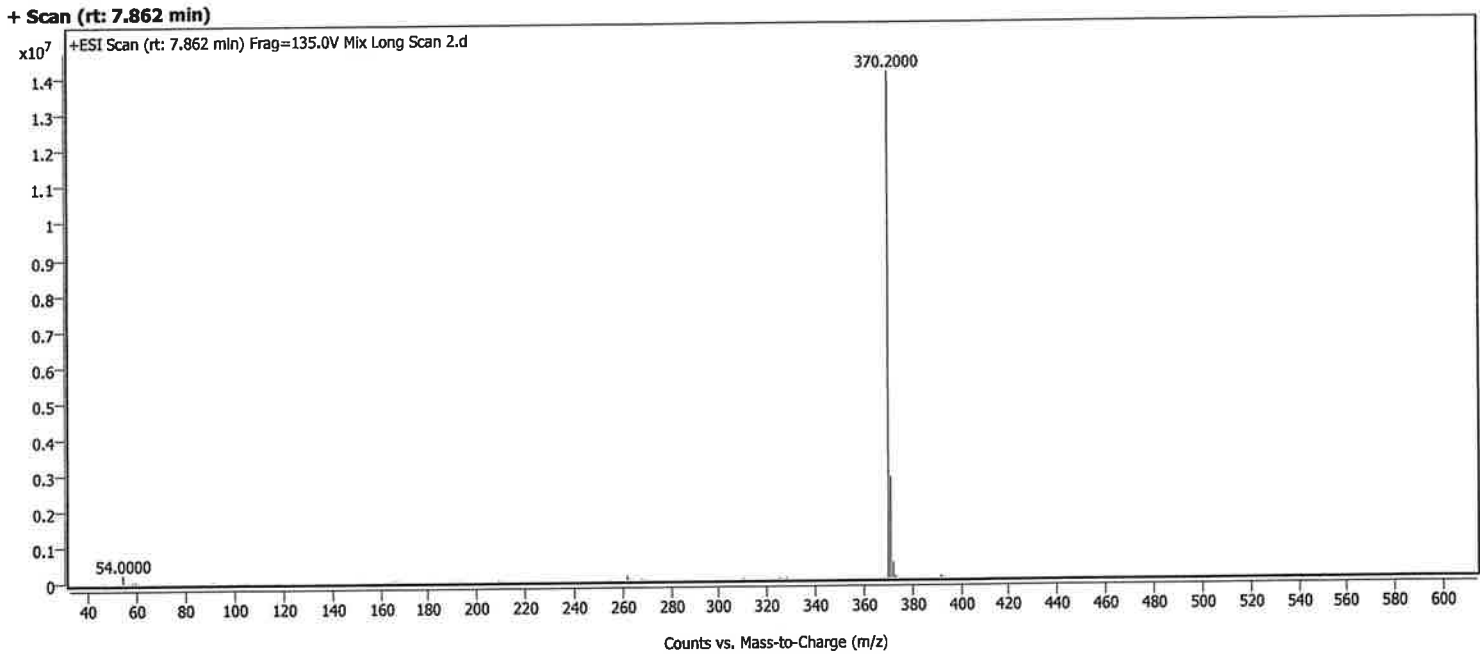
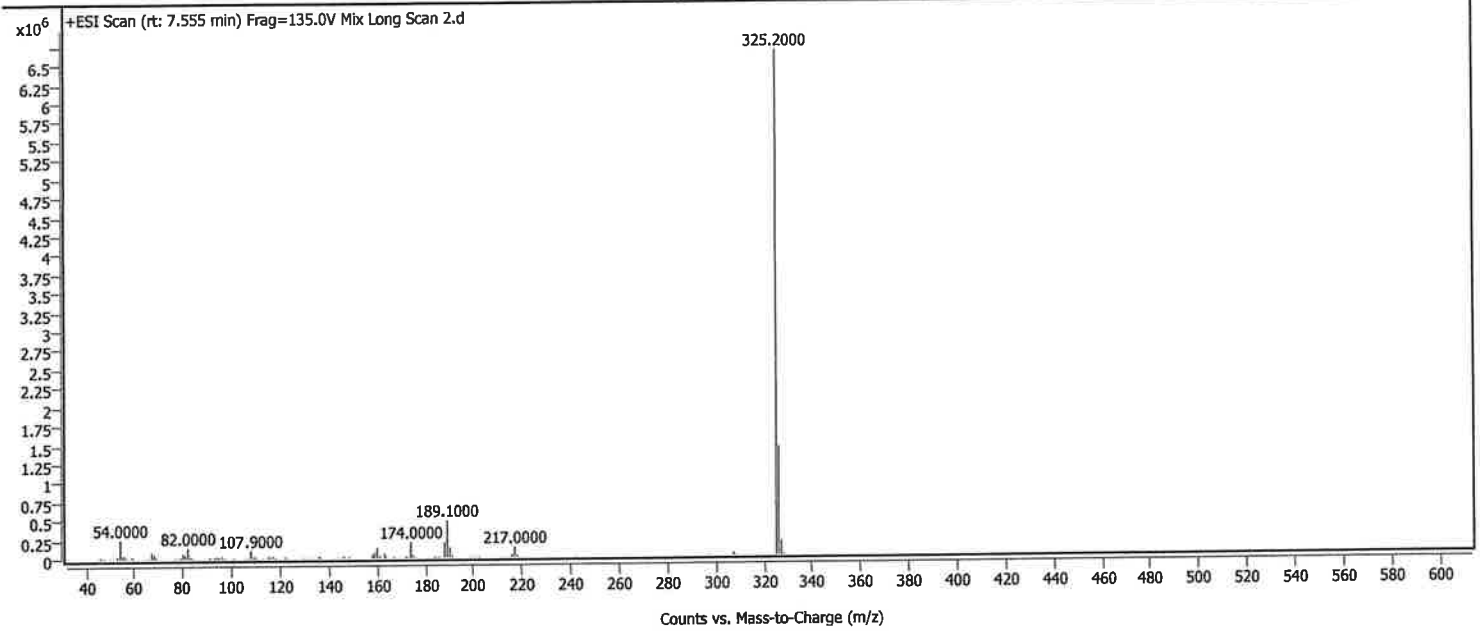
Sample Spectra

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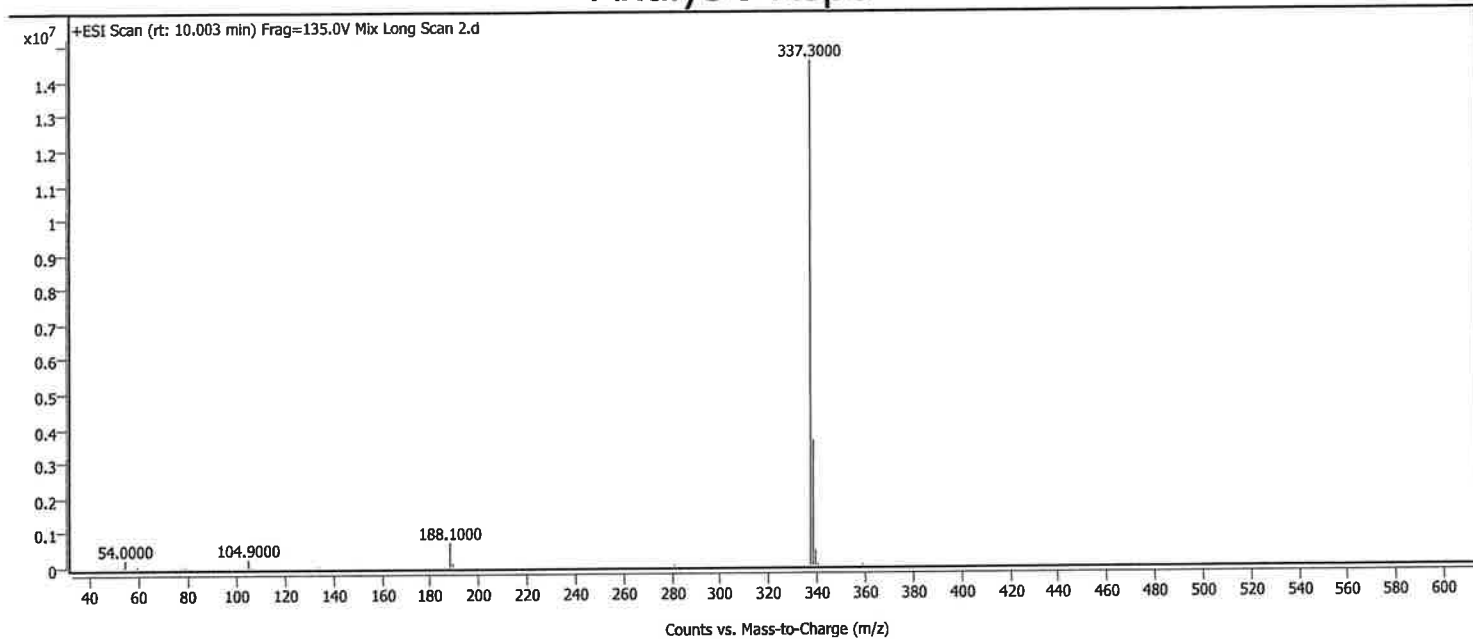
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Analysis Report



+ Scan (rt: 10.003 min)

Analysis Report



MassHunter Qual 10.0
(End of Report)

Analysis Report

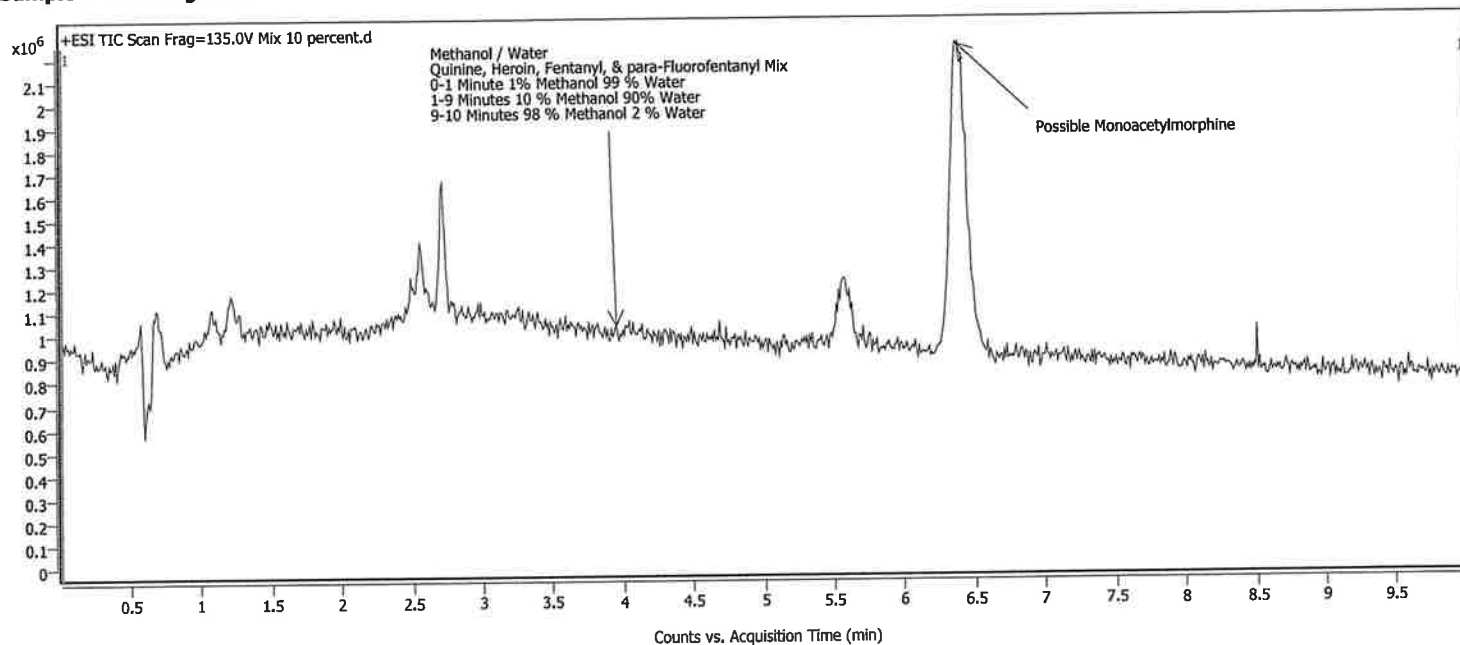
Sample Information

Name Mix Standard
Sample ID
Instrument Ocoee
MS Type QQQ
Inj. Vol. (ul) 5
Position P2-F2
Plate Pos.
Operator

Data File Path
Acq. Time (Local)
Method Path (Acq)
Version (Acq SW)
IRM Status
Method Path (DA)
Target Source Path
Result Summary

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Ultivo LC/TQ 1.2 (23)

Sample Chromatograms



MassHunter Qual 10.0
(End of Report)

Analysis Report

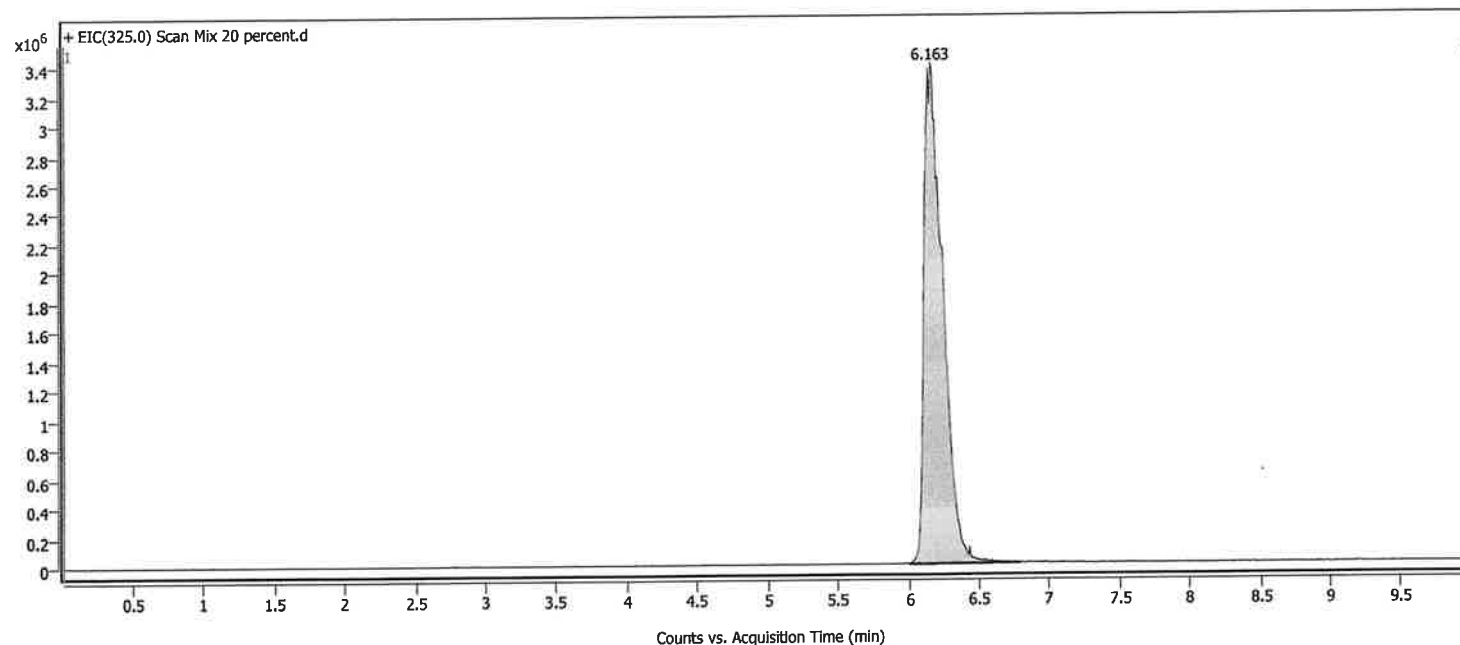
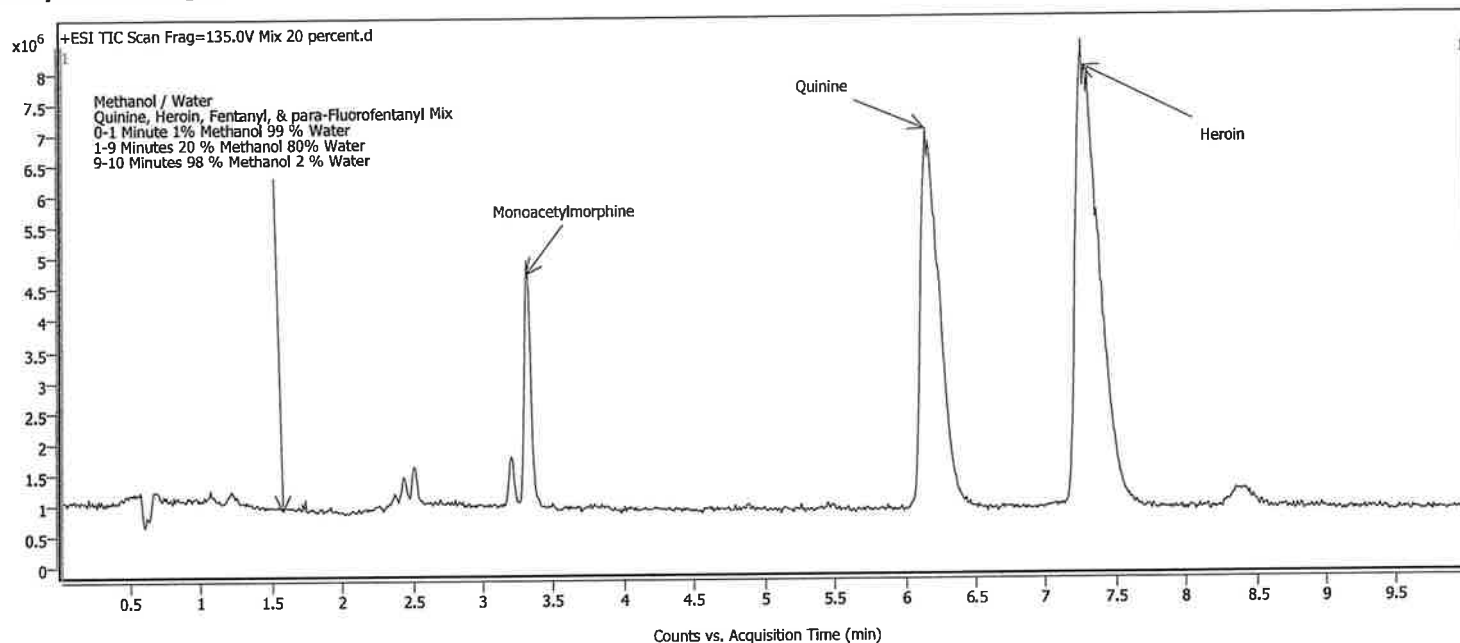
Sample Information

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Sample ID
Instrument Ocoee
MS Type QQQ
Inj. Vol. (ul) 5
Position P2-F2
Plate Pos.
Operator

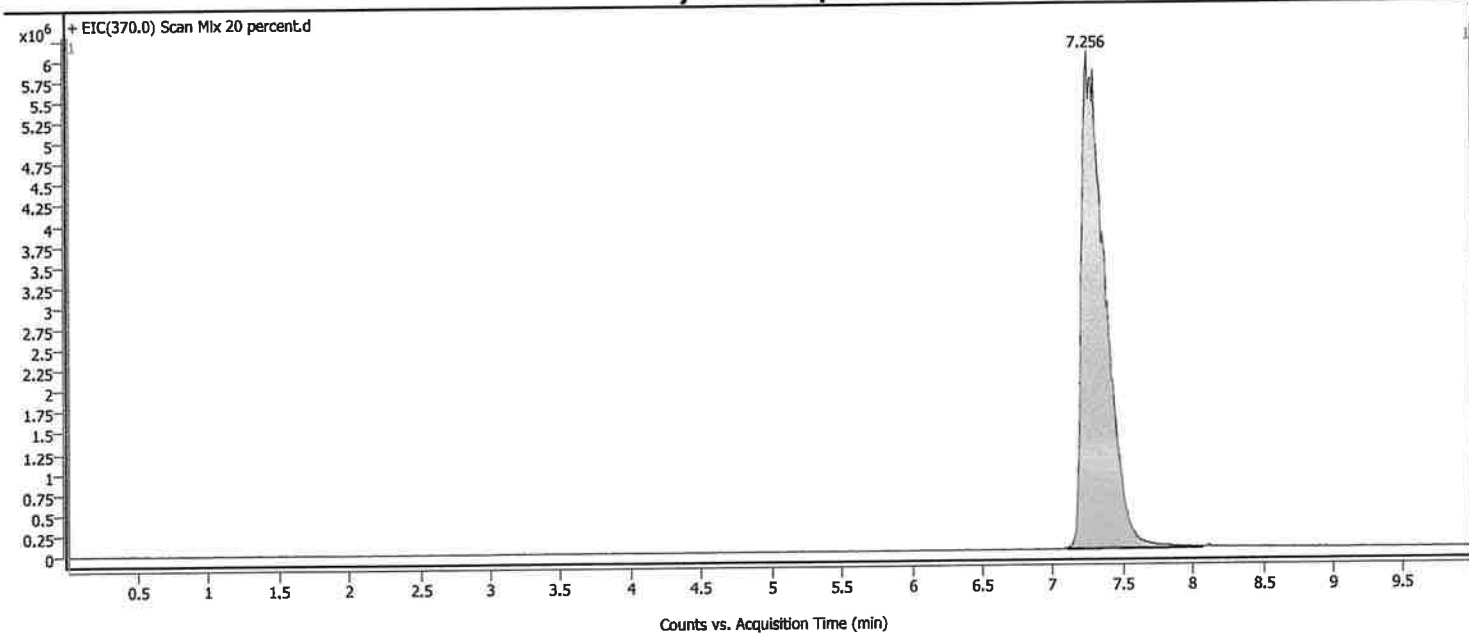
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Version (Acq SW)
IRM Status
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Result Summary

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Ultivo LC/TQ 1.2 (23)

Sample Chromatograms

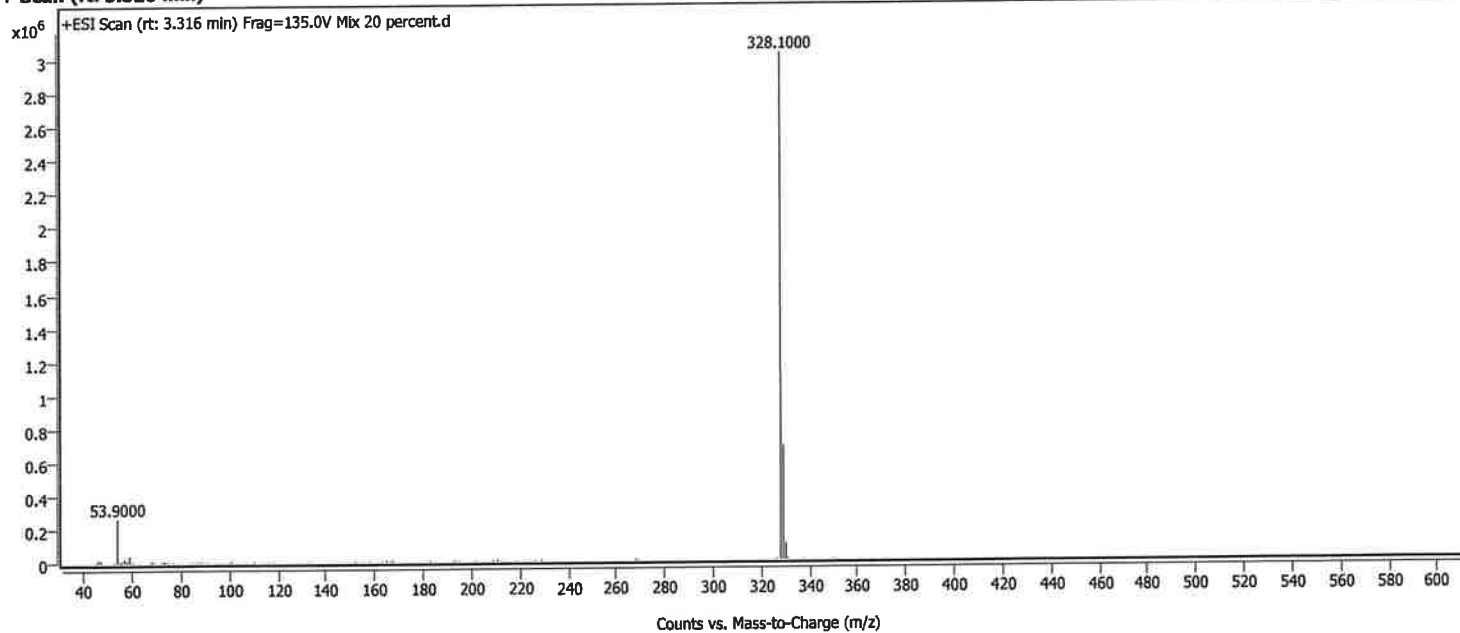


Analysis Report



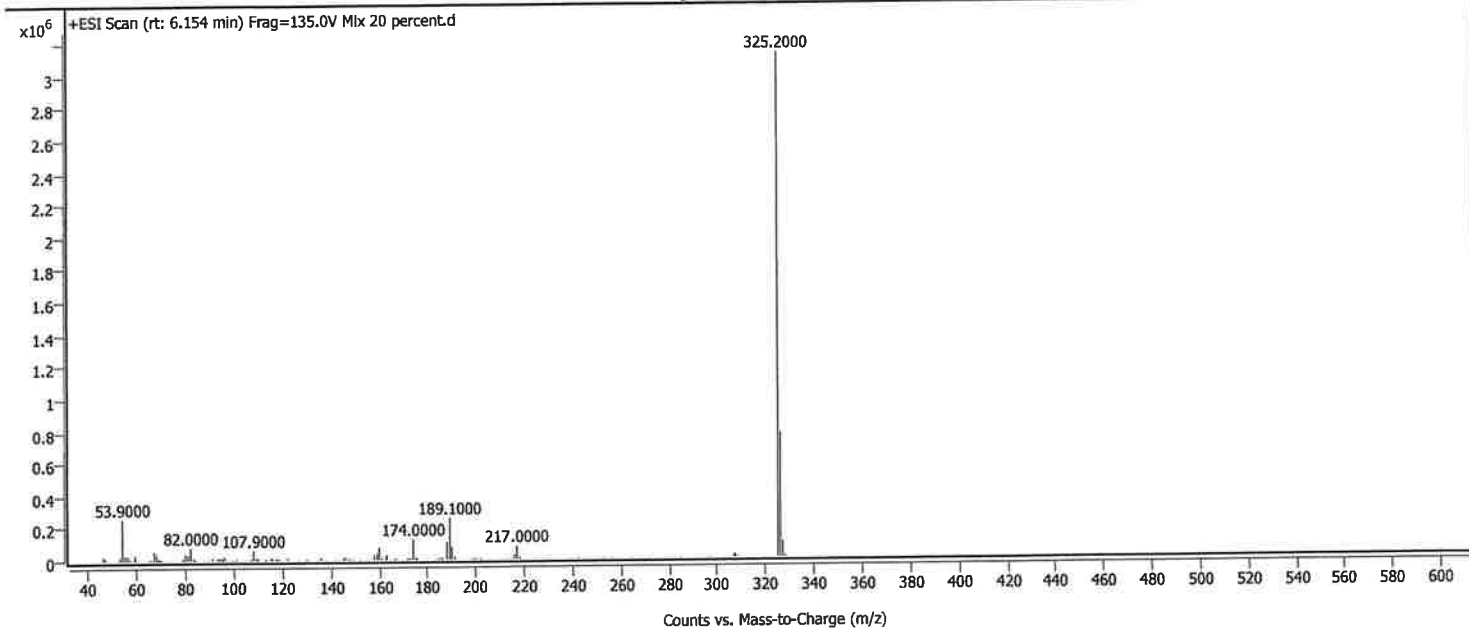
Sample Spectra

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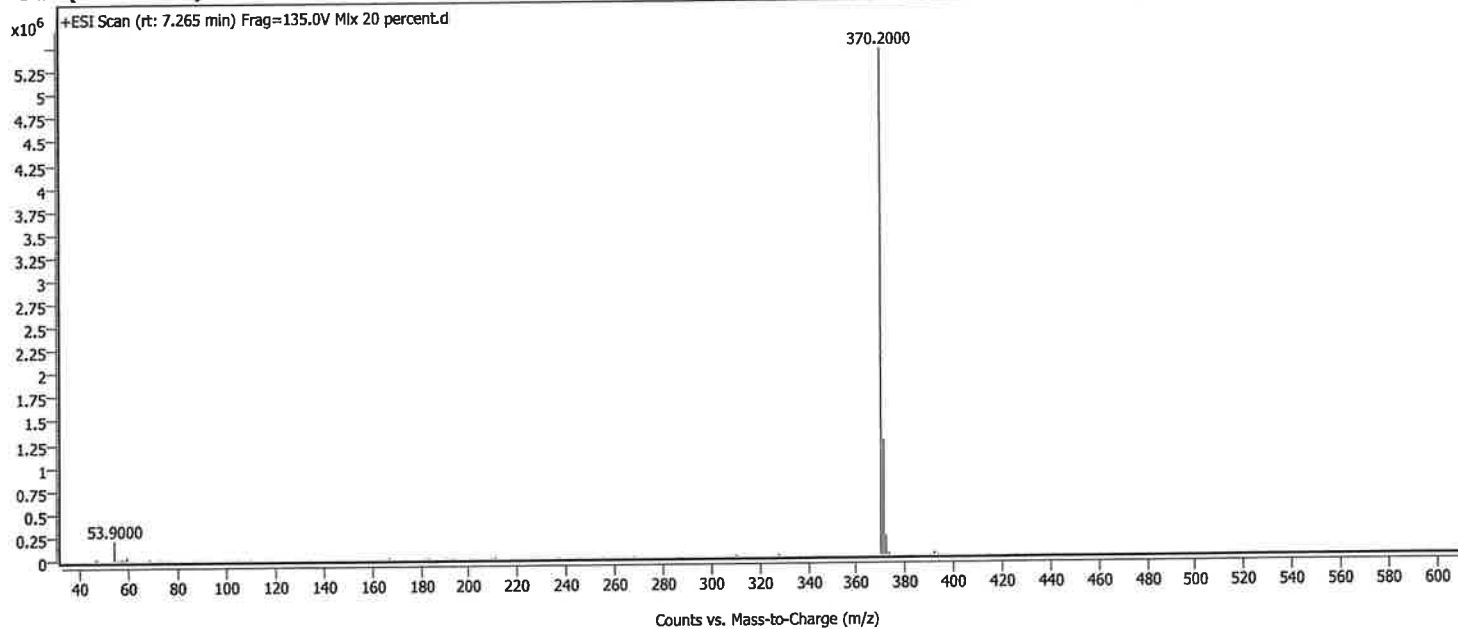


+ Scan (rt: 6.154 min)

Analysis Report



+ Scan (rt: 7.265 min)



MassHunter Qual 10.0
(End of Report)

Analysis Report

Sample Information

Name
Sample ID
Instrument
MS Type
Inj. Vol. (ul)
Position
Plate Pos.
Operator

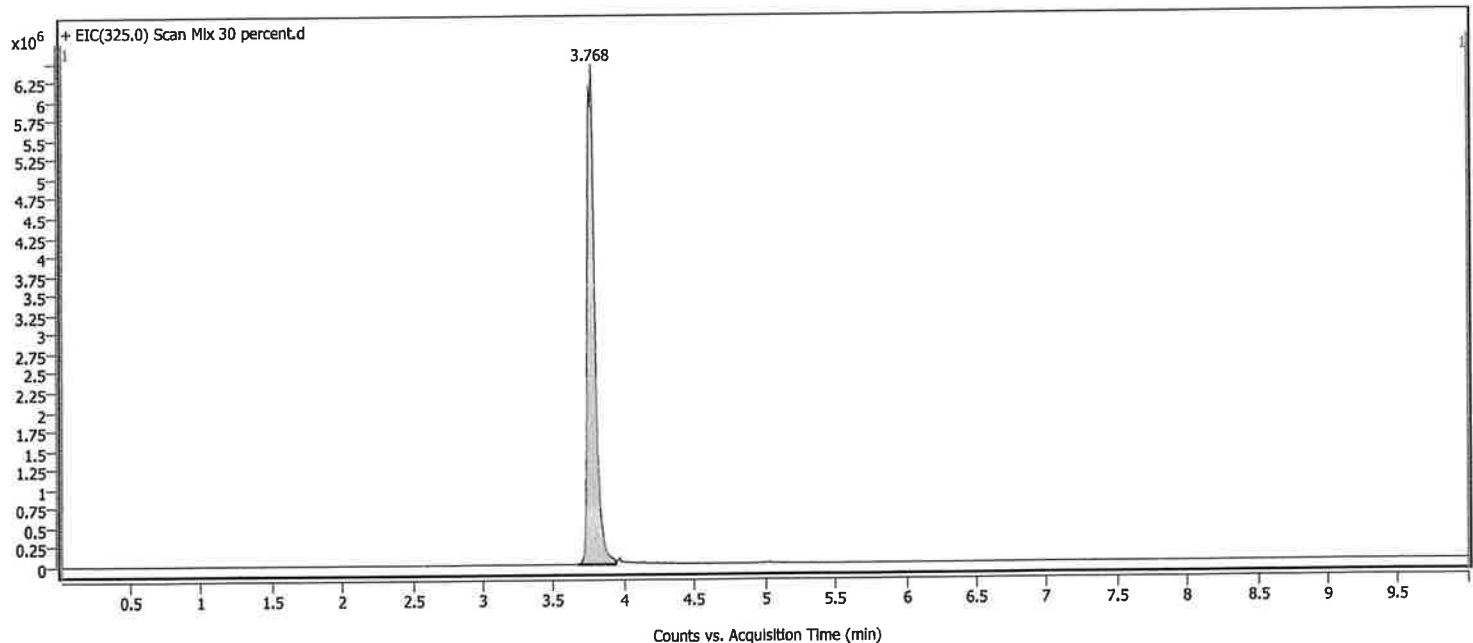
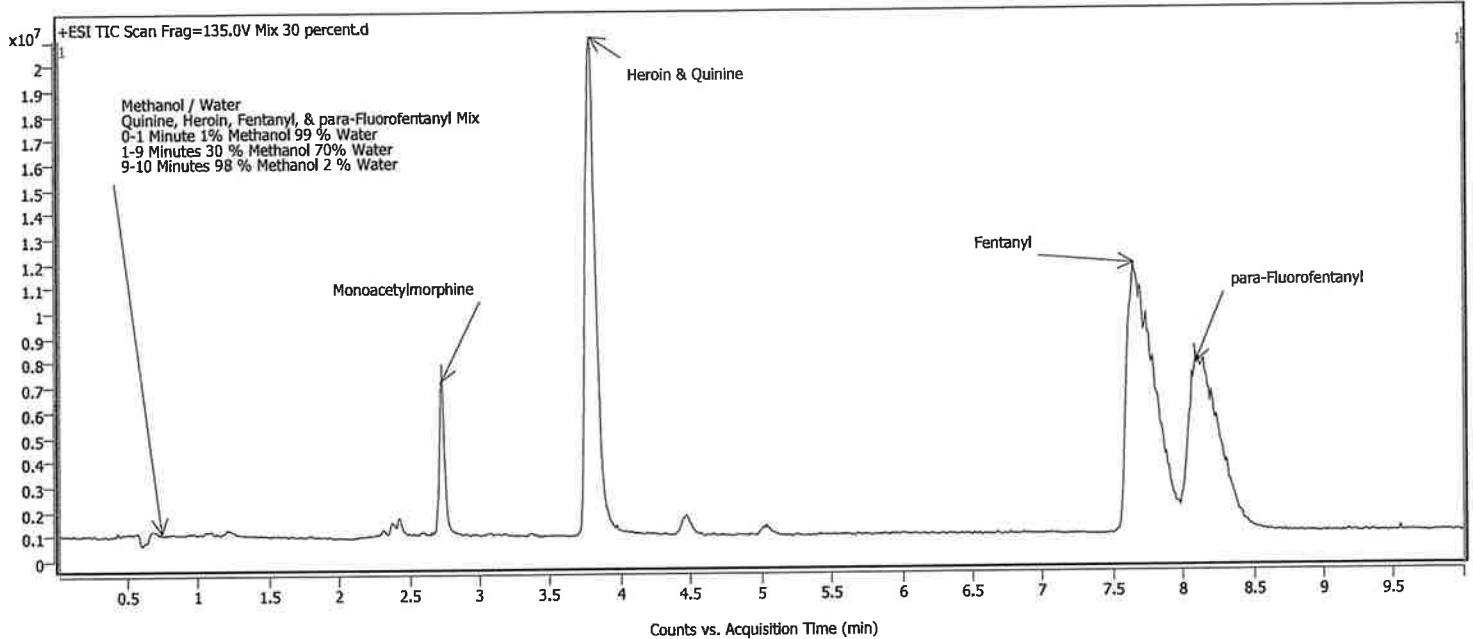
Mix Standard

Ocoee
QQQ
5
P2-F2

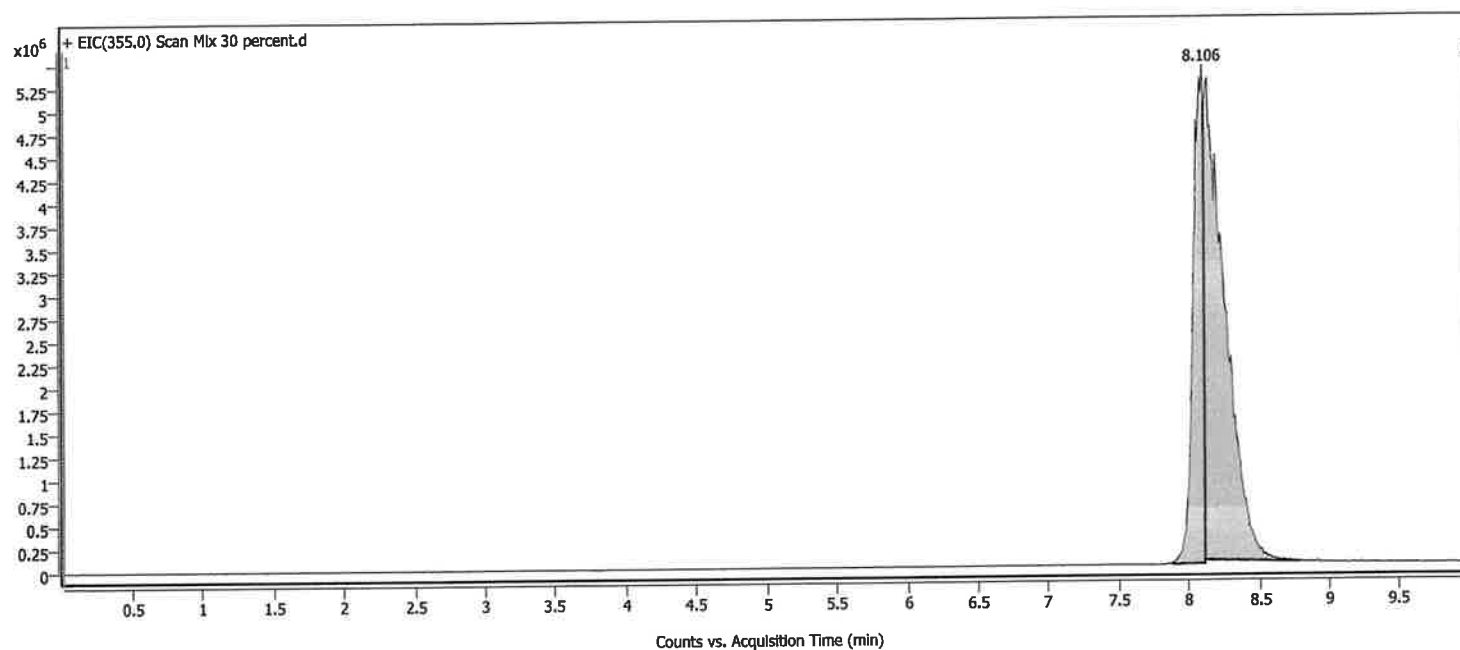
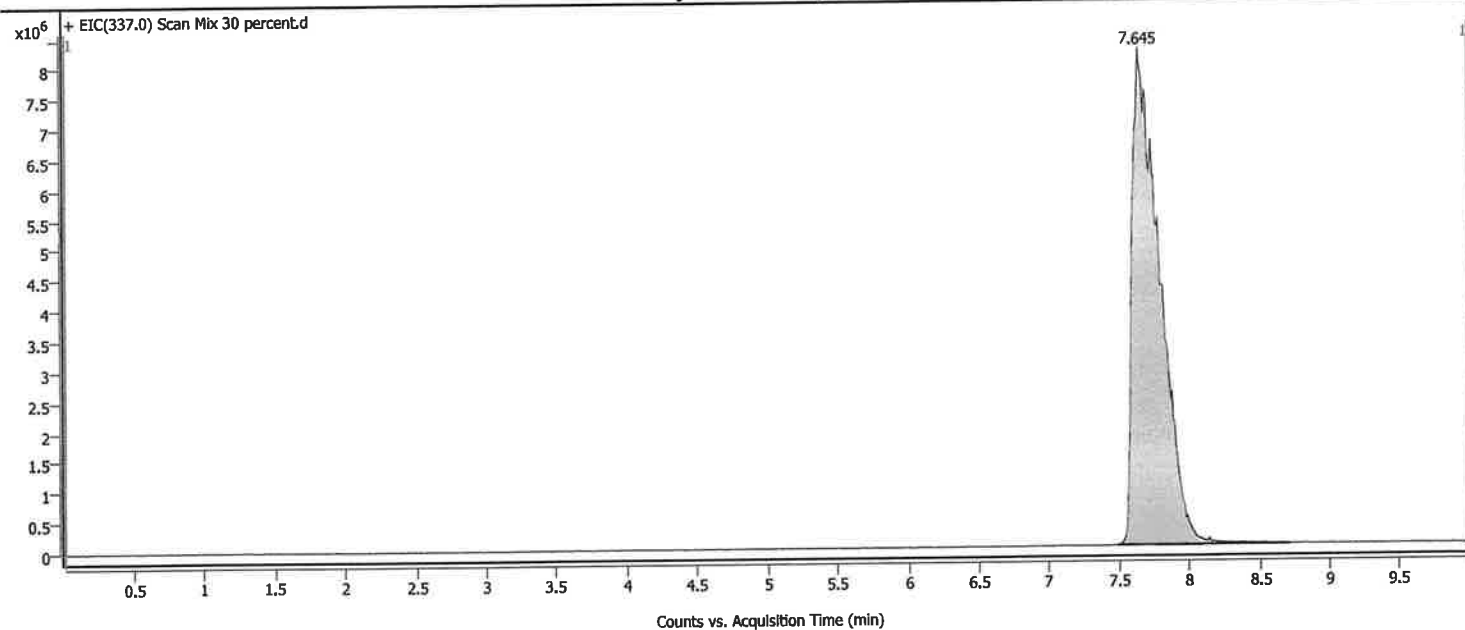
Data File Path
Acq. Time (Local)
Method Path (Acq)
Version (Acq SW)
IRM Status
Method Path (DA)
Target Source Path
Result Summary

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Ultivo LC/TQ 1.2 (23)

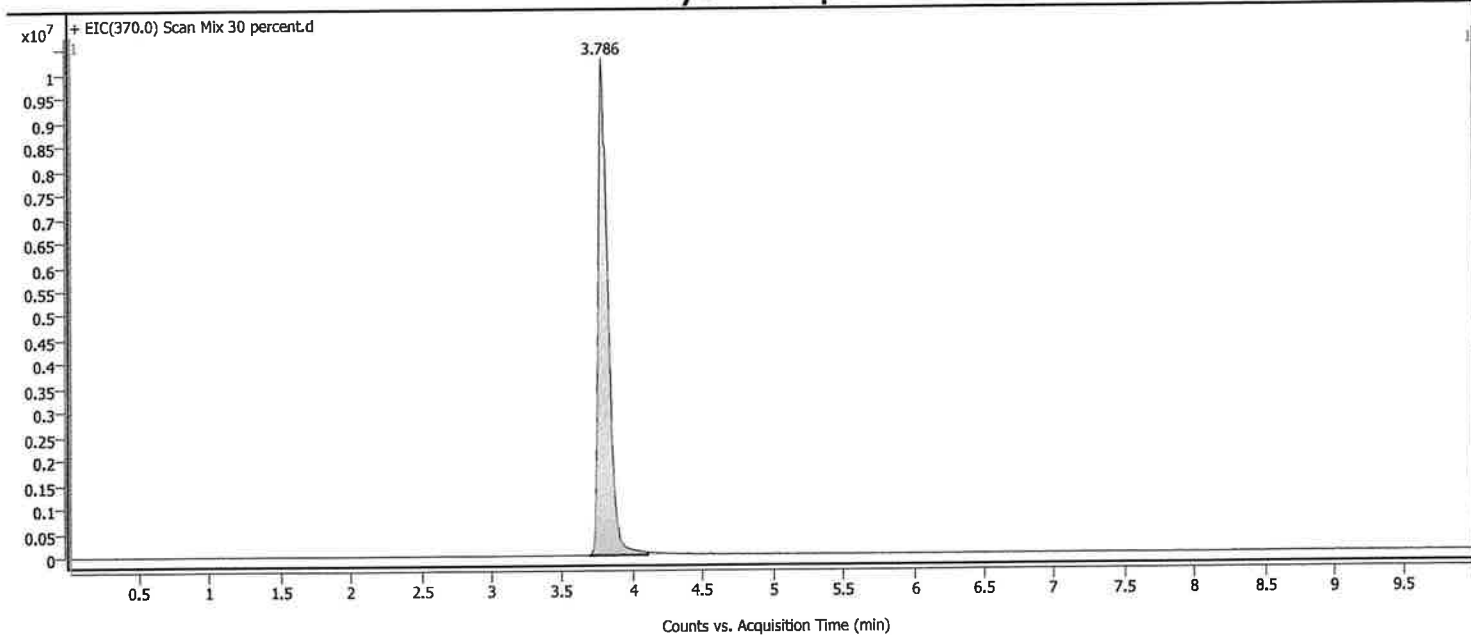
Sample Chromatograms



Analysis Report

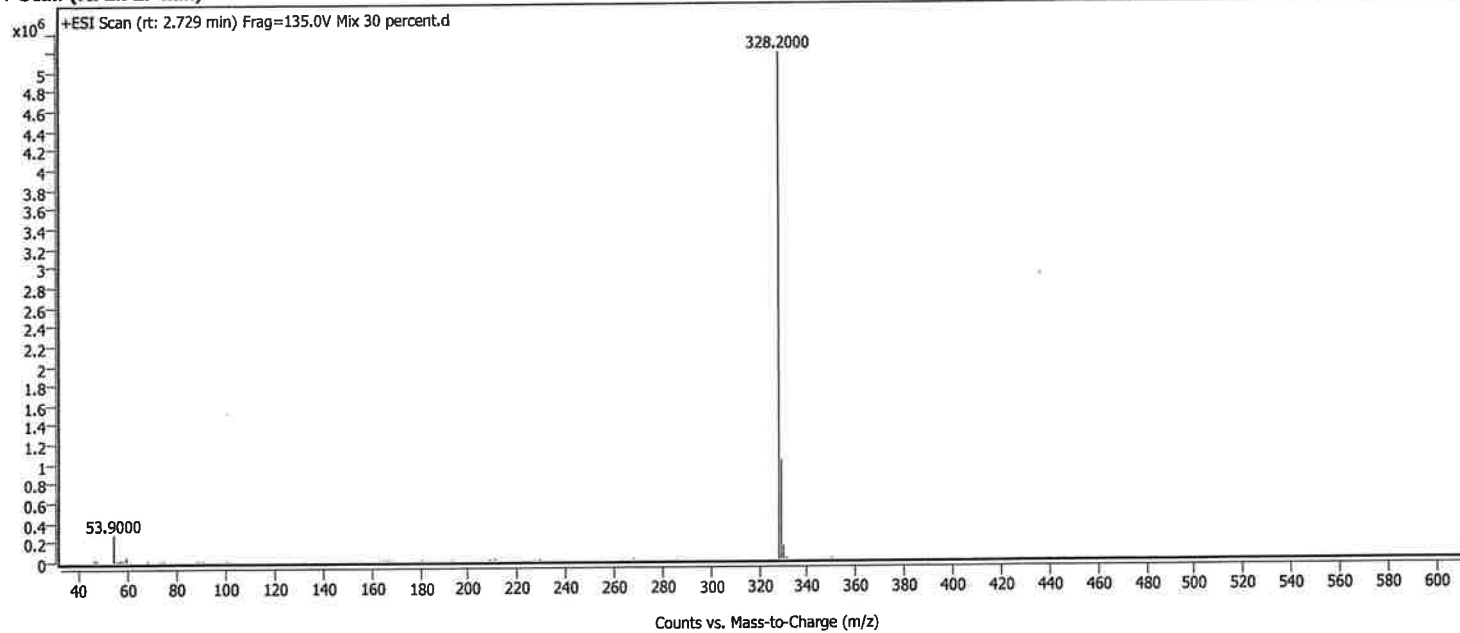


Analysis Report



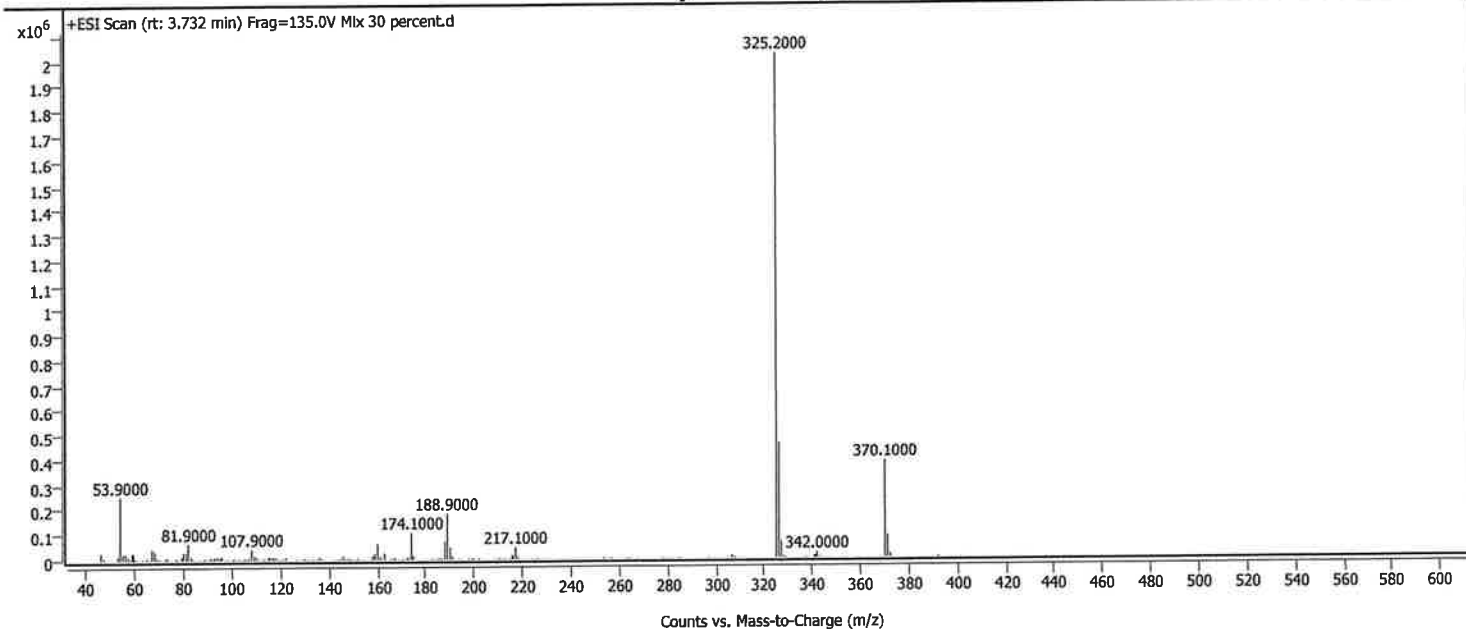
Sample Spectra

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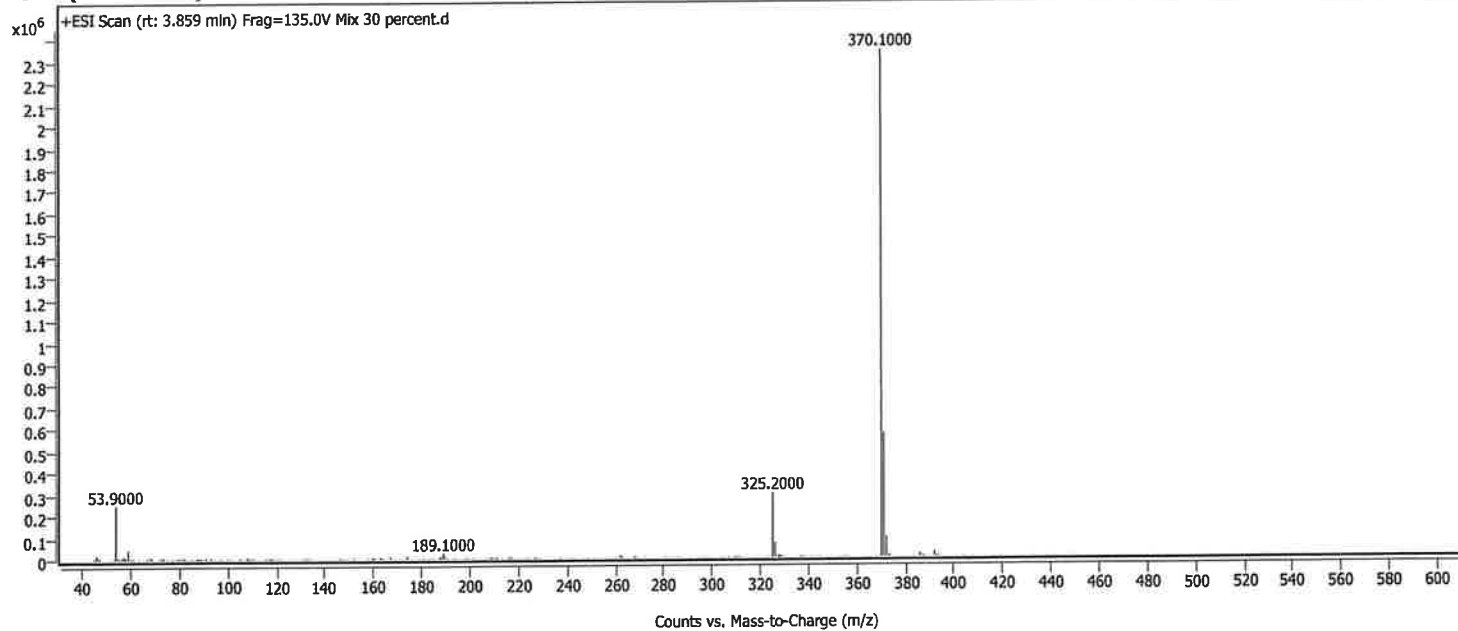


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Analysis Report

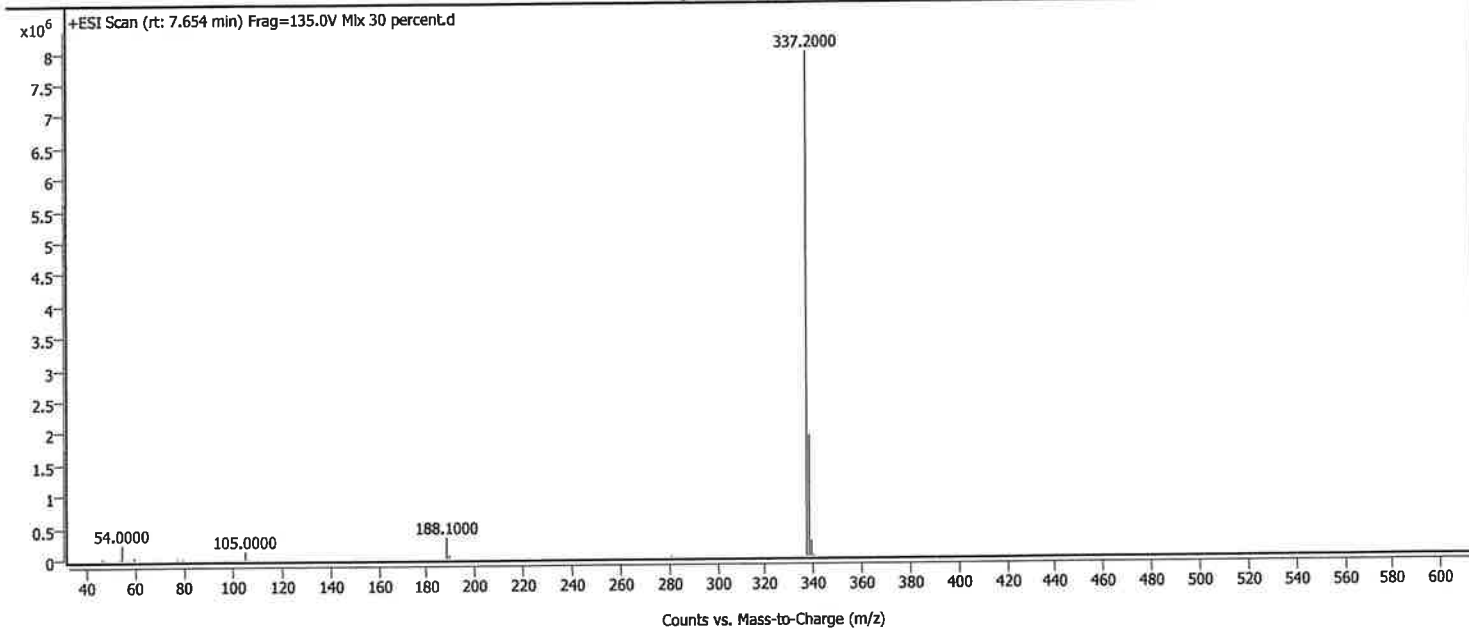


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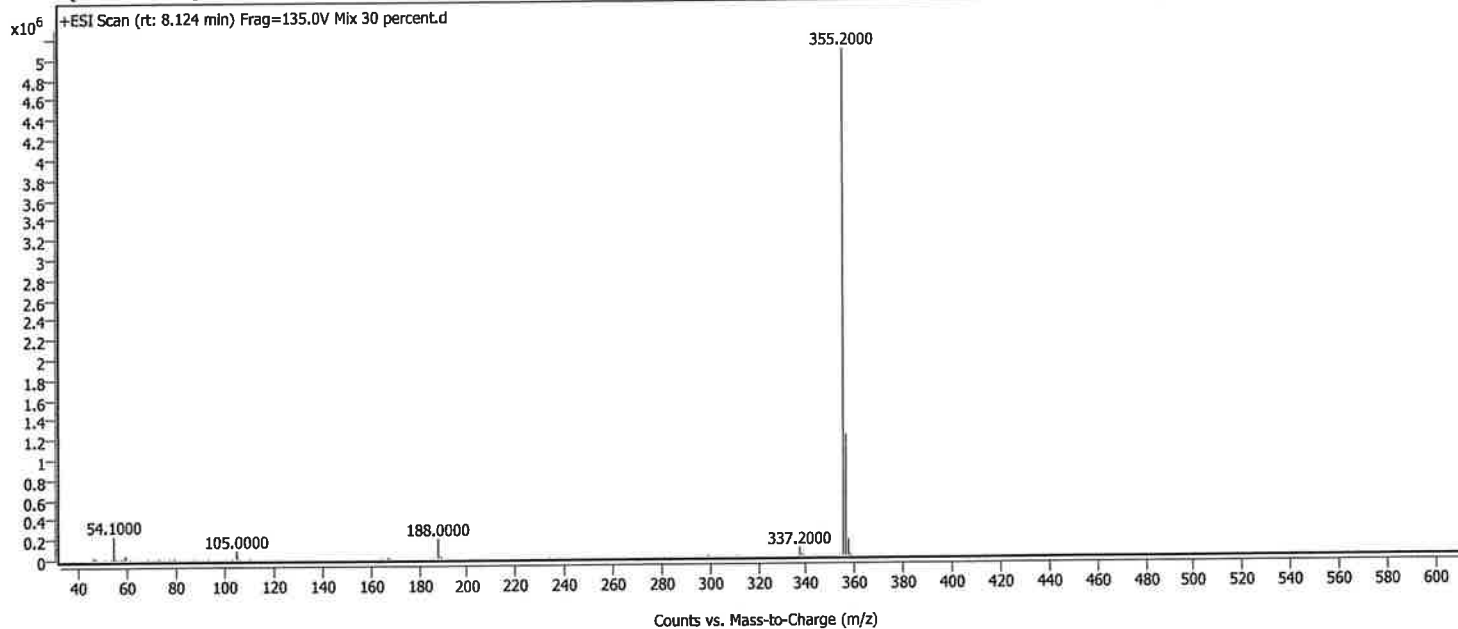


+ Scan (rt: 7.654 min)

Analysis Report



+ Scan (rt: 8.124 min)



MassHunter Qual 10.0
(End of Report)

Analysis Report

Sample Information

Name
Sample ID
Instrument
MS Type
Inj. Vol. (ul)
Position
Plate Pos.
Operator

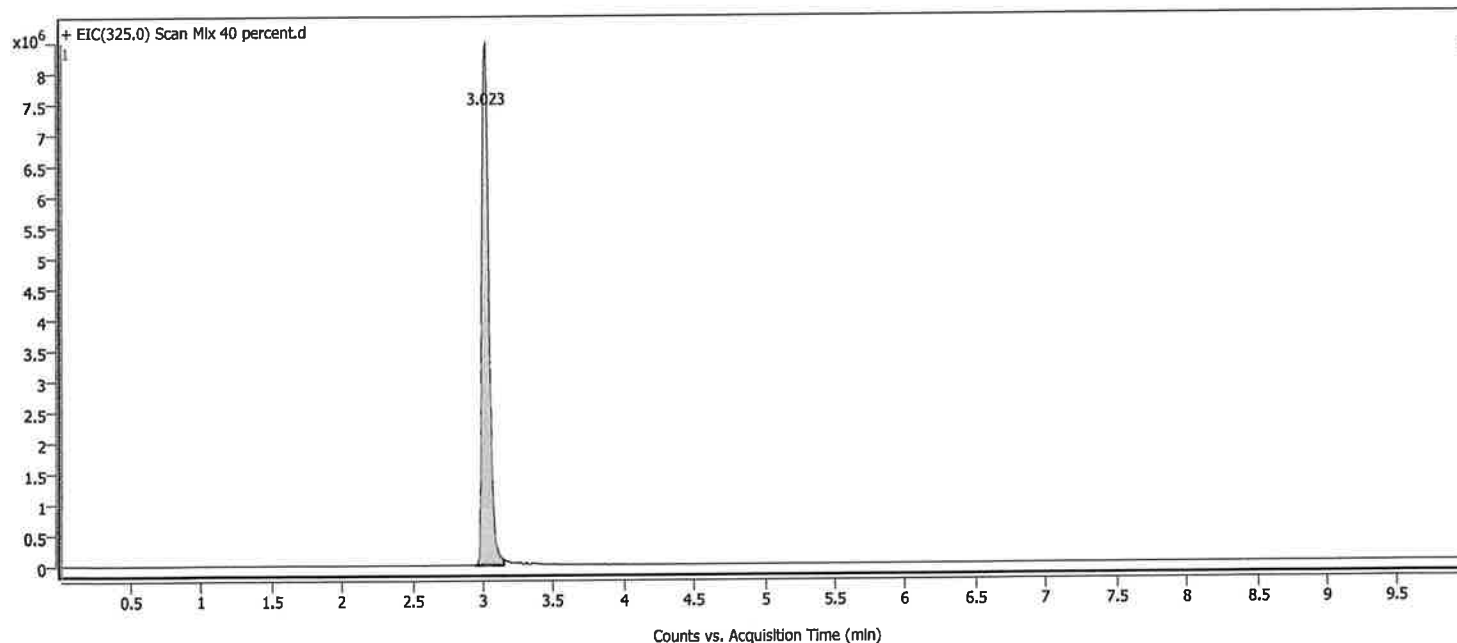
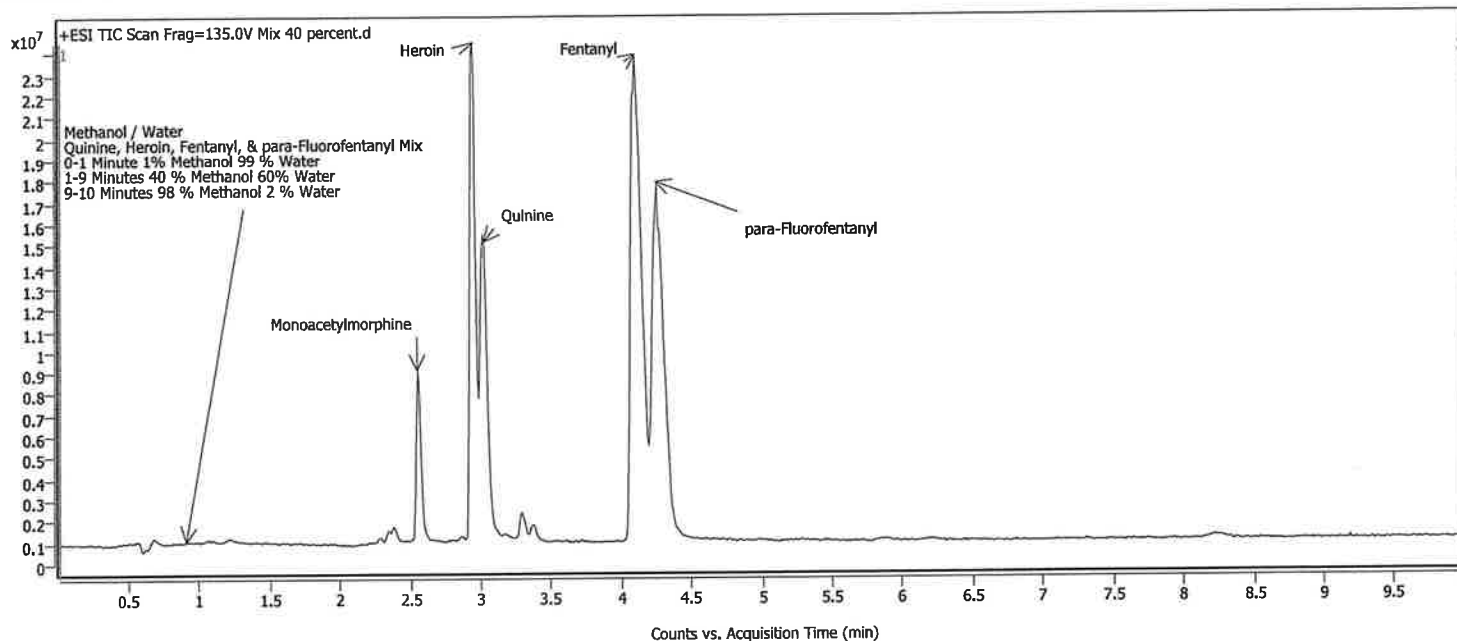
Mix Standard

Ocoee
QQQ
5
P2-F2

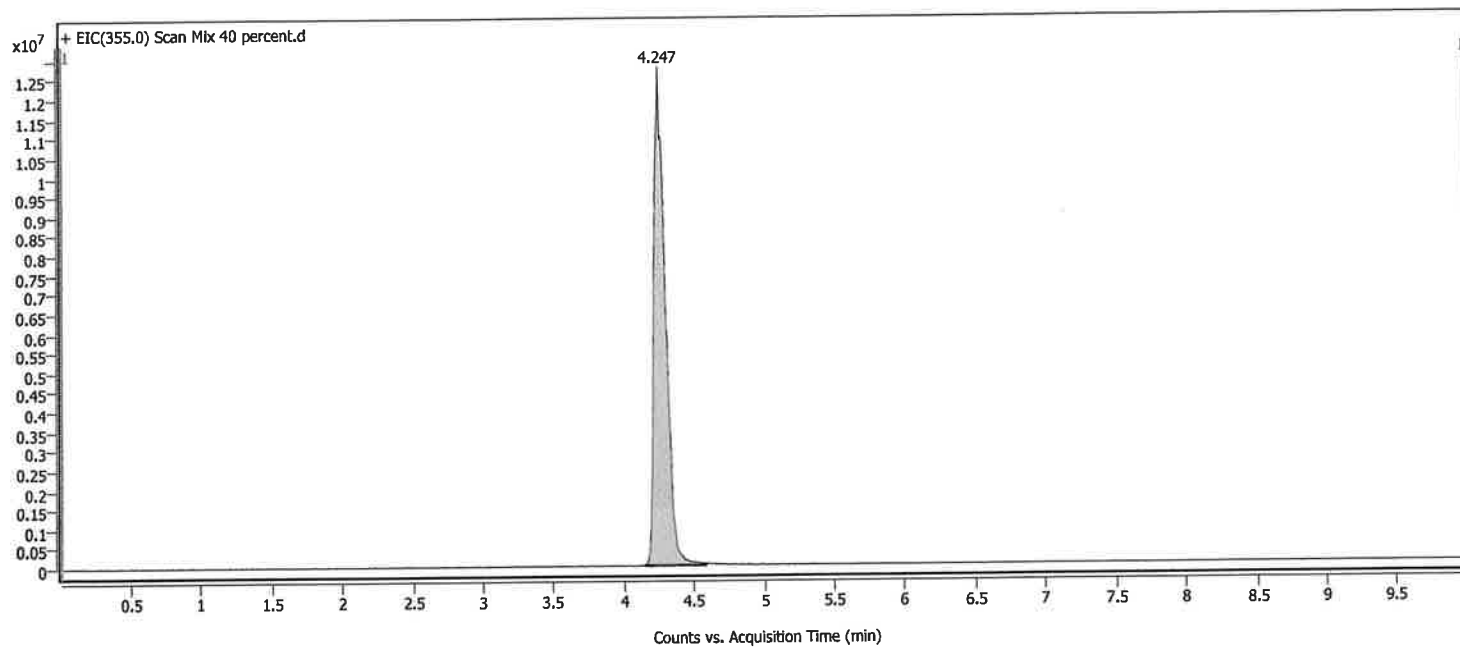
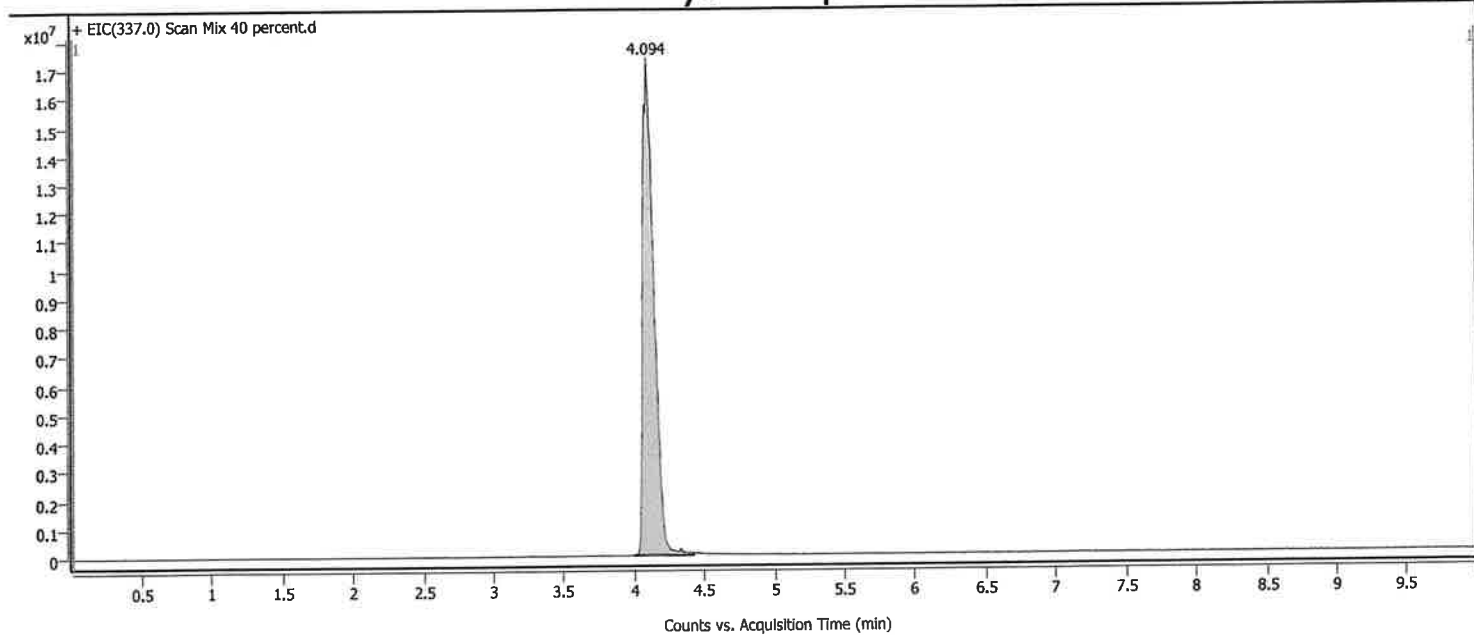
Data File Path
Acq. Time (Local)
Method Path (Acq)
Version (Acq SW)
IRM Status
Method Path (DA)
Target Source Path
Result Summary

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Ultivo LC/TQ 1.2 (23)

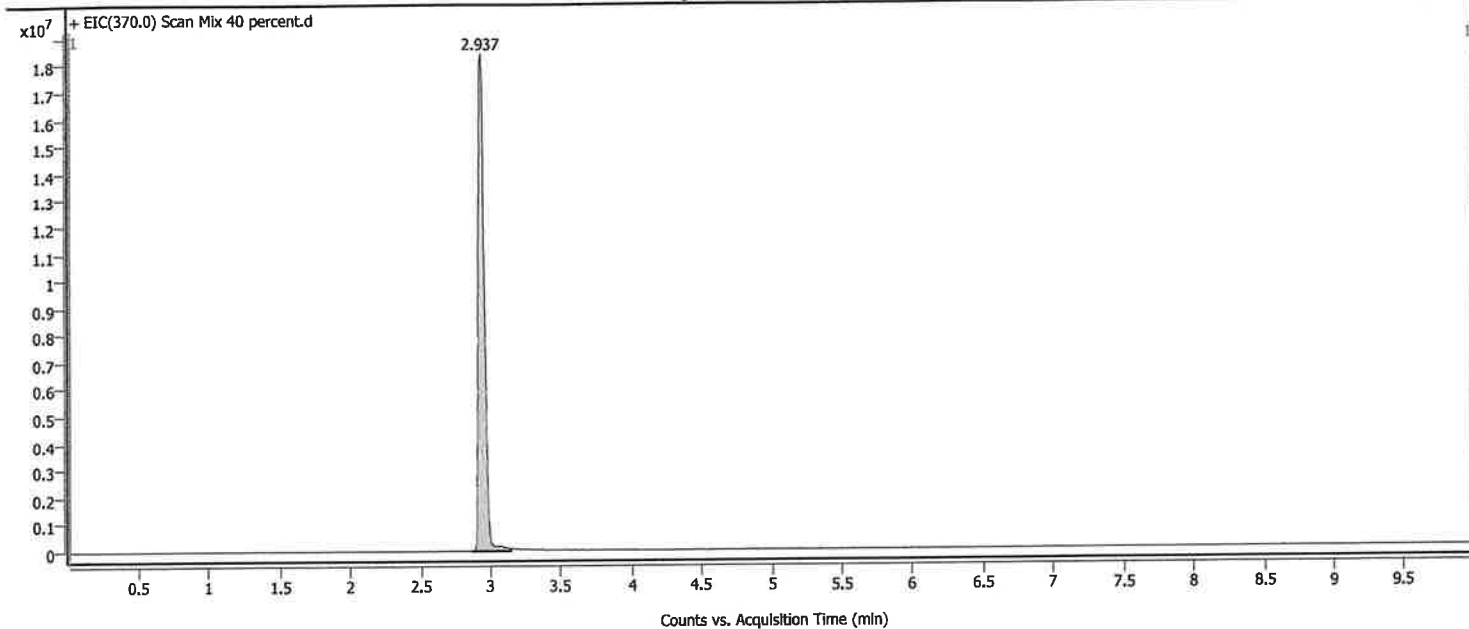
Sample Chromatograms



Analysis Report

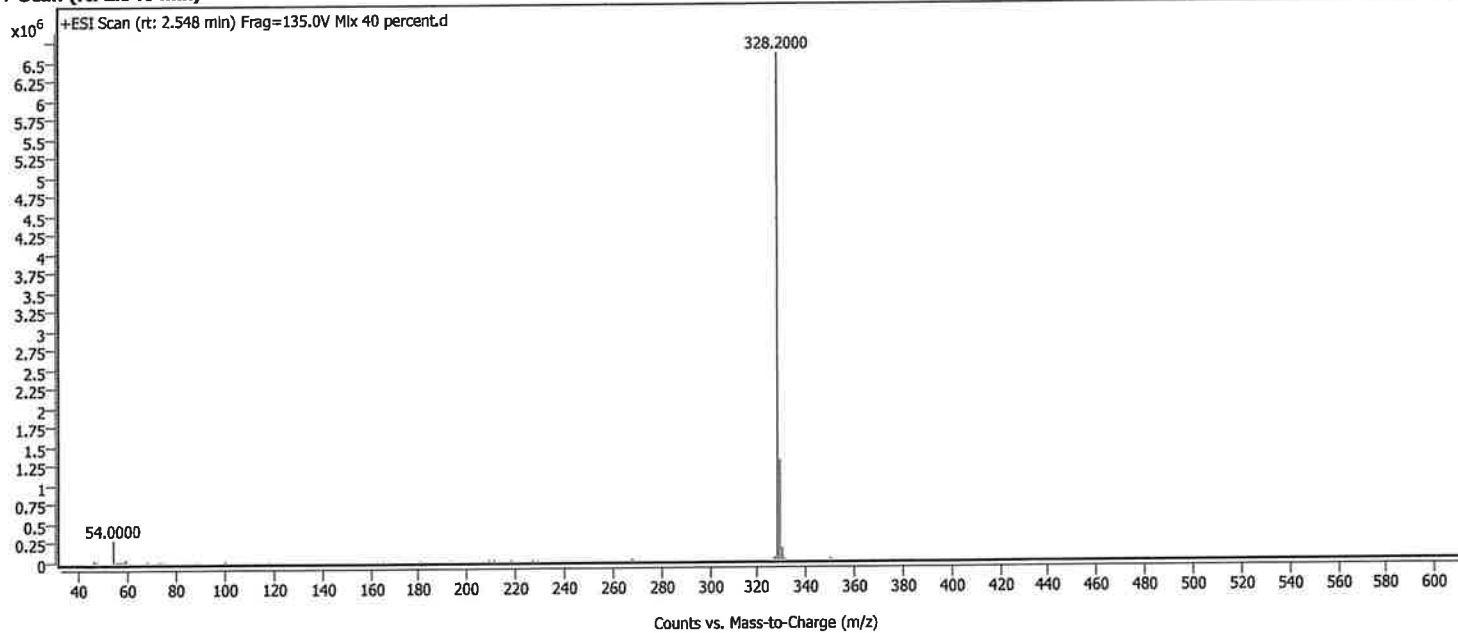


Analysis Report



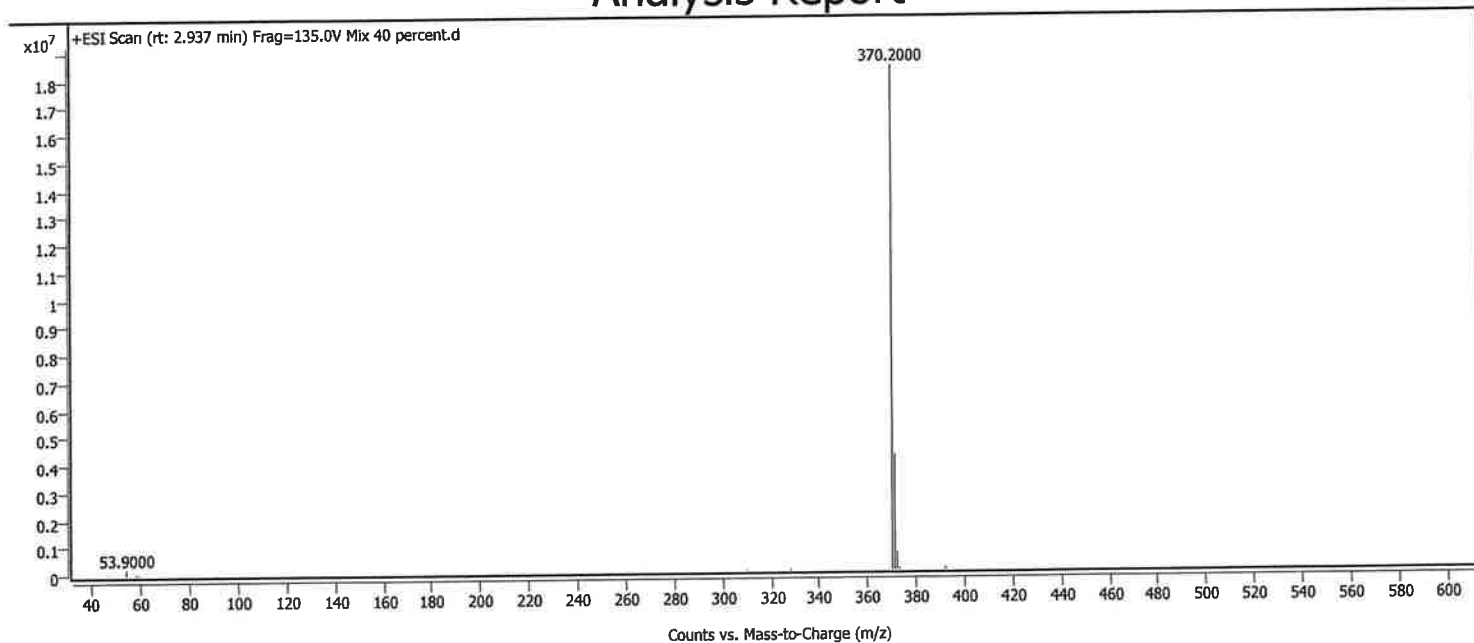
Sample Spectra

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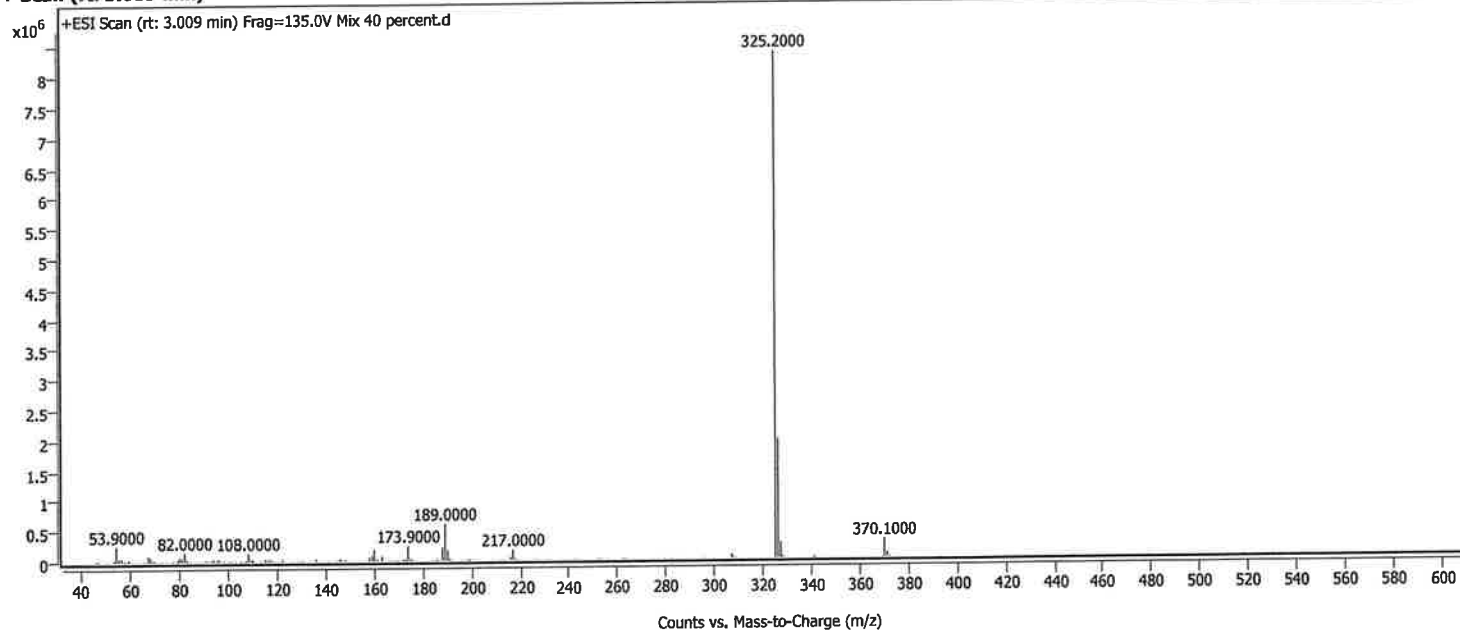


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Analysis Report

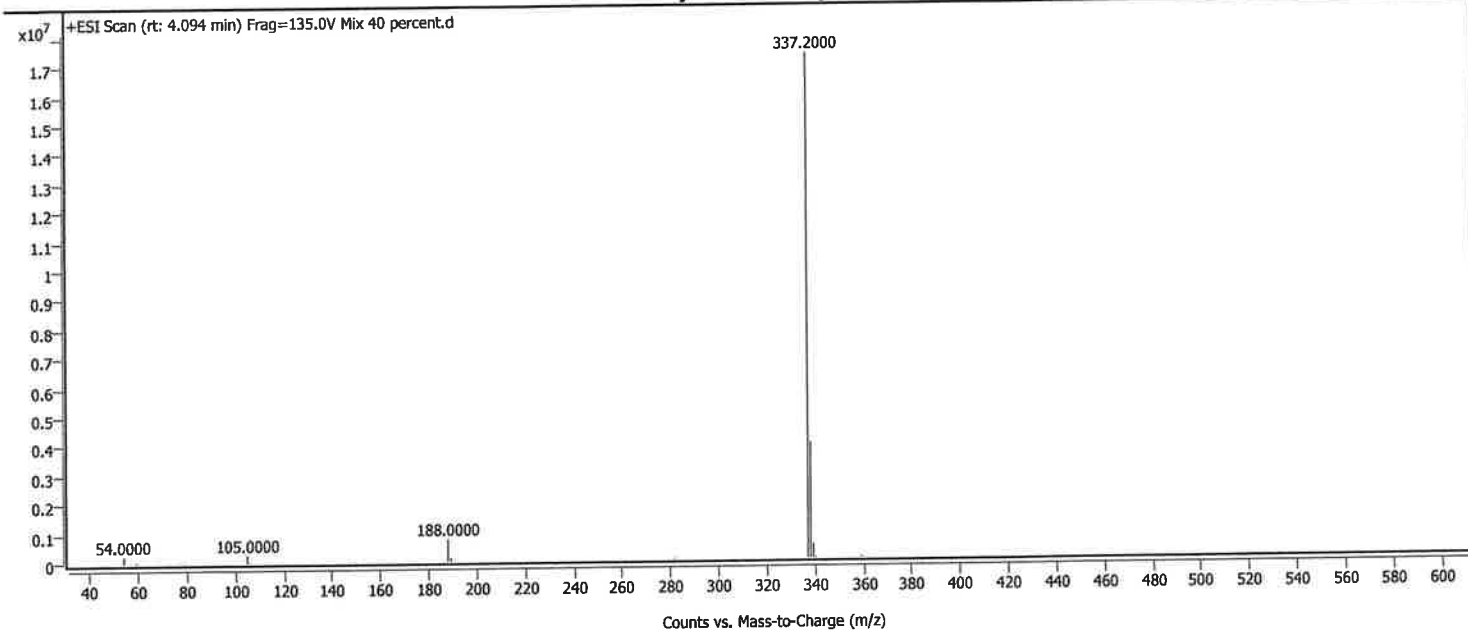


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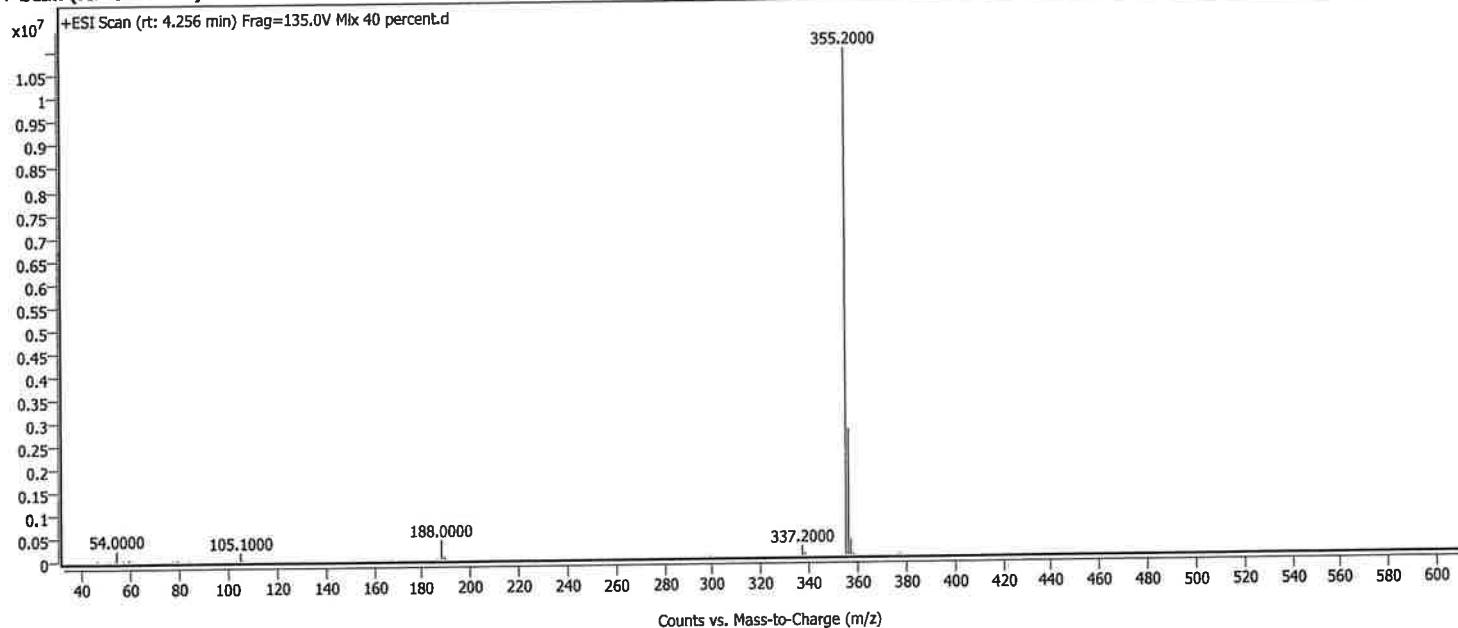


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Analysis Report



+ Scan (rt: 4.256 min)



MassHunter Qual 10.0
(End of Report)

Analysis Report

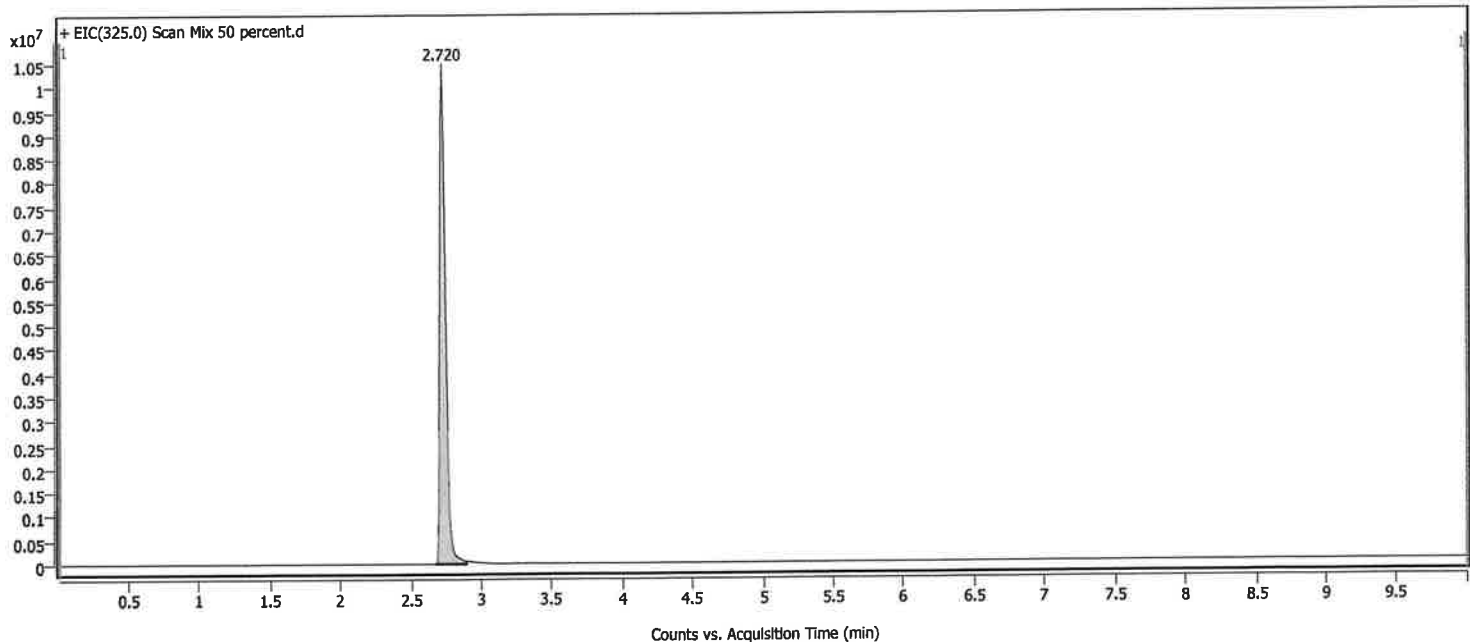
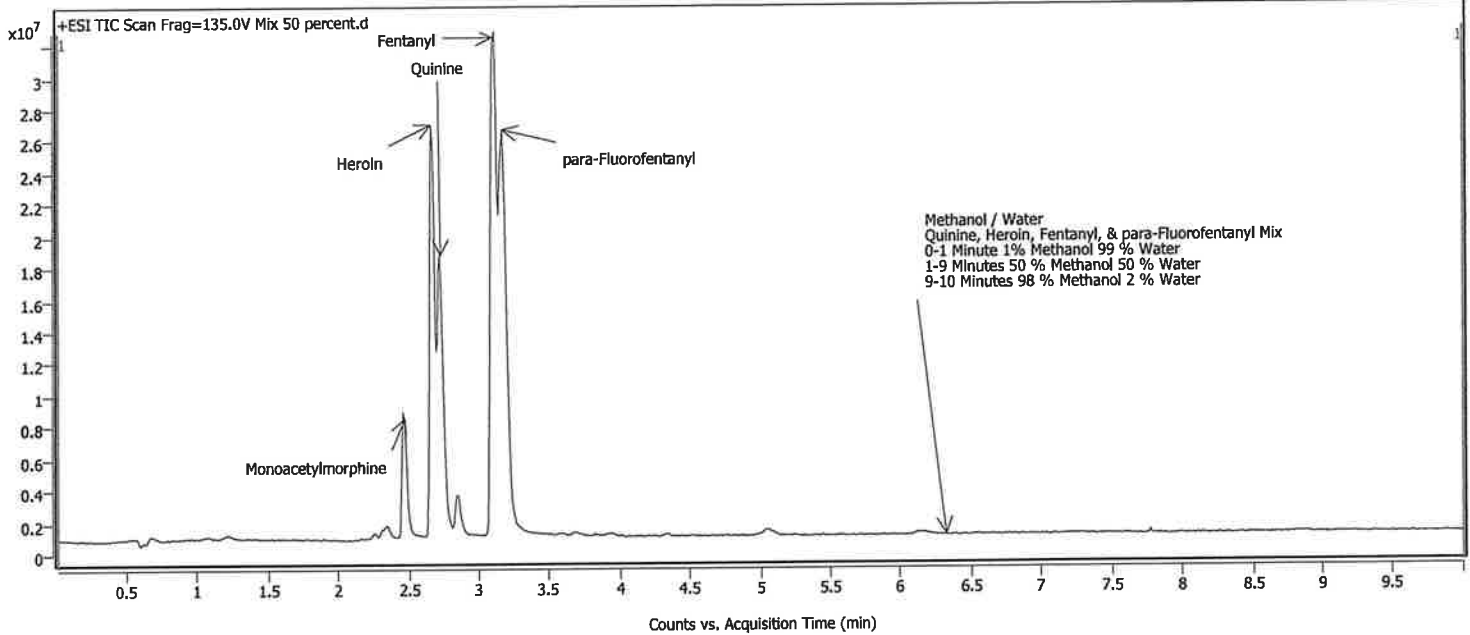
Sample Information

Name Mix Standard
Sample ID
Instrument Ocoee
MS Type QQQ
Inj. Vol. (ul) 5
Position P2-F2
Plate Pos.
Operator

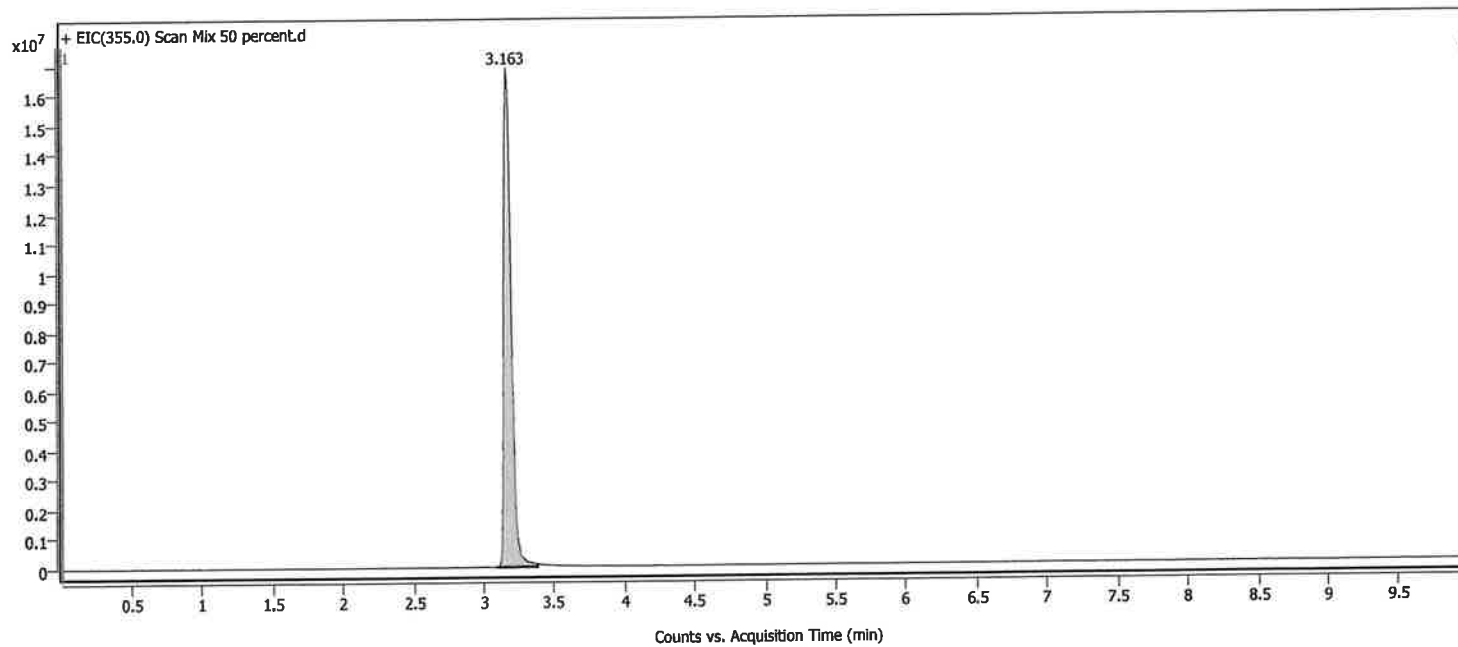
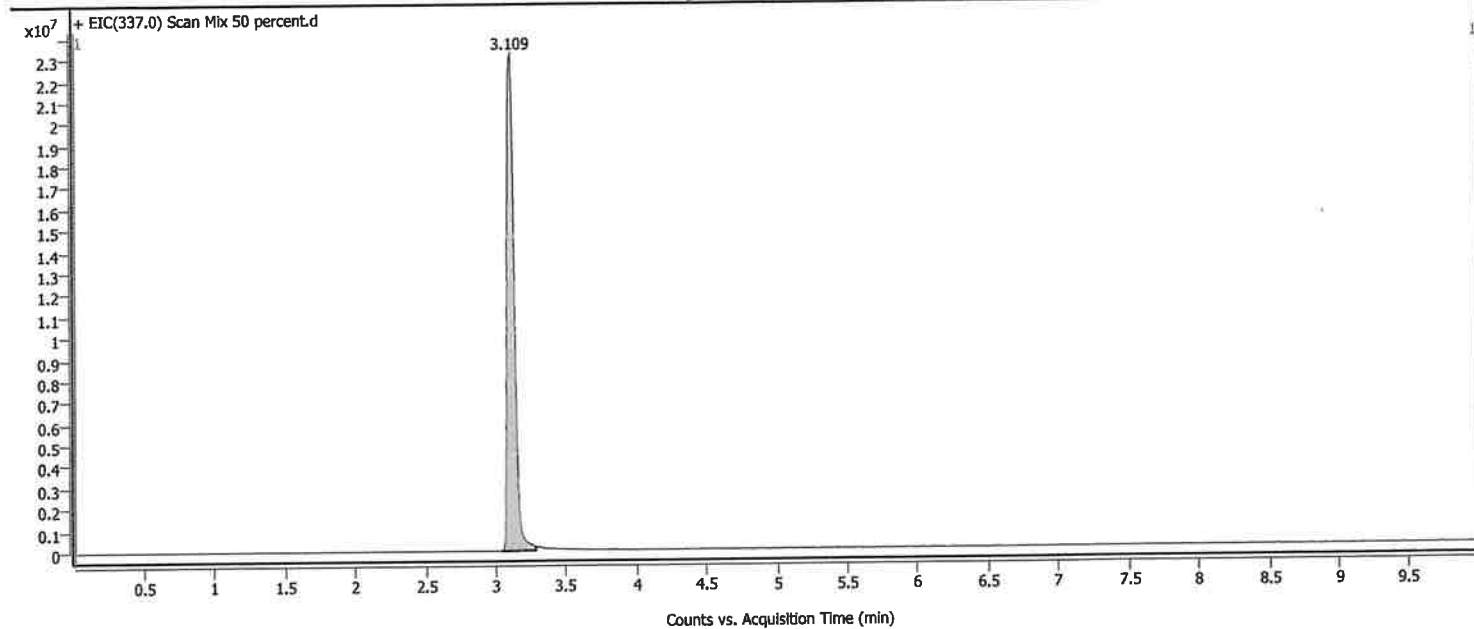
Data File Path
Acq. Time (Local)
Method Path (Acq)
Version (Acq SW)
IRM Status
Method Path (DA)
Target Source Path
Result Summary

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Ultivo LC/TQ 1.2 (23)

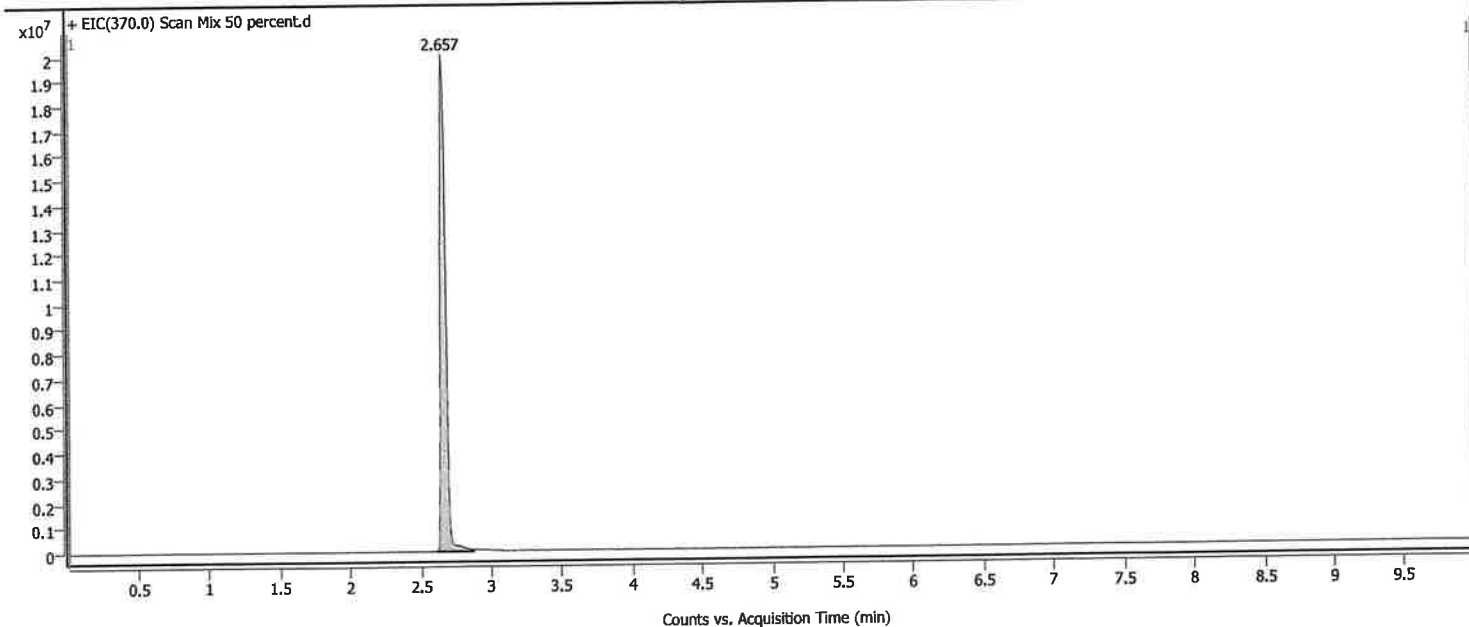
Sample Chromatograms



Analysis Report

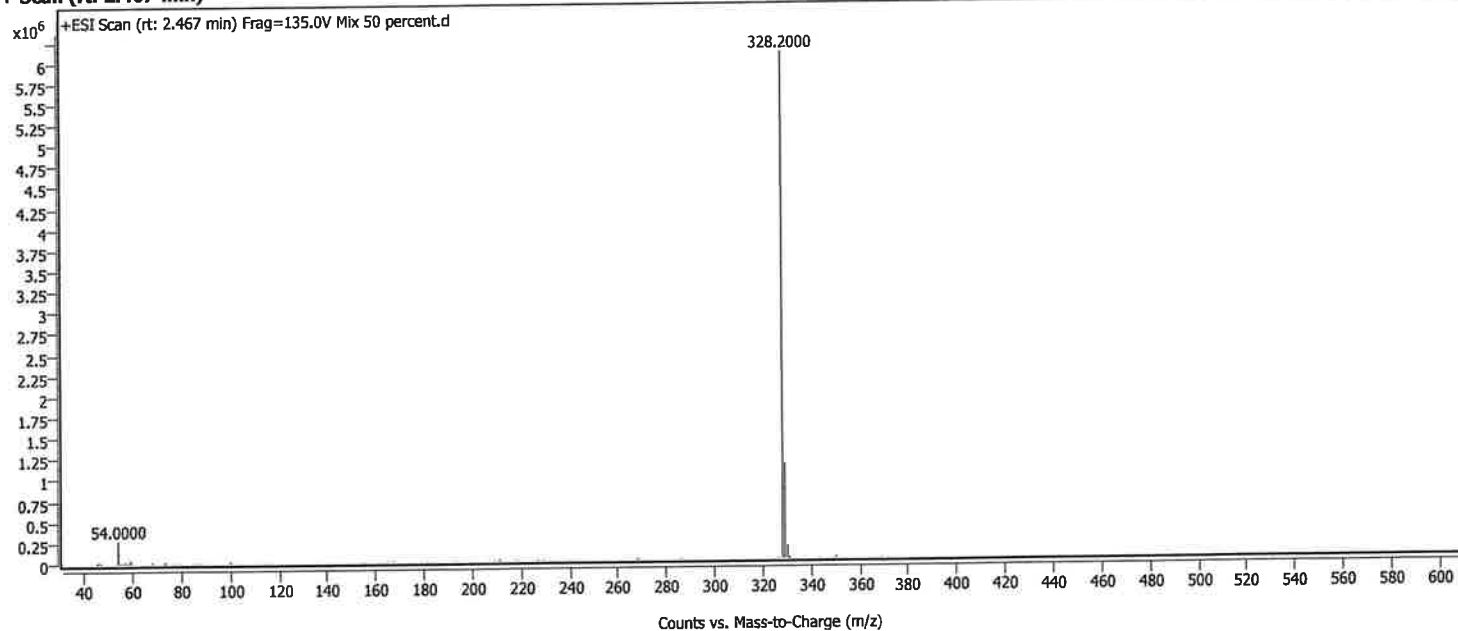


Analysis Report



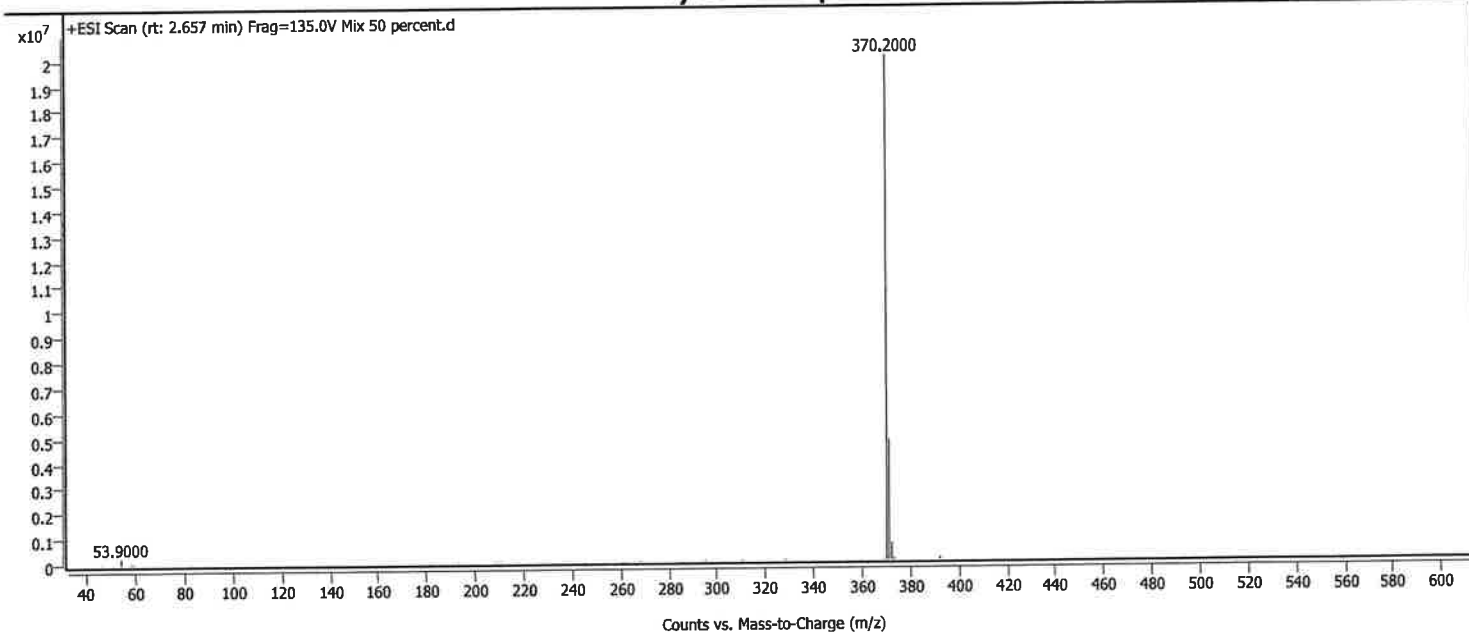
Sample Spectra

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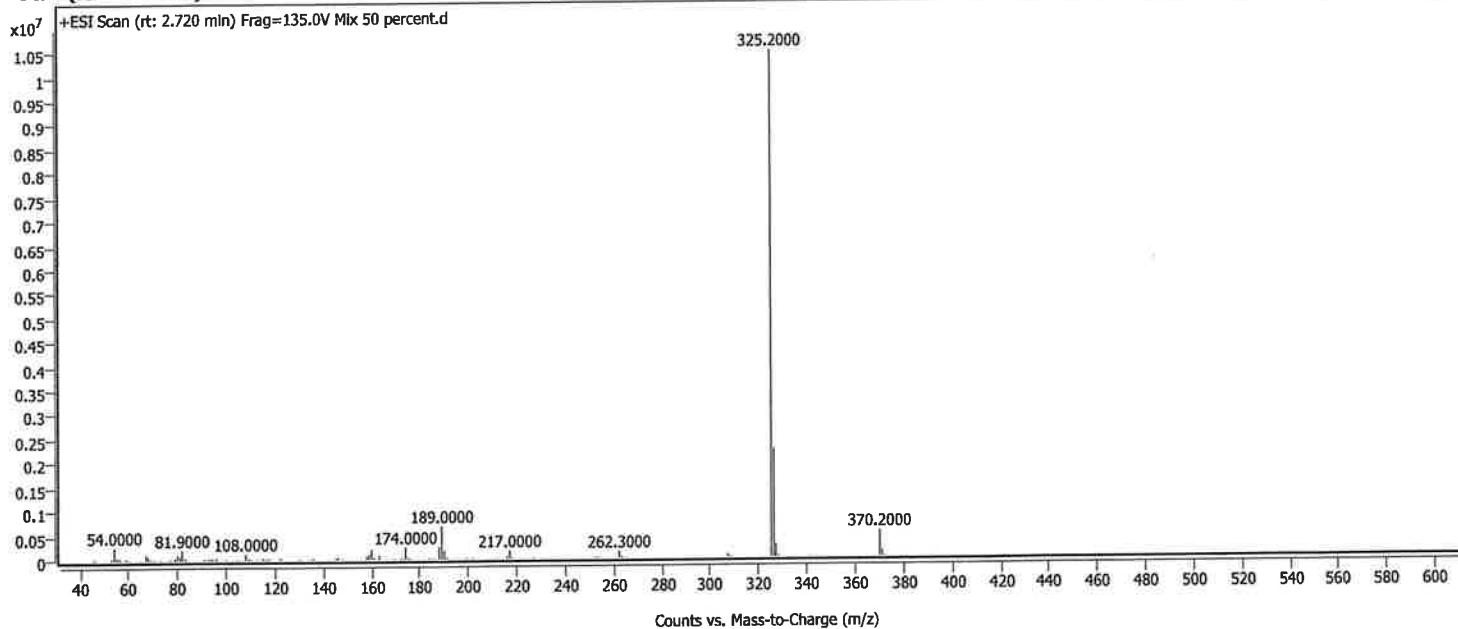


+ Scan (rt: 2.657 min)

Analysis Report

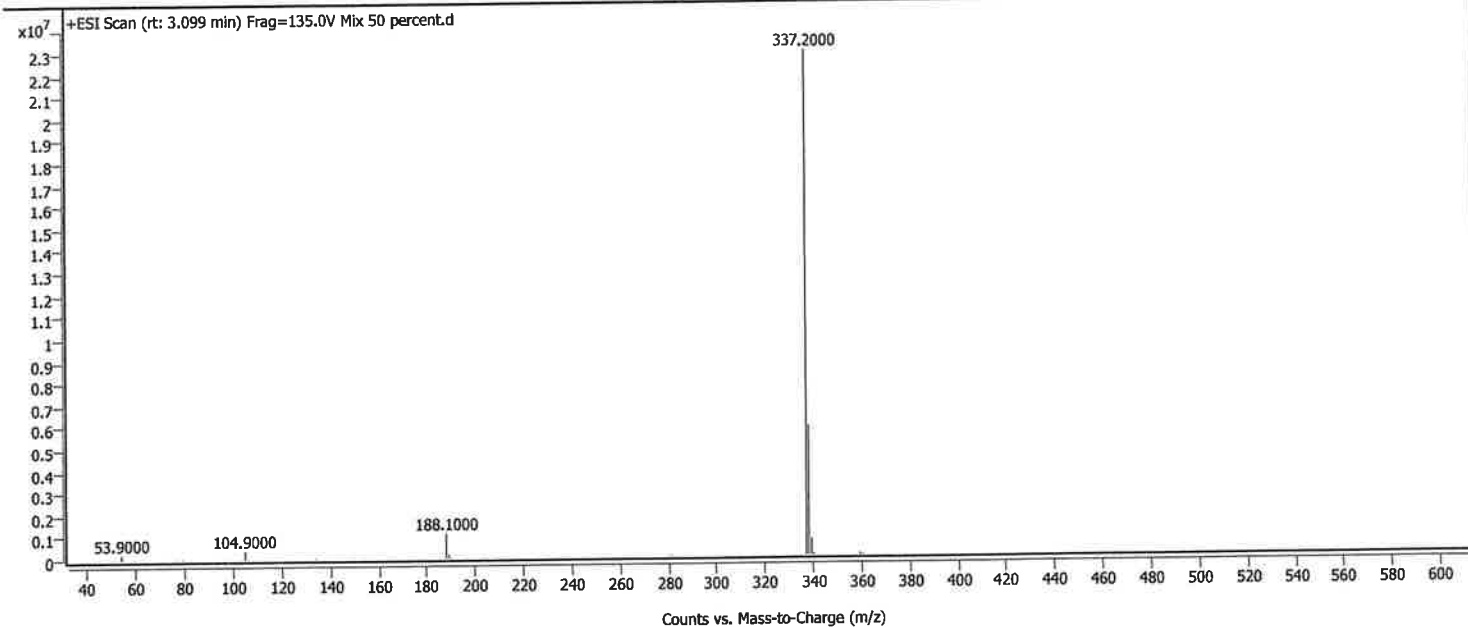


+ Scan (rt: 2.720 min)

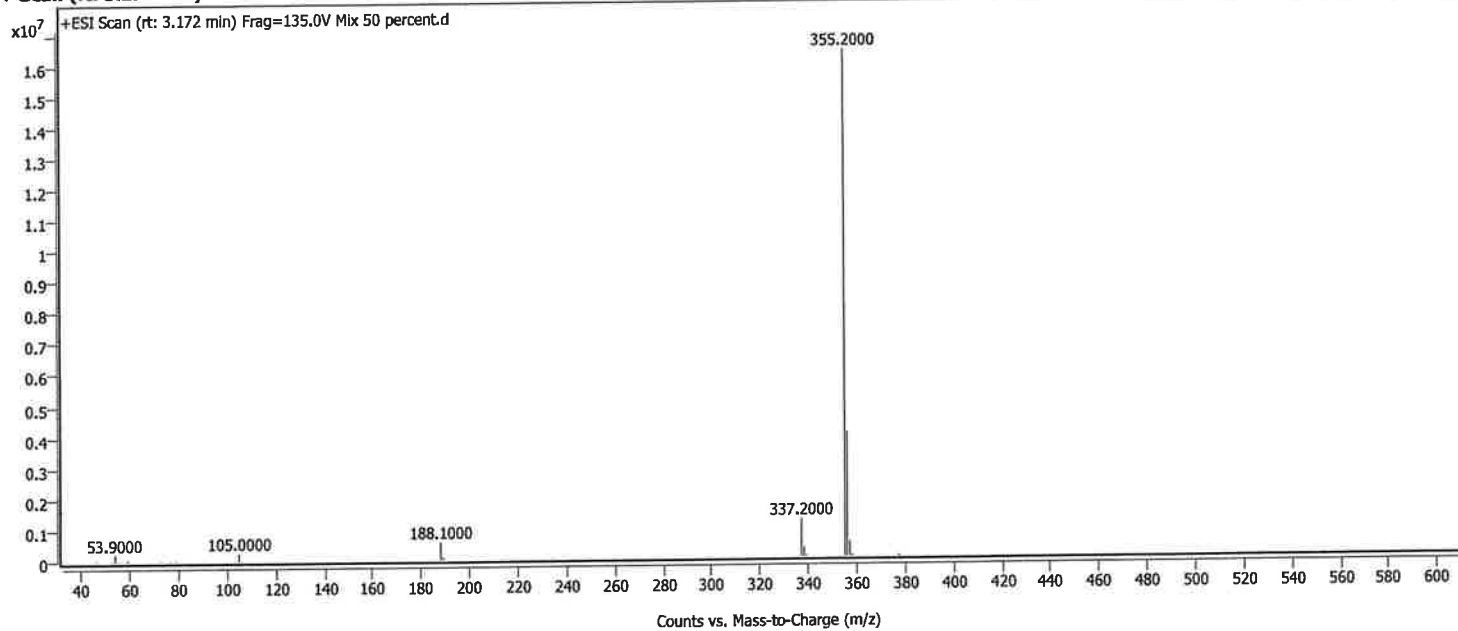


+ Scan (rt: 3.099 min)

Analysis Report



+ Scan (rt: 3.172 min)



MassHunter Qual 10.0
(End of Report)

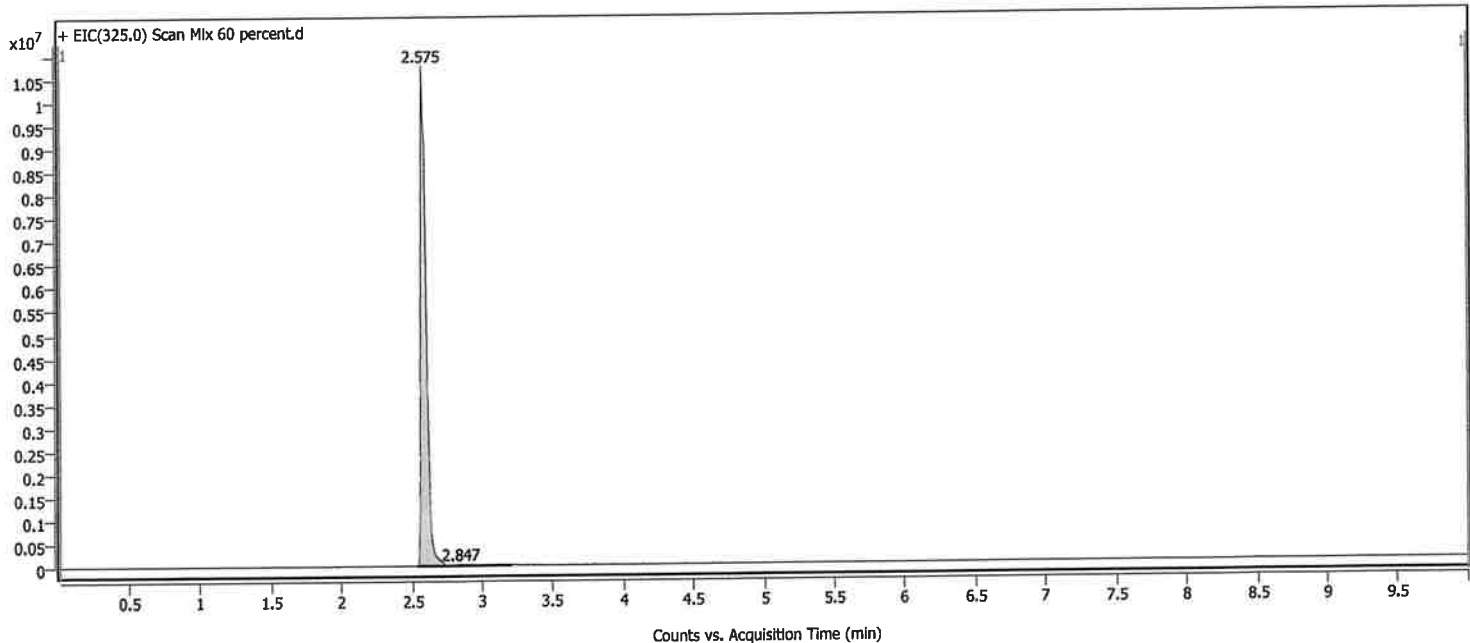
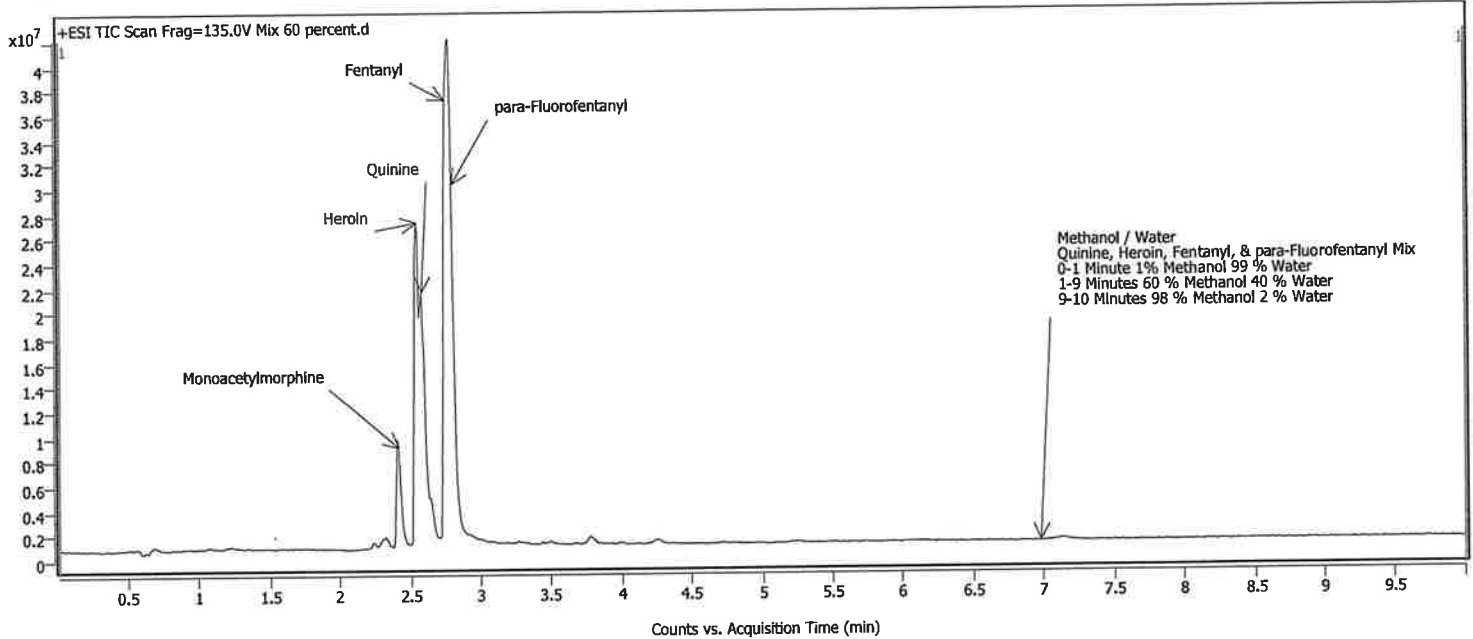
Analysis Report

Sample Information

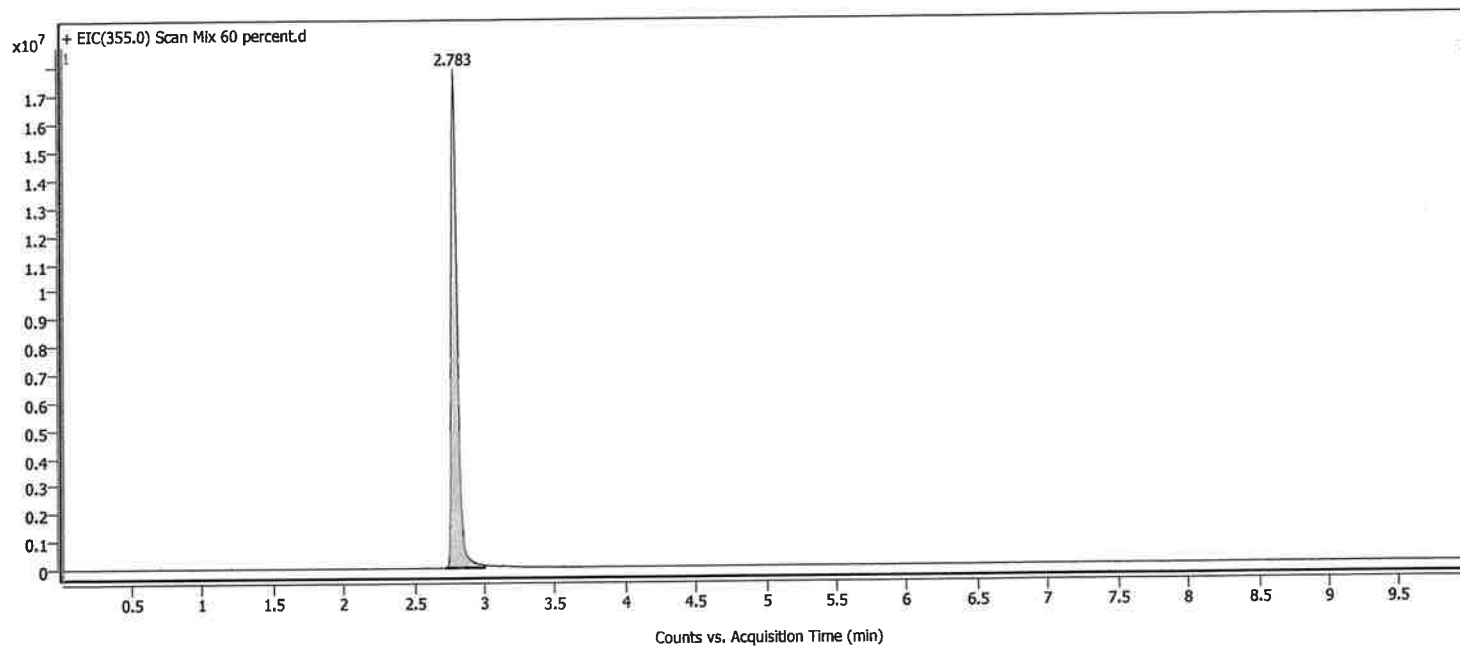
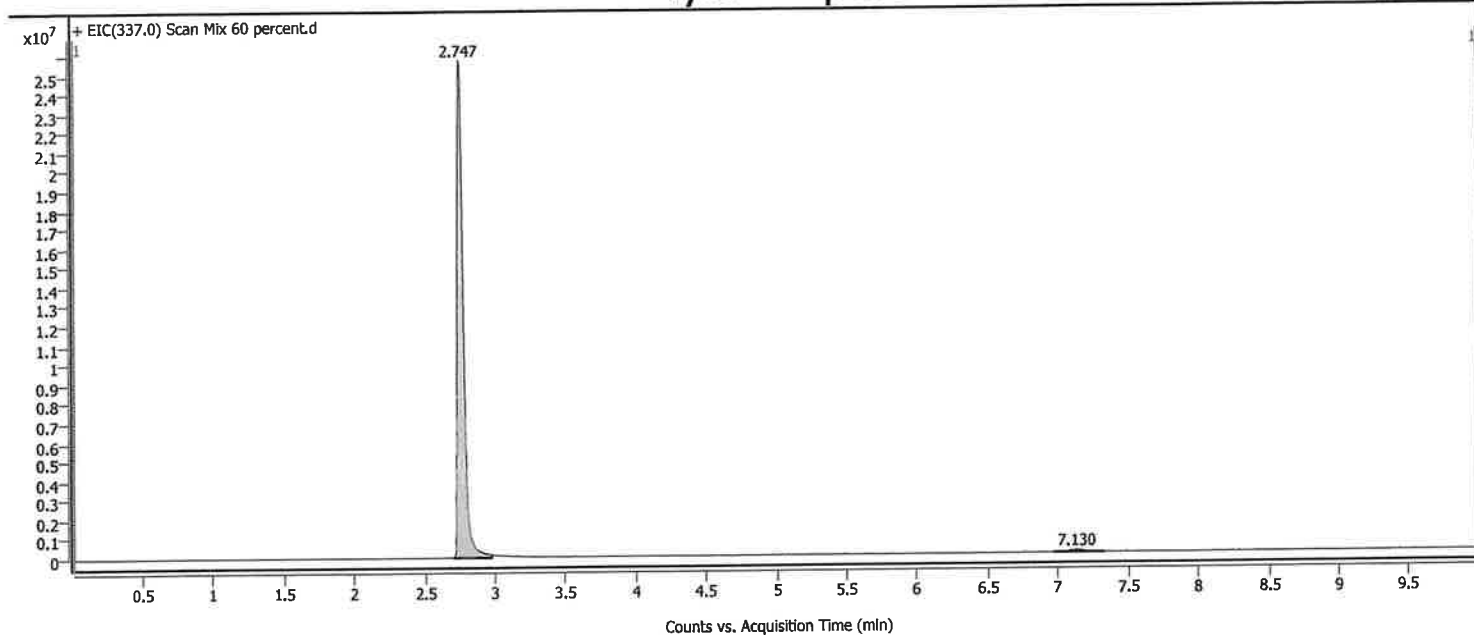
Name	Mix Standard	Data File Path
Sample ID		Acq. Time (Local)
Instrument	Ocoee	Method Path (Acq)
MS Type	QQQ	Version (Acq SW)
Inj. Vol. (ul)	5	IRM Status
Position	P2-F2	Method Path (DA)
Plate Pos.		Target Source Path
Operator		Result Summary

D:\MassHunter\Data\2022\BTT\Feb\2-10-22\New Method Test\Mix 60 percent.d
2/10/2022 2:16:42 PM (UTC-06:00)
D:\MassHunter\Methods\Isocratic M60W40.m
Ultivo LC/TQ 1.2 (23)

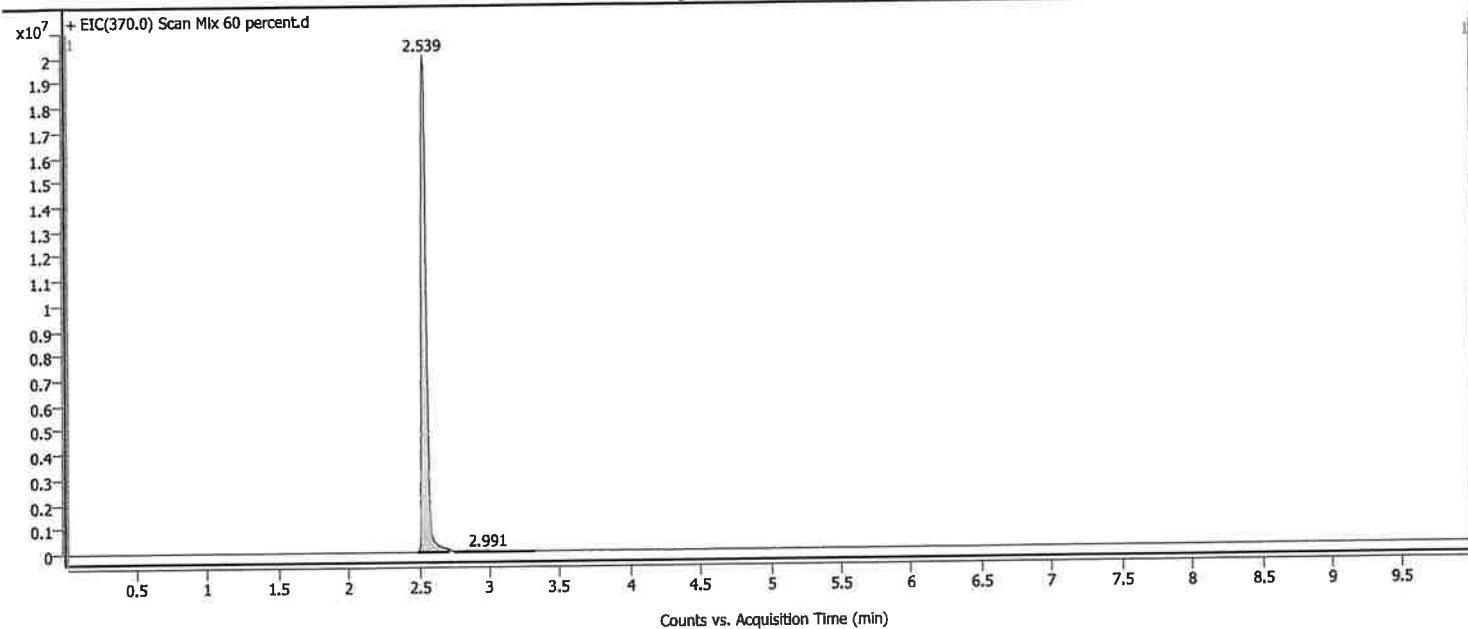
Sample Chromatograms



Analysis Report

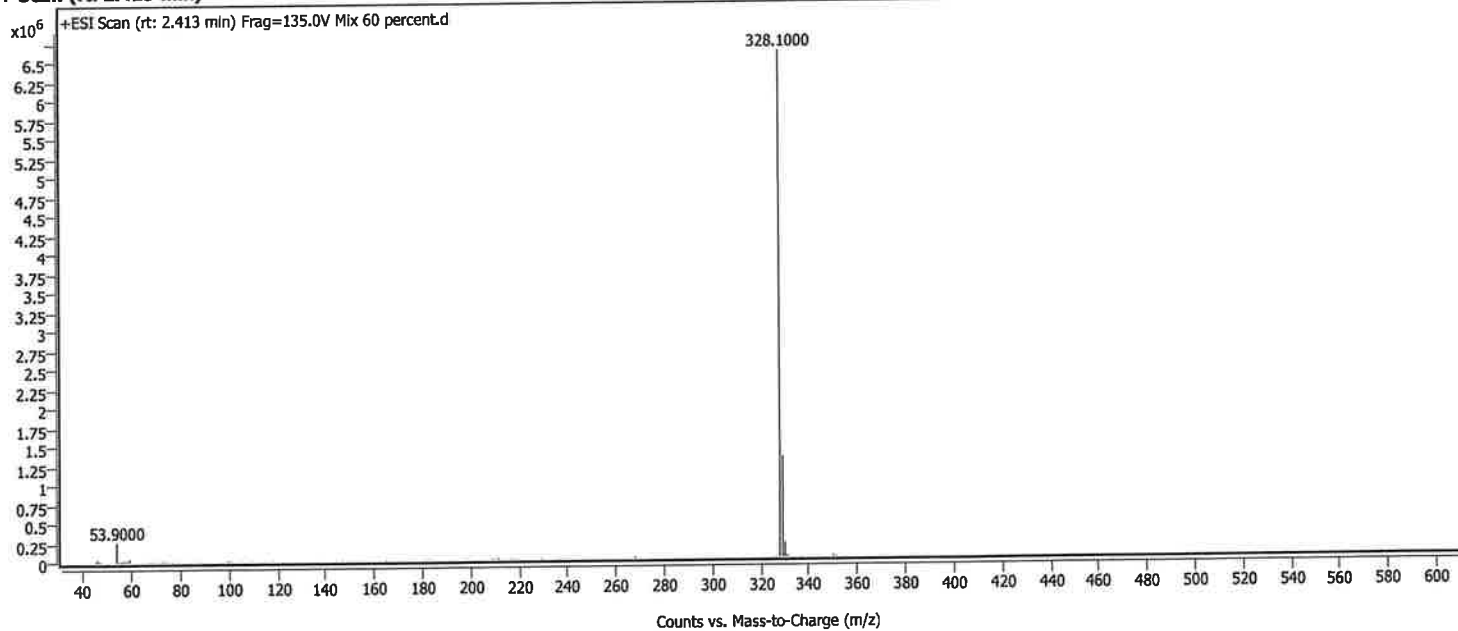


Analysis Report



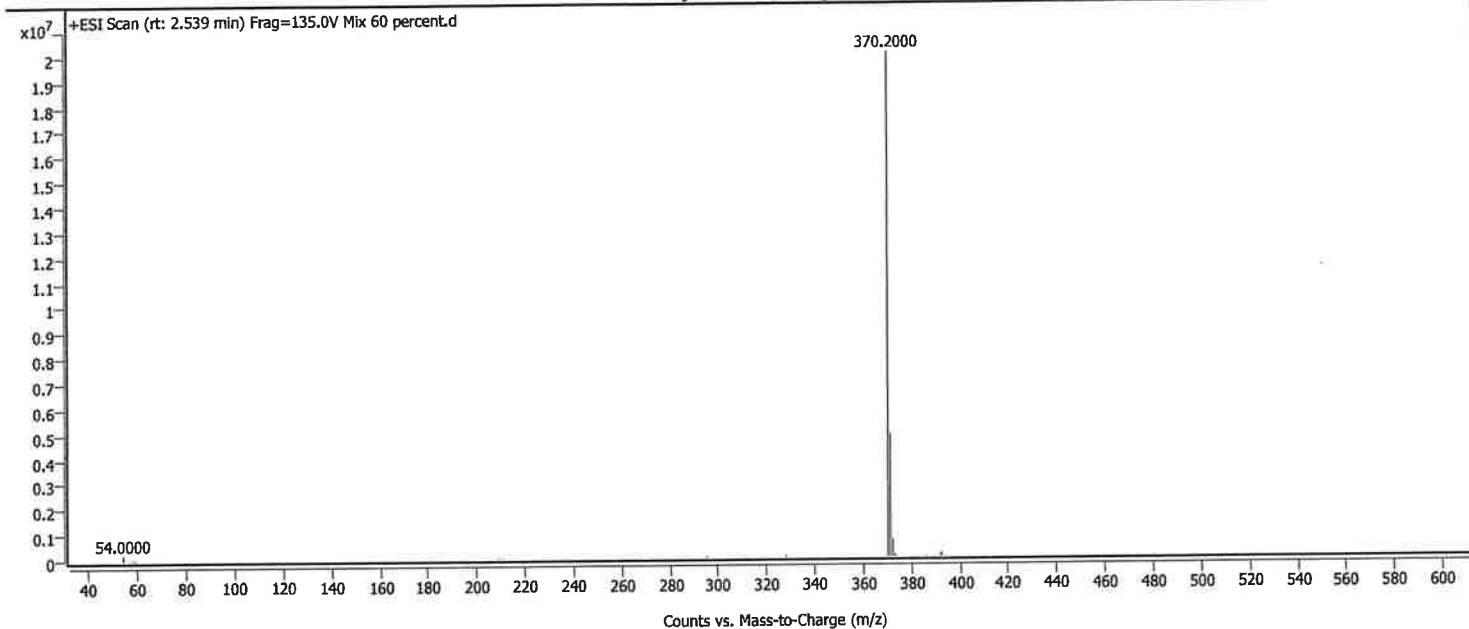
Sample Spectra

+ Scan (rt: 2.413 min)

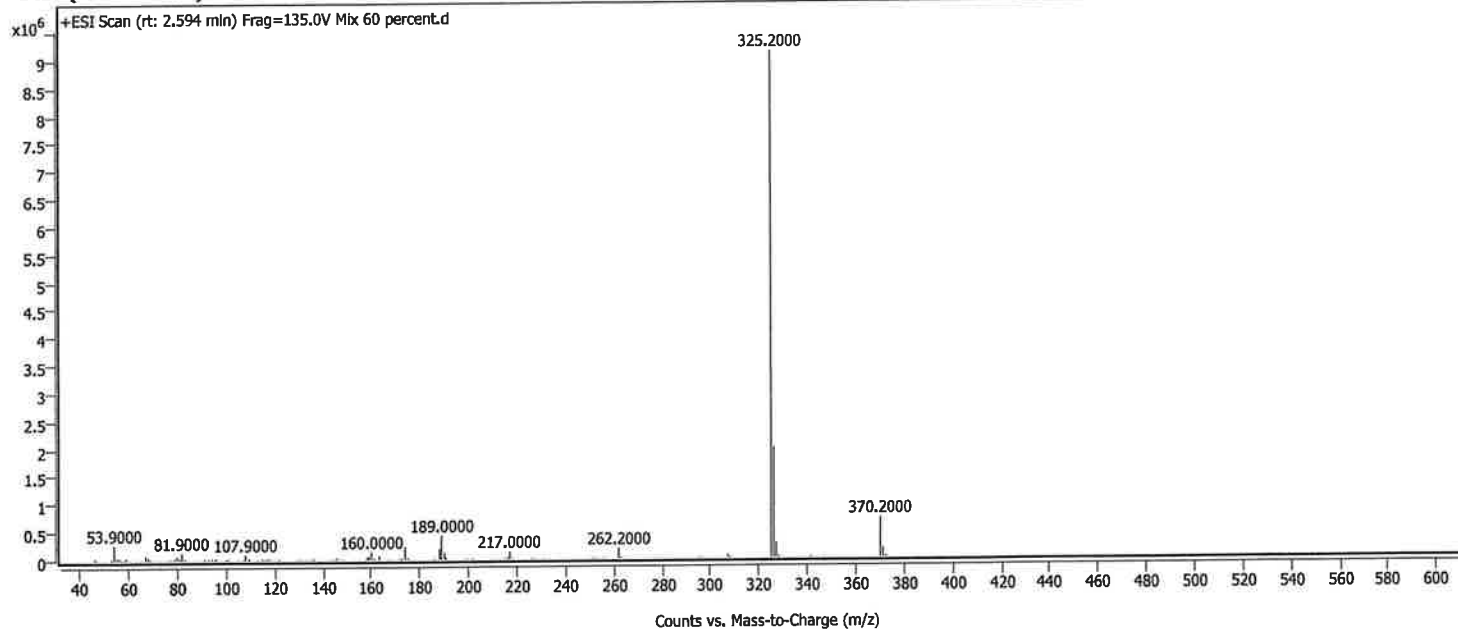


+ Scan (rt: 2.539 min)

Analysis Report

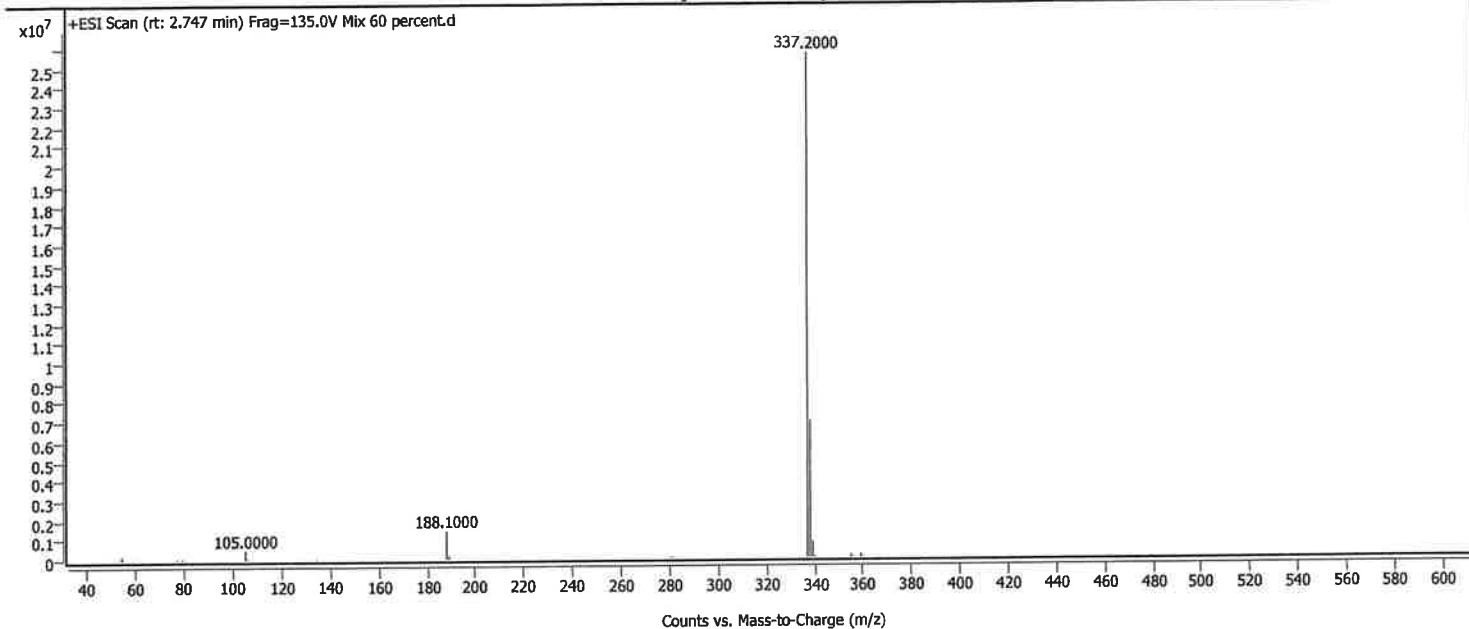


+ Scan (rt: 2.594 min)

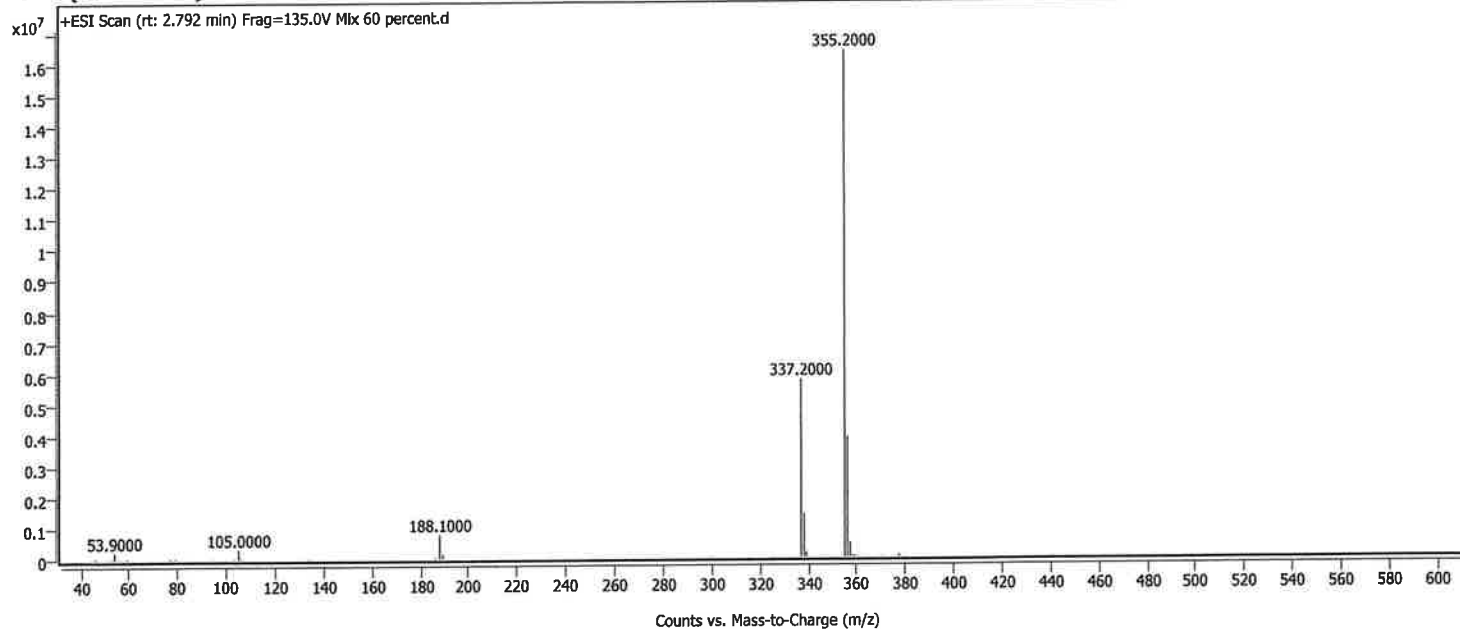


+ Scan (rt: 2.747 min)

Analysis Report



+ Scan (rt: 2.792 min)



MassHunter Qual 10.0
(End of Report)

Analysis Report

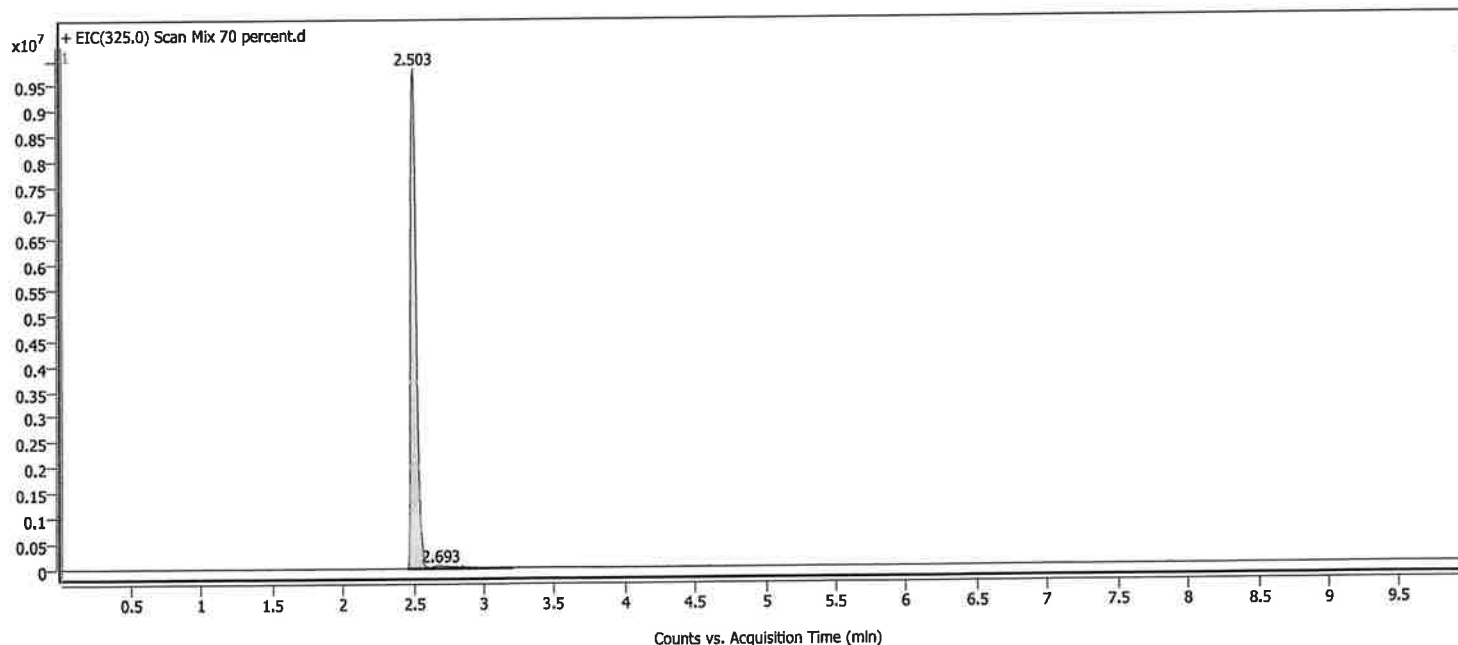
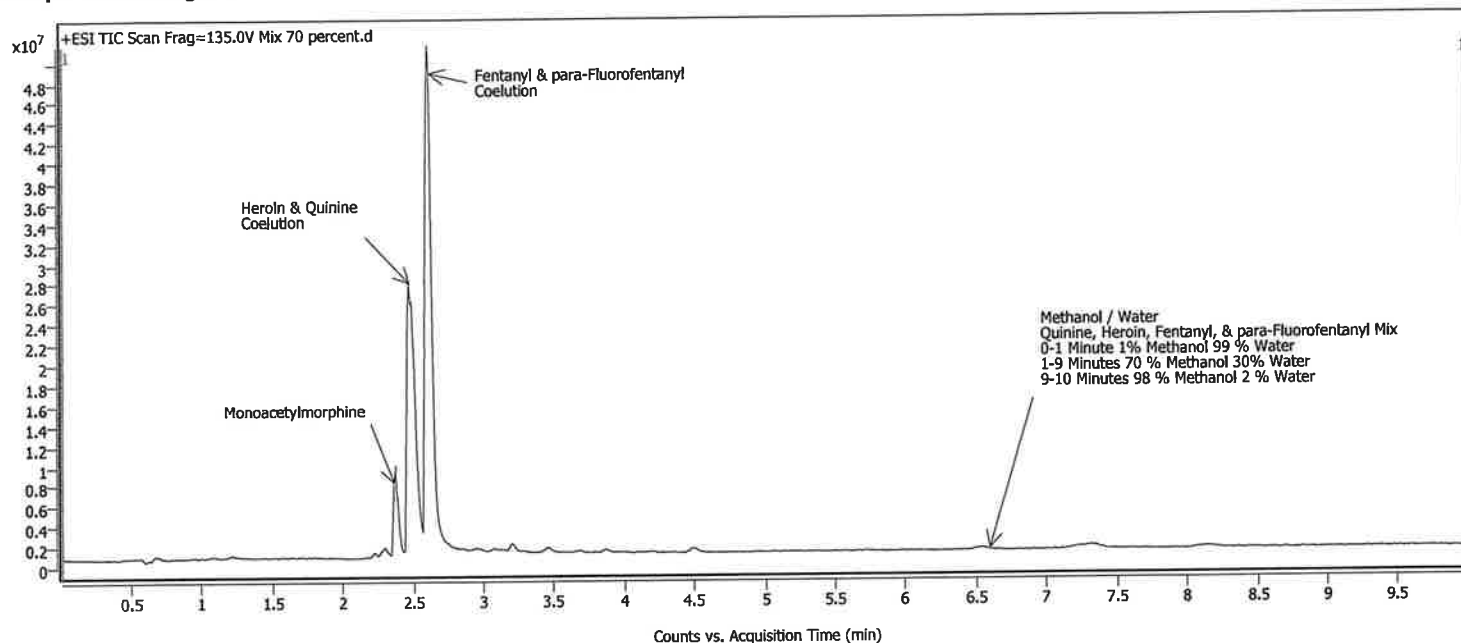
Sample Information

Name Mix Standard
Sample ID
Instrument Ocoee
MS Type QQQ
Inj. Vol. (ul) 5
Position P2-F2
Plate Pos.
Operator

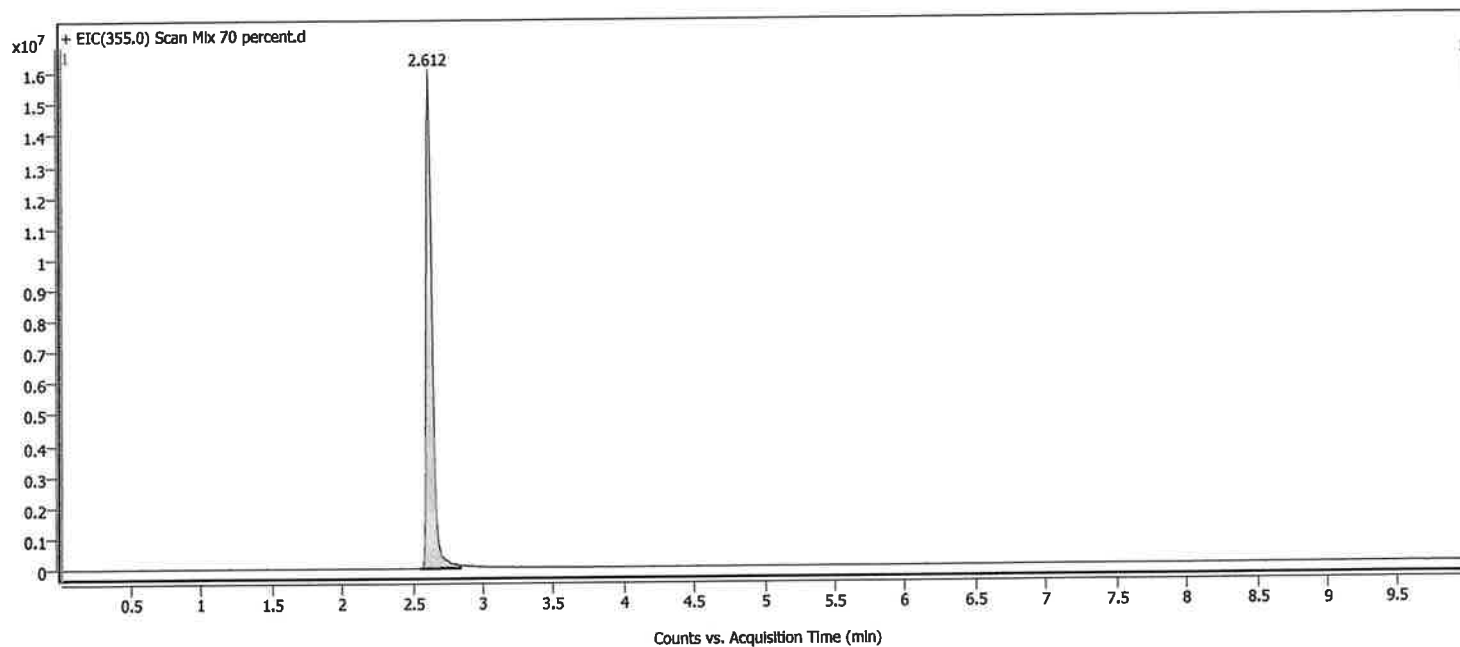
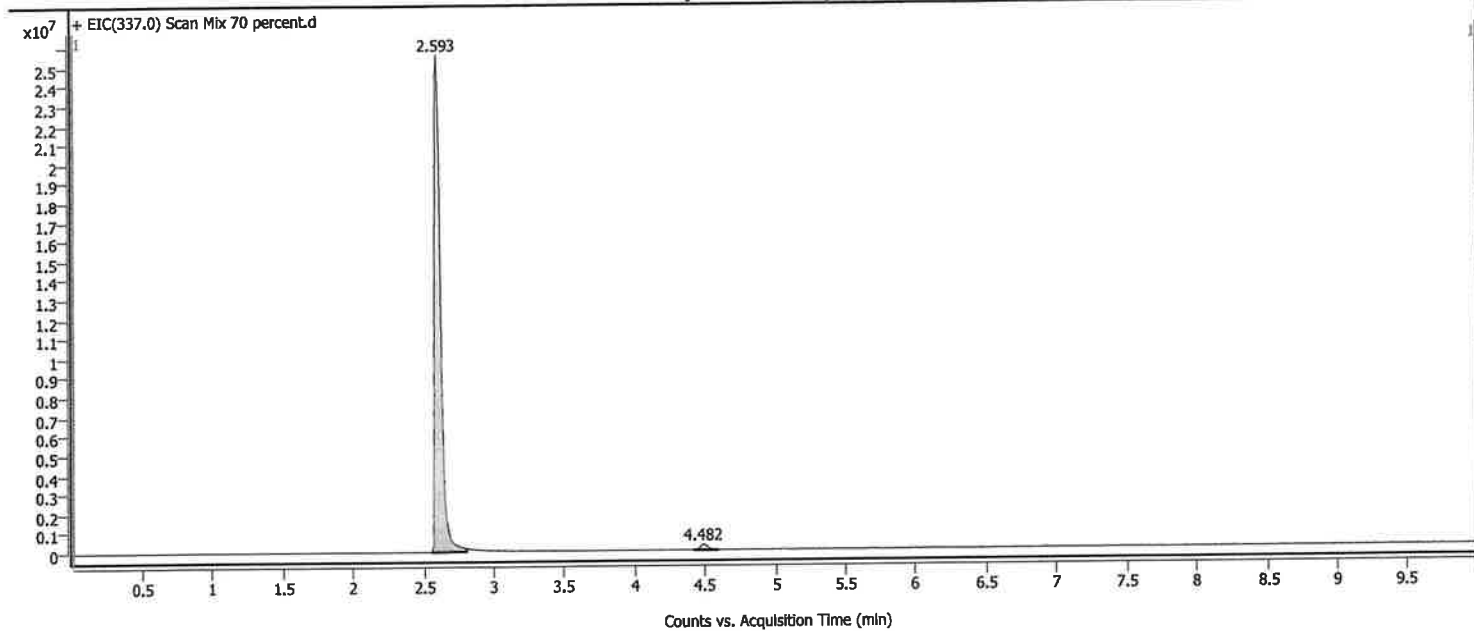
Data File Path
Acq. Time (Local)
Method Path (Acq)
Version (Acq SW)
IRM Status
Method Path (DA)
Target Source Path
Result Summary

D:\MassHunter\Data\2022\BTT\Feb\2-10-22\New Method Test\Mix 70 percent.d
2/10/2022 2:33:28 PM (UTC-06:00)
D:\MassHunter\Methods\Isocratic M70W30.m
Ultivo LC/TQ 1.2 (23)

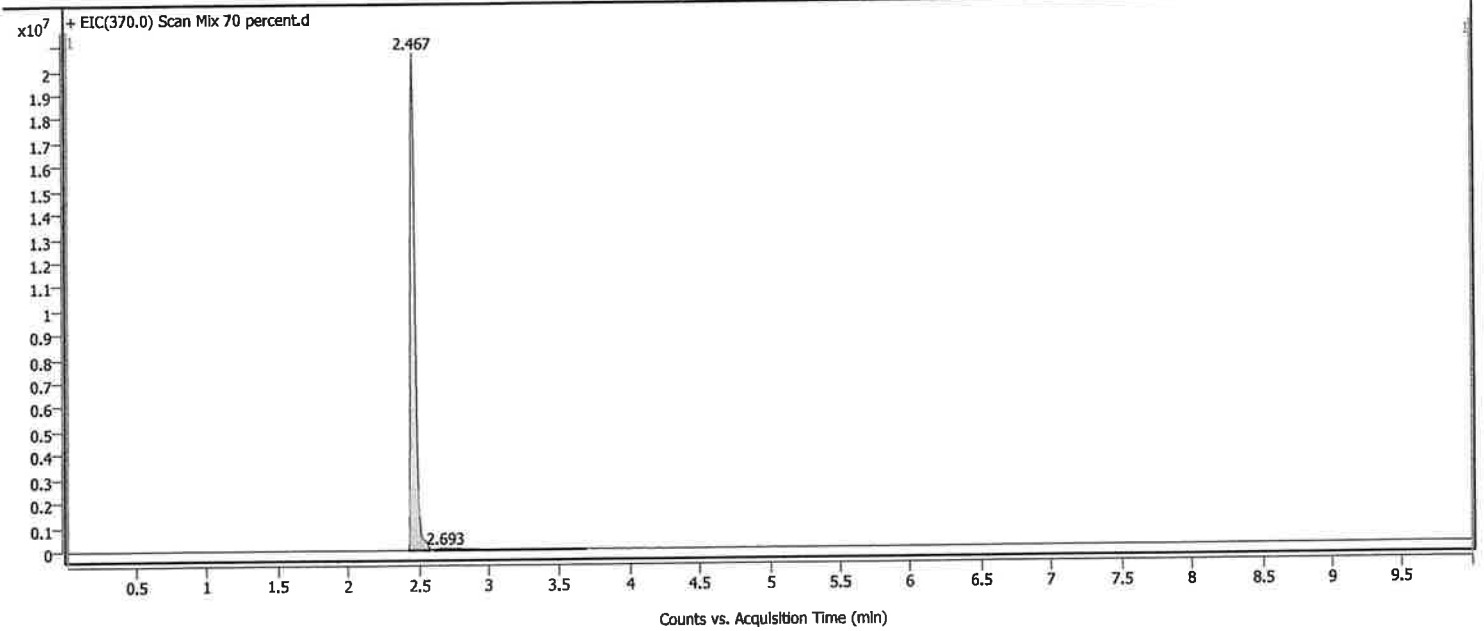
Sample Chromatograms



Analysis Report

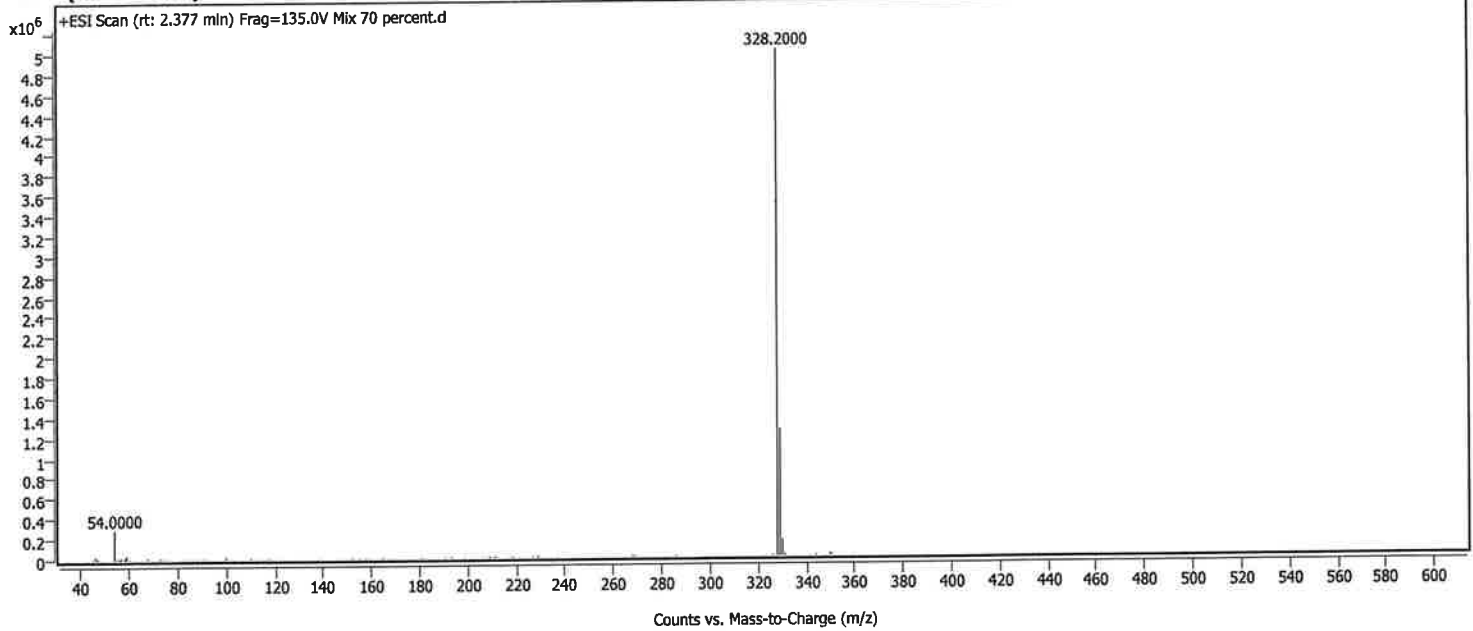


Analysis Report



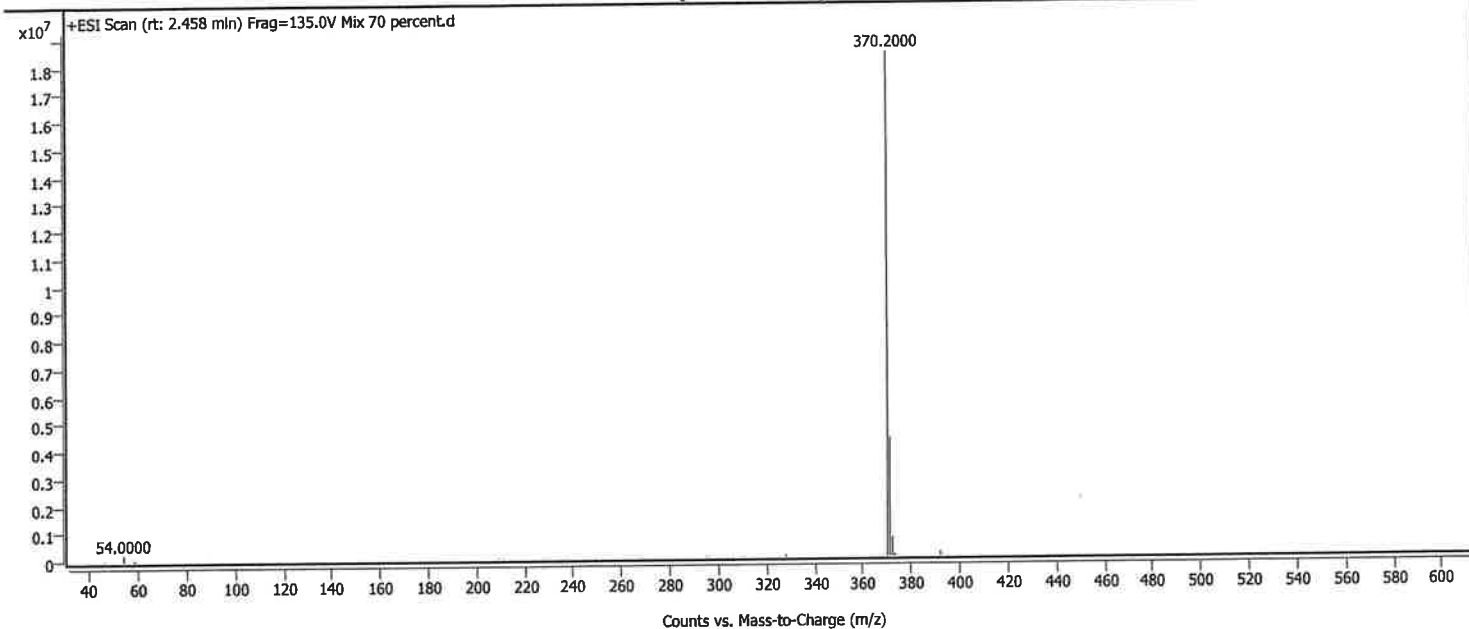
Sample Spectra

+ Scan (rt: 2.377 min)

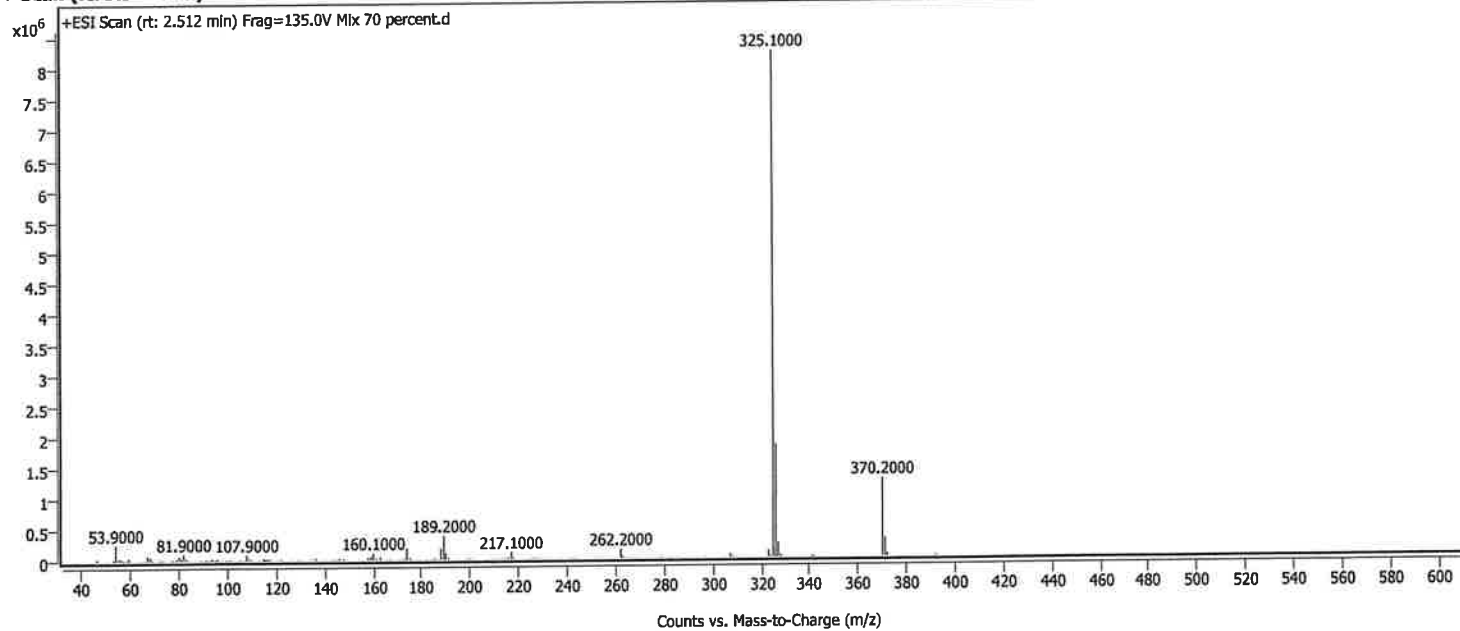


+ Scan (rt: 2.458 min)

Analysis Report

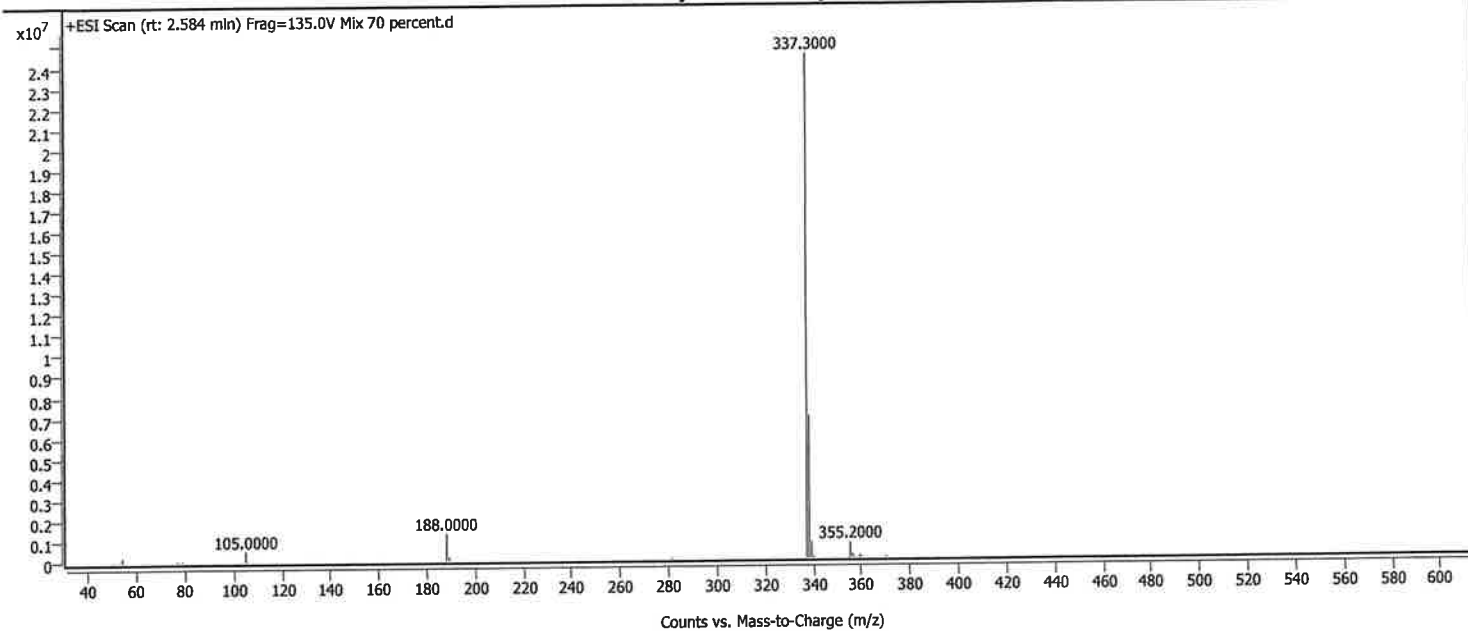


+ Scan (rt: 2.512 min)

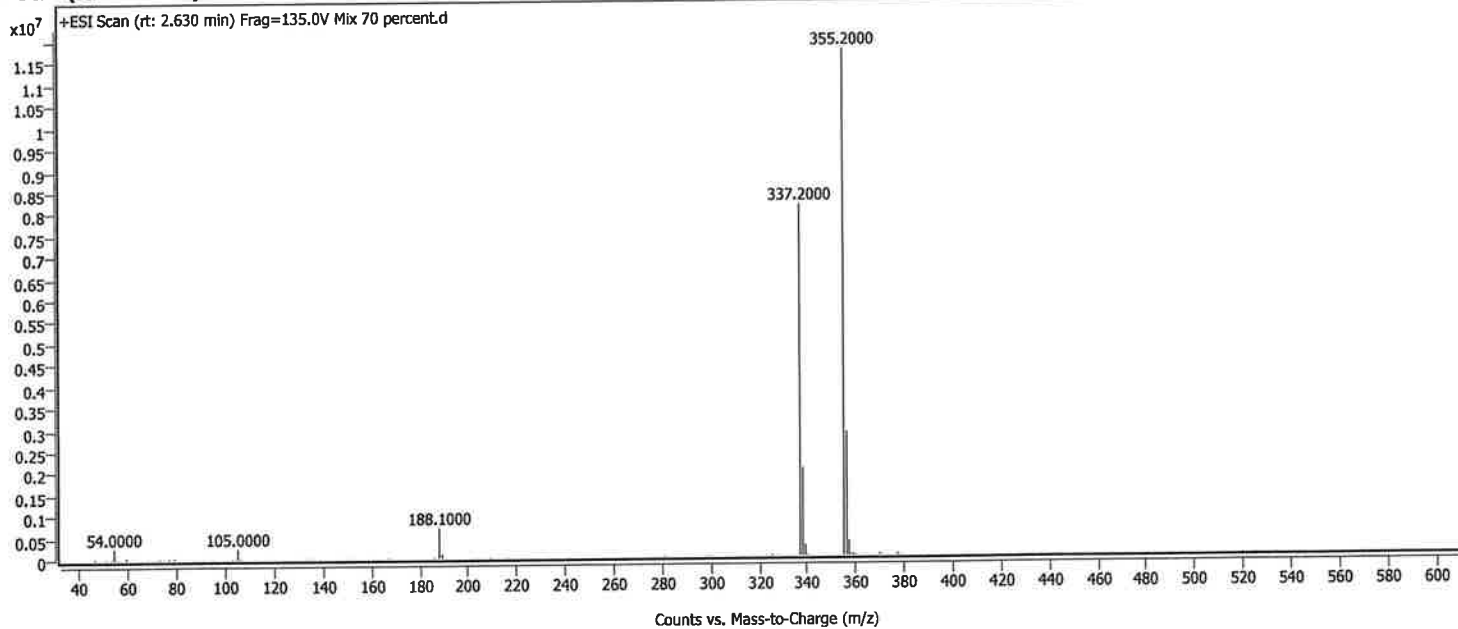


+ Scan (rt: 2.584 min)

Analysis Report



+ Scan (rt: 2.630 min)



MassHunter Qual 10.0
(End of Report)

Analysis Report

Sample Information

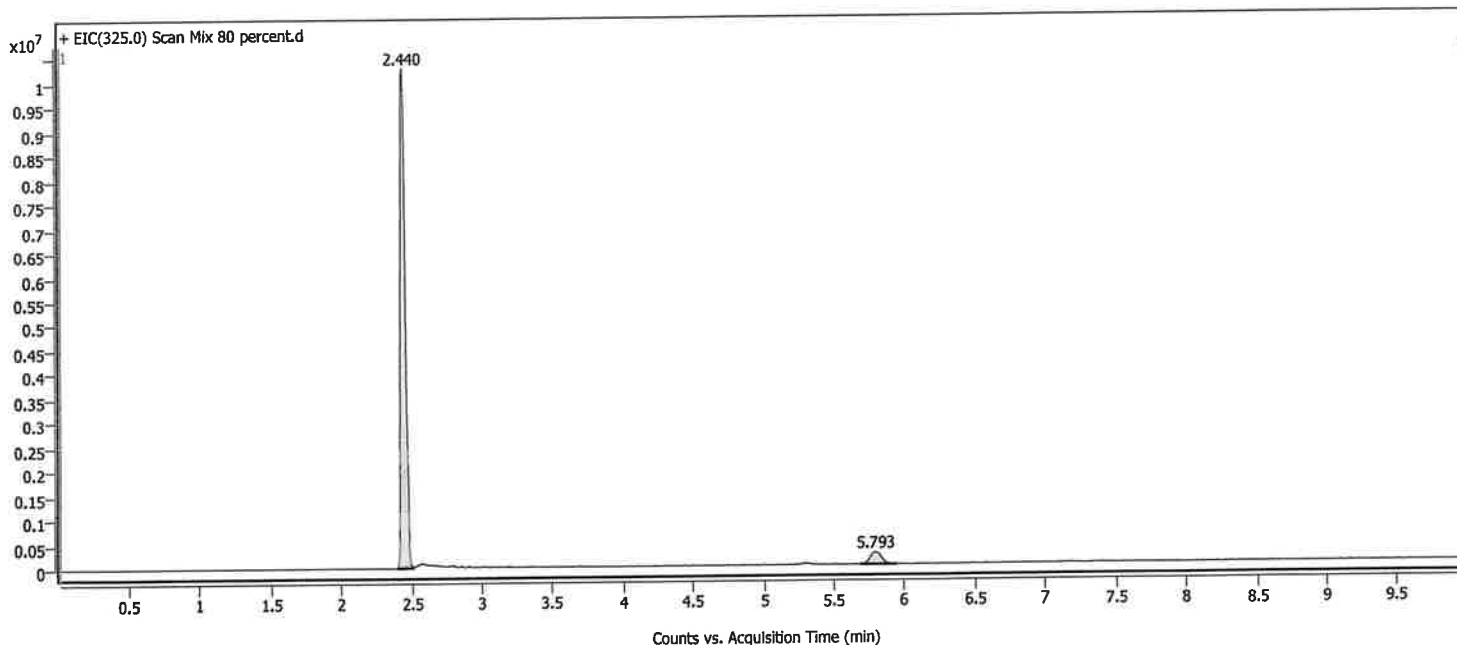
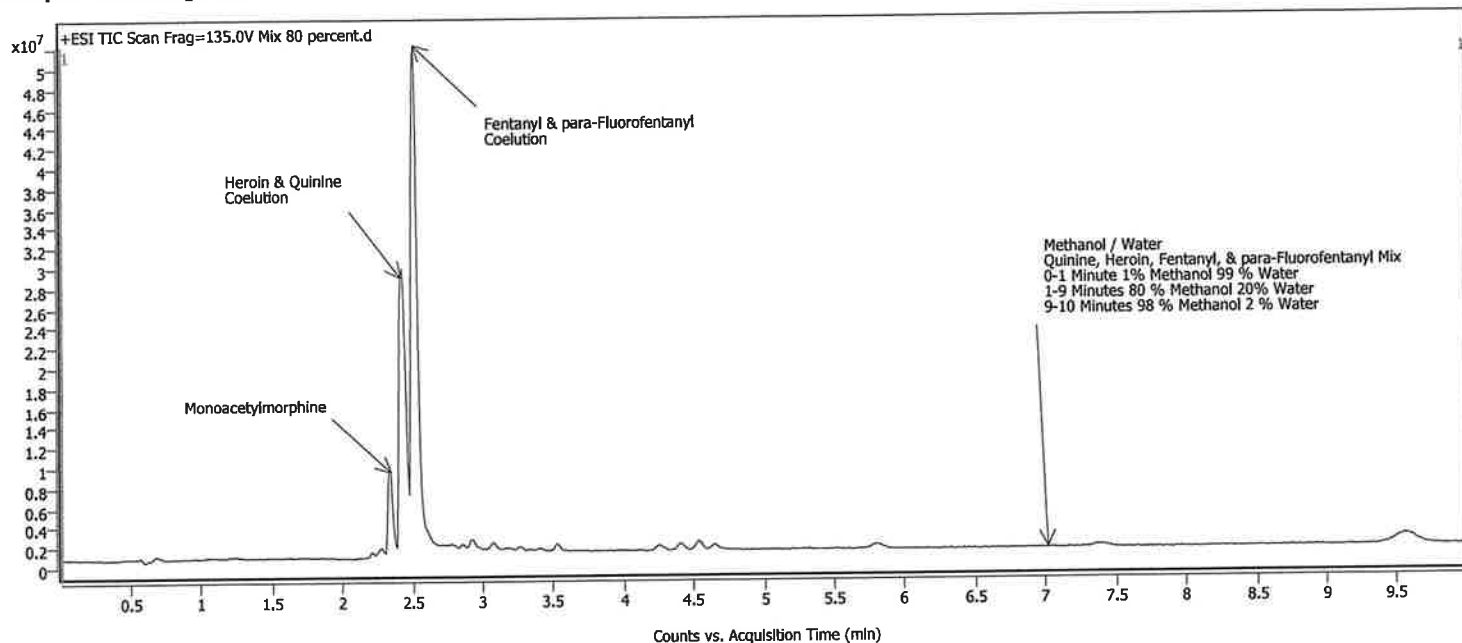
Name
Sample ID
Instrument
MS Type
Inj. Vol. (ul)
Position
Plate Pos.
Operator

Mix Standard
Ocoee
QQQ
5
P2-F2

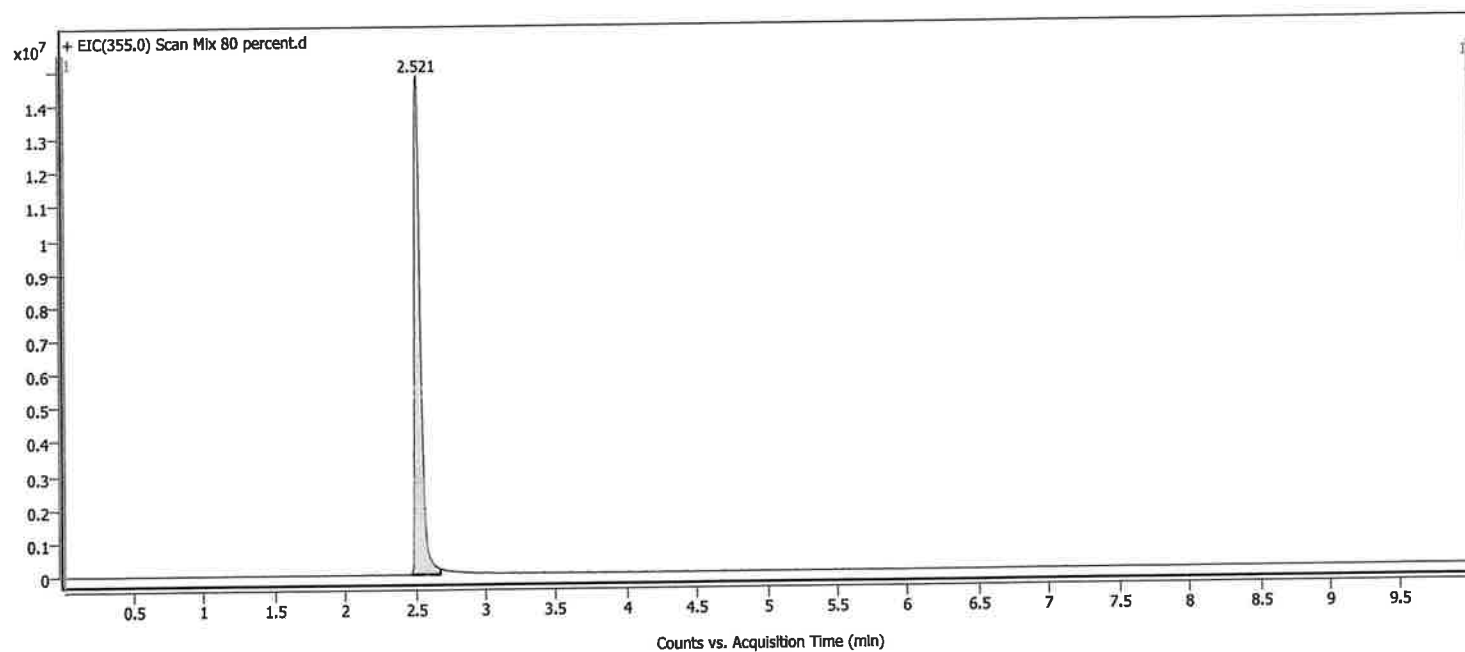
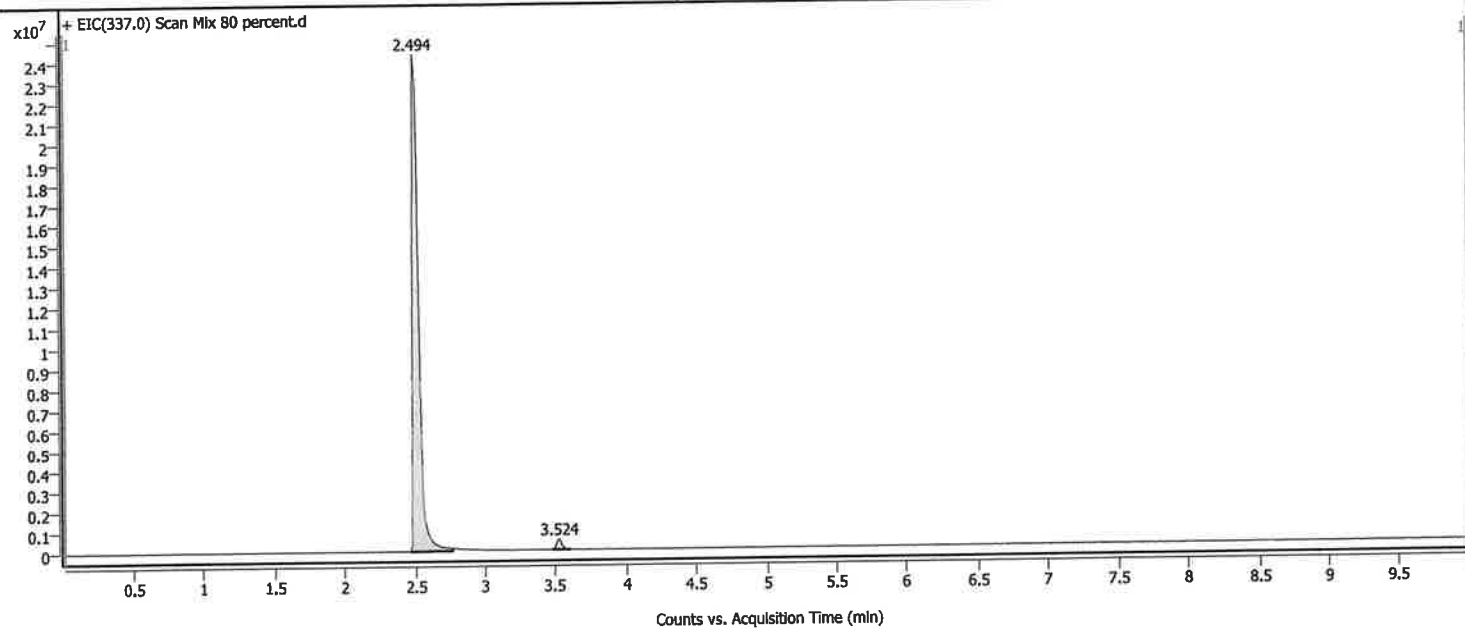
Data File Path
Acq. Time (Local)
Method Path (Acq)
Version (Acq SW)
IRM Status
Method Path (DA)
Target Source Path
Result Summary

D:\MassHunter\Data\2022\BTT\Feb\2-10-22\New Method Test\Mix 80 percent.d
2/10/2022 2:50:11 PM (UTC-06:00)
D:\MassHunter\Methods\Isocratic M80W20.m
Ultivo LC/TQ 1.2 (23)

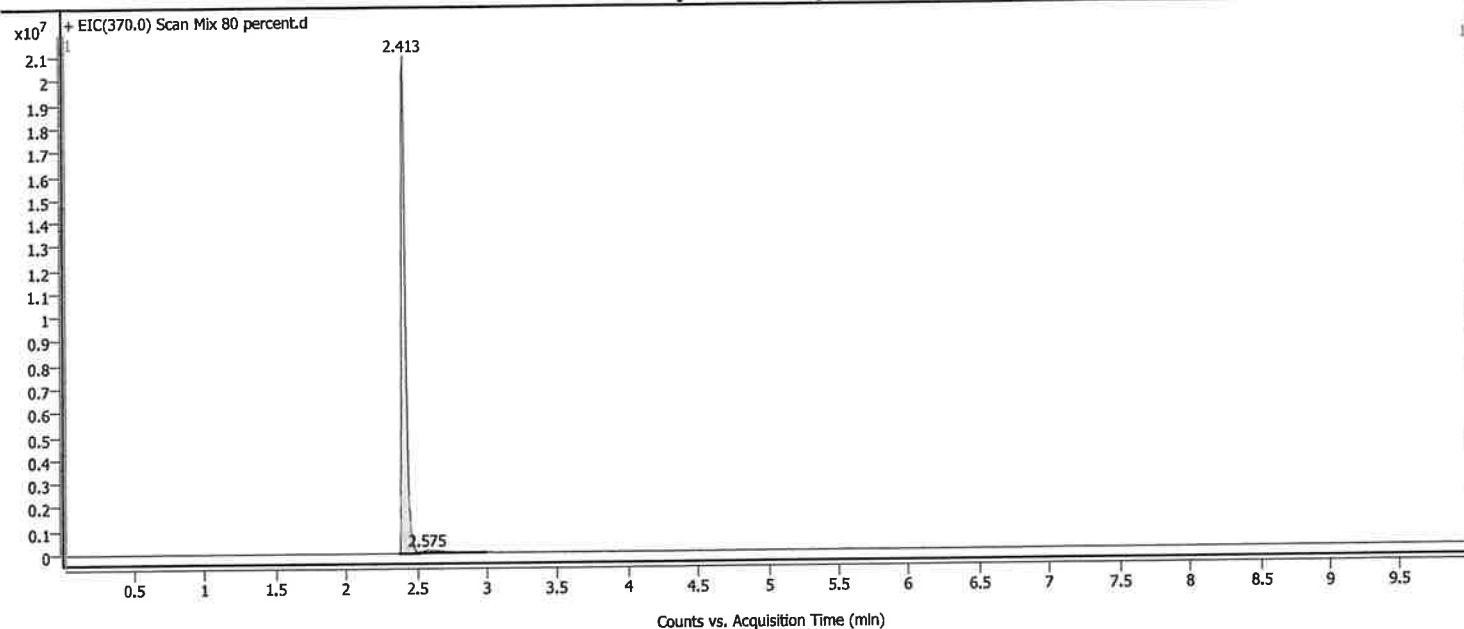
Sample Chromatograms



Analysis Report

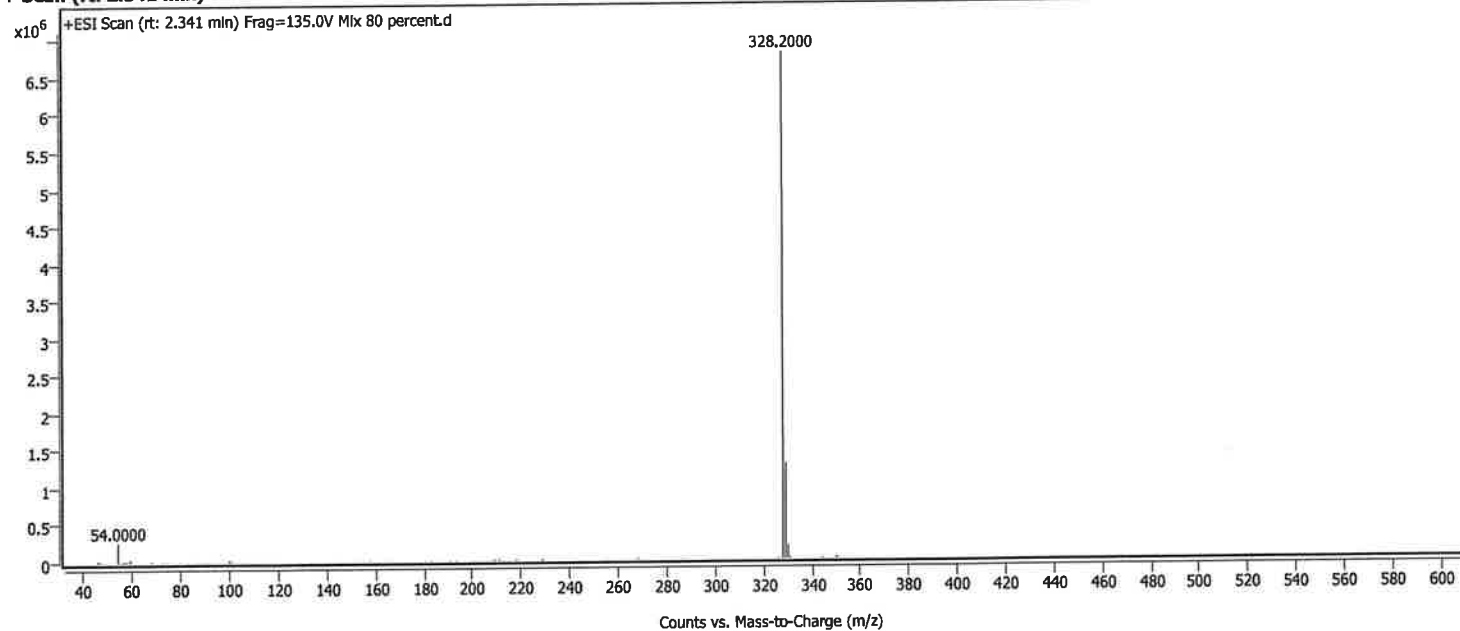


Analysis Report



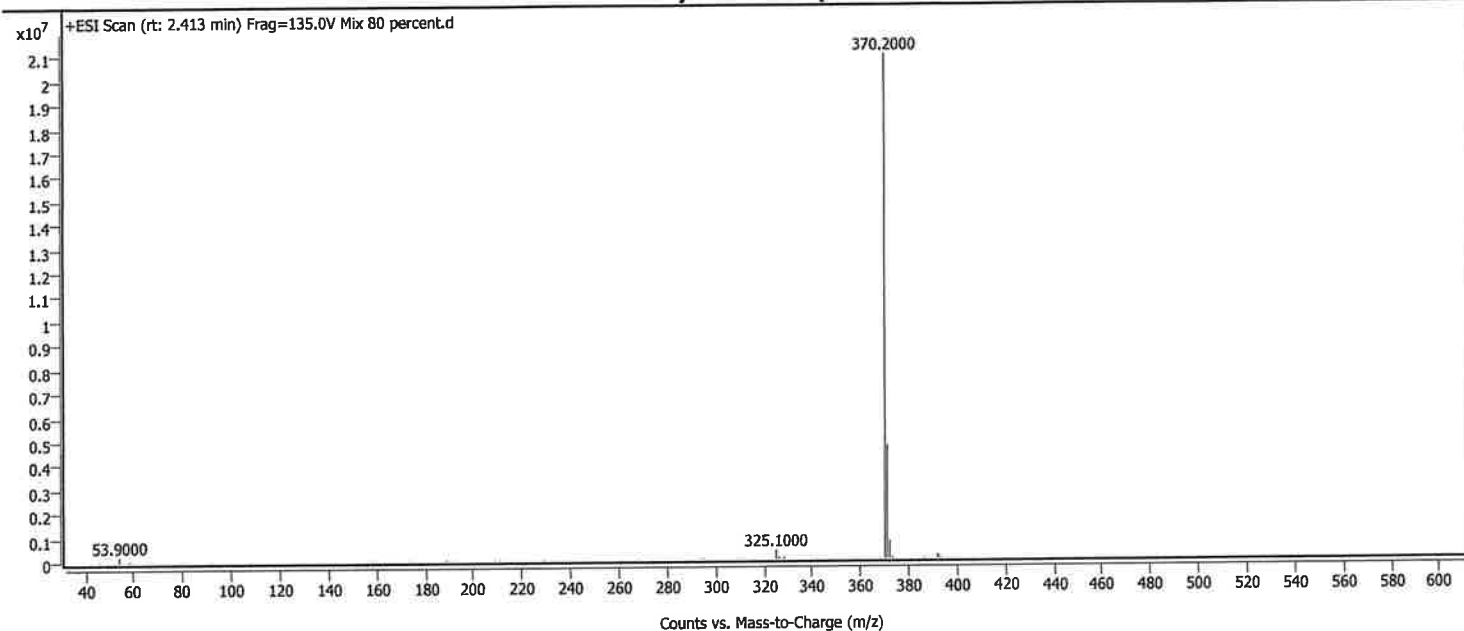
Sample Spectra

+ Scan (rt: 2.341 min)

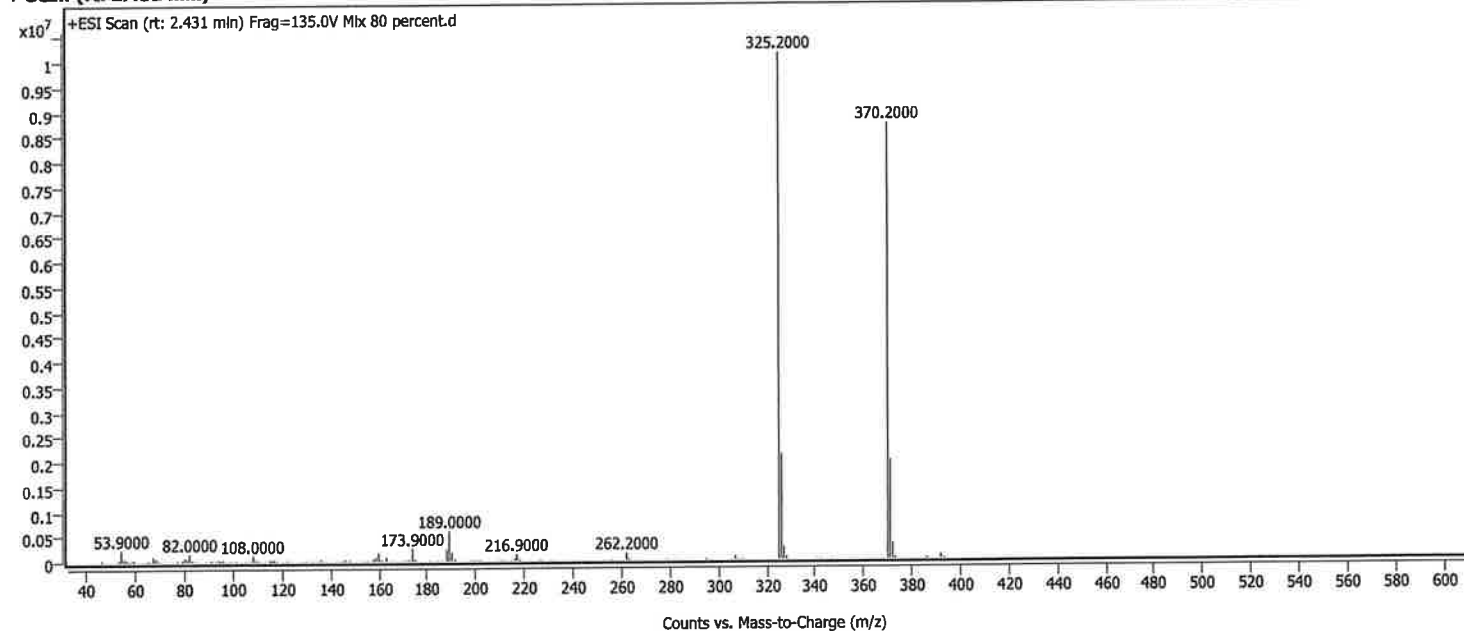


+ Scan (rt: 2.413 min)

Analysis Report

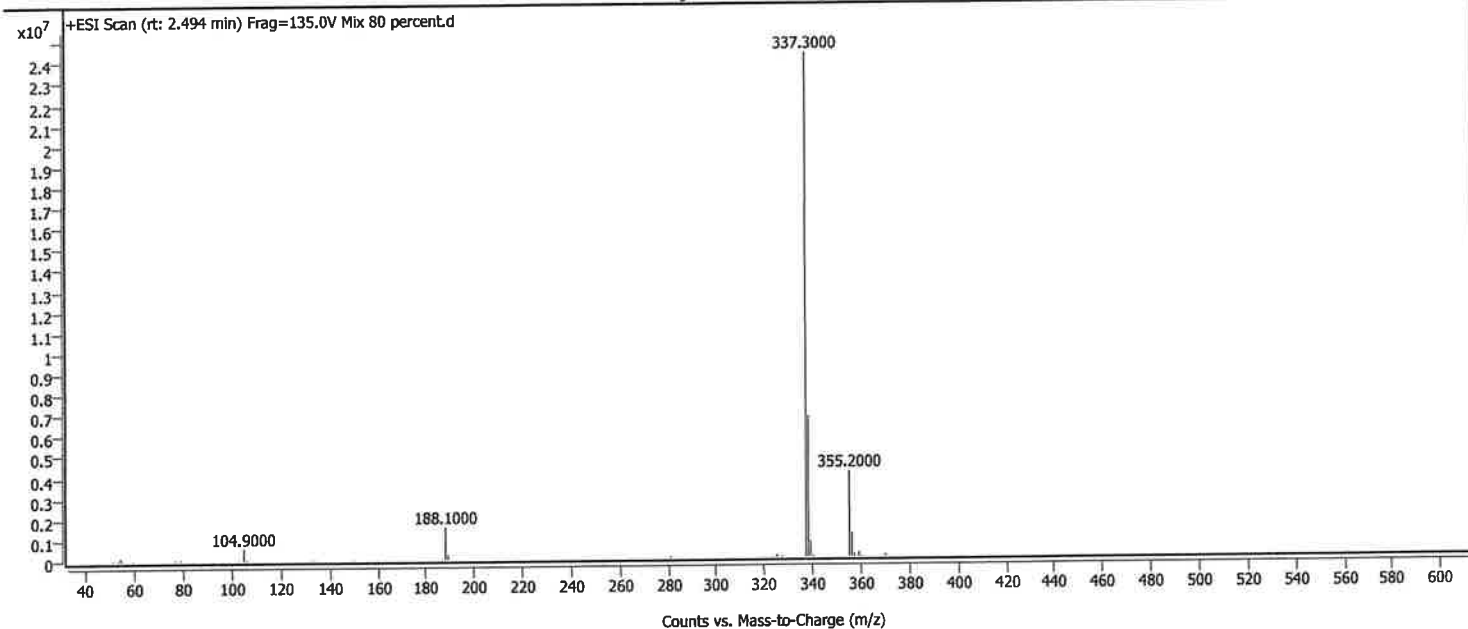


+ Scan (rt: 2.431 min)

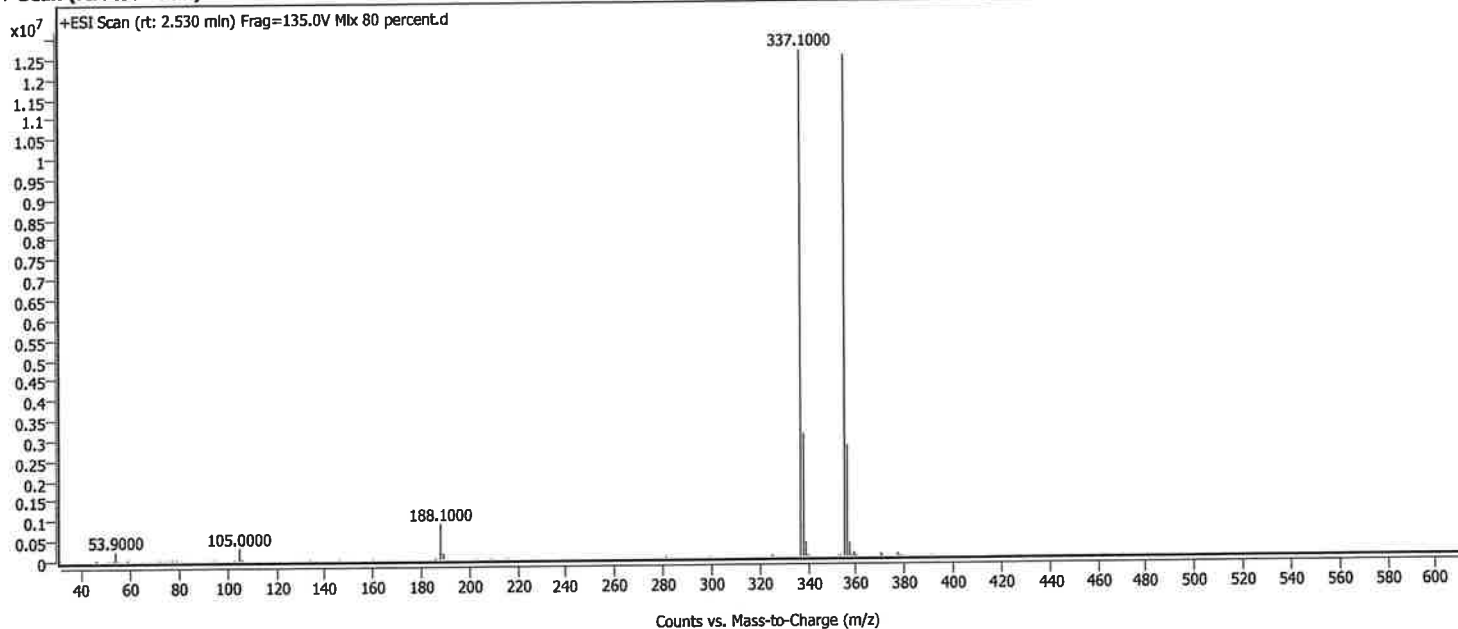


+ Scan (rt: 2.494 min)

Analysis Report



+ Scan (rt: 2.530 min)



MassHunter Qual 10.0
(End of Report)

Analysis Report

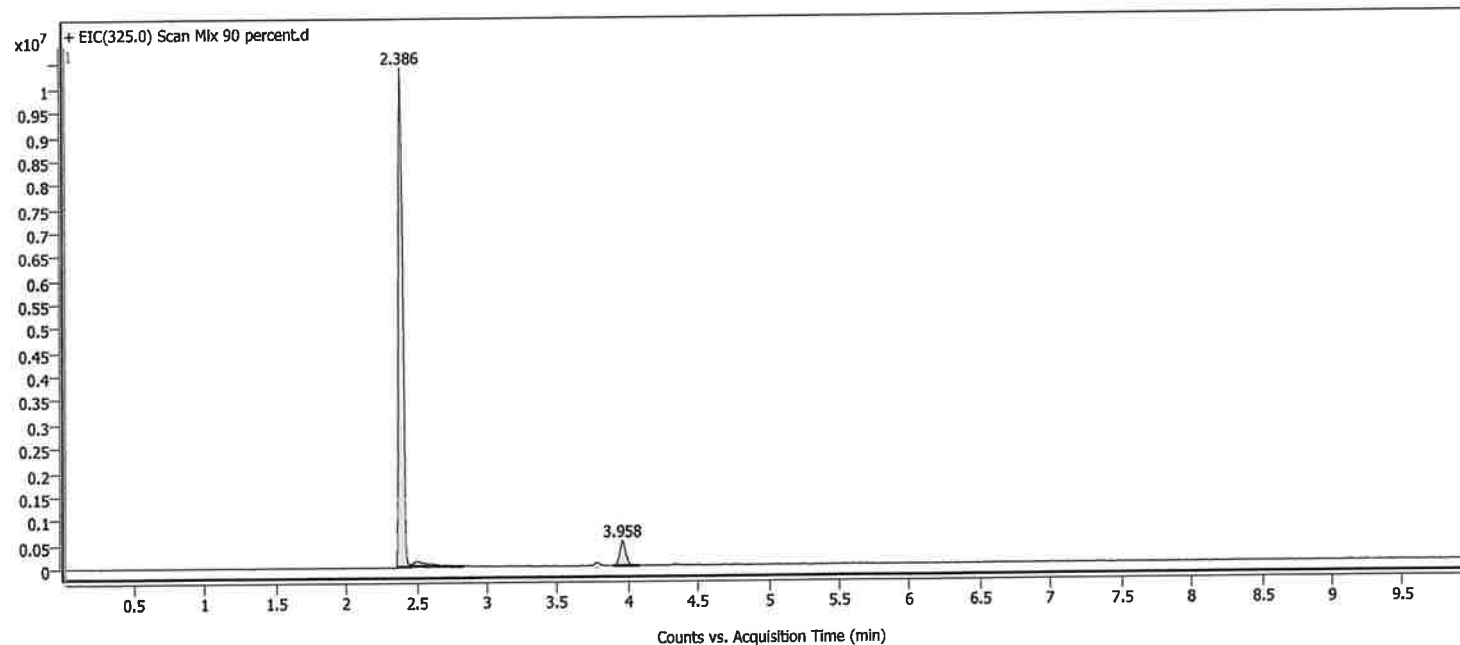
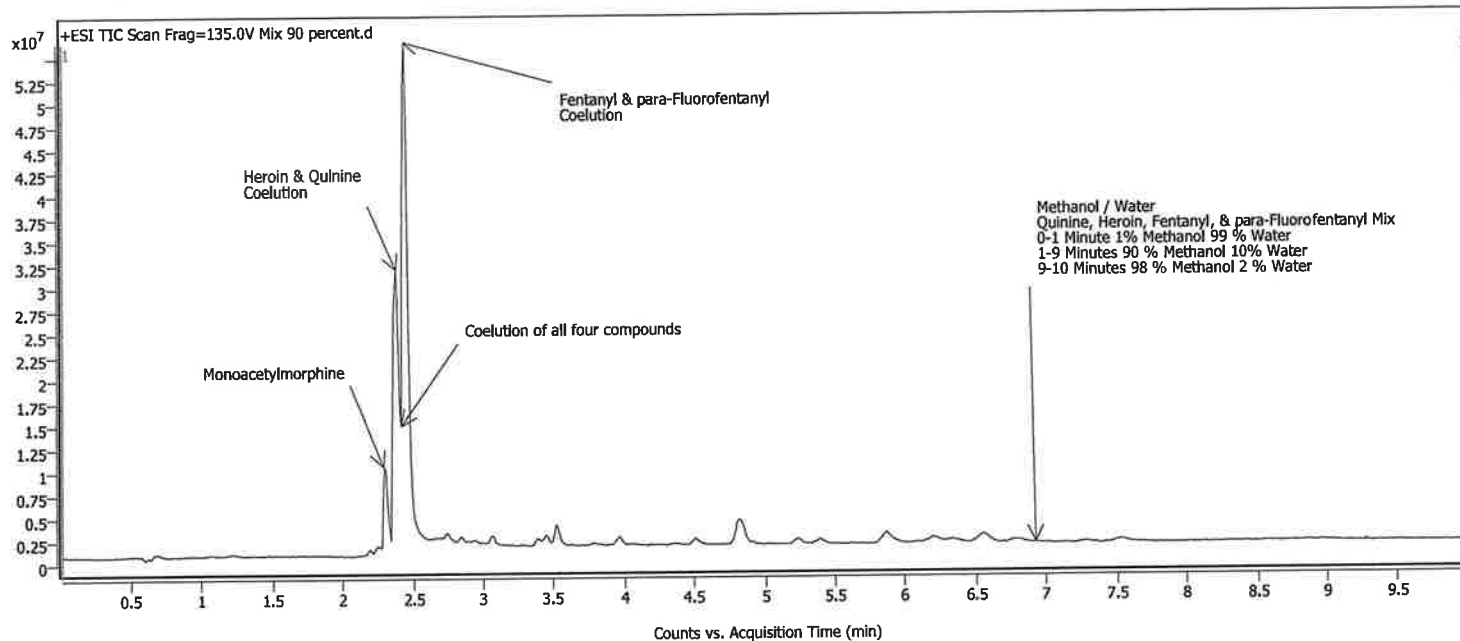
Sample Information

Name Mix Standard
Sample ID
Instrument Ocoee
MS Type QQQ
Inj. Vol. (ul) 5
Position P2-F2
Plate Pos.
Operator

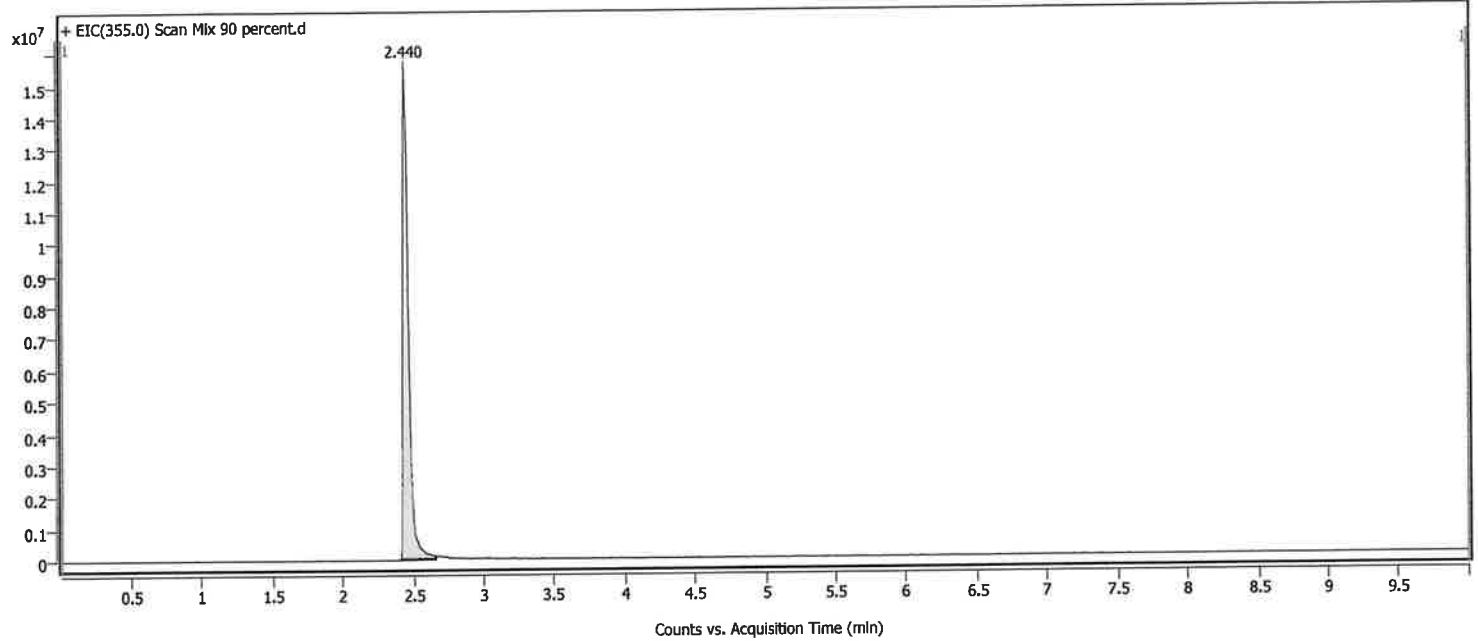
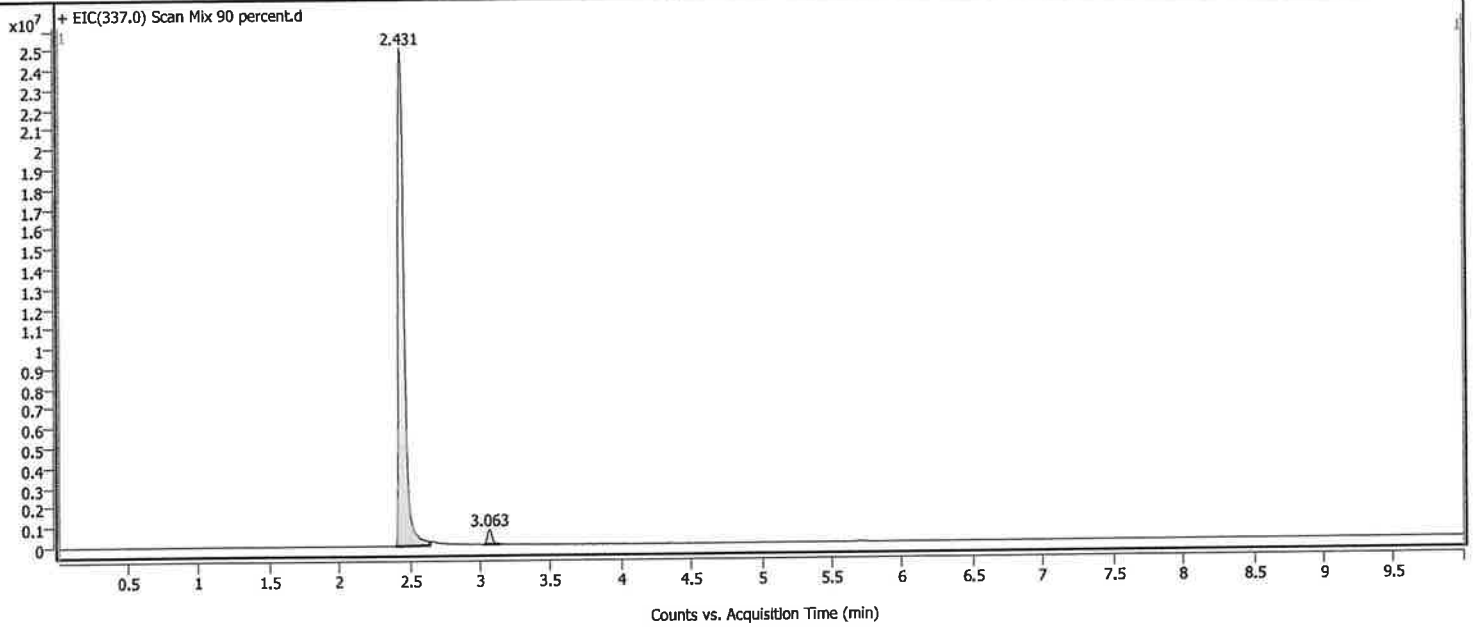
Data File Path
Acq. Time (Local)
Method Path (Acq)
Version (Acq SW)
IRM Status
Method Path (DA)
Target Source Path
Result Summary

D:\MassHunter\Data\2022\BTT\Feb\2-10-22\New Method Test\Mix 90 percent.d
2/10/2022 3:06:55 PM (UTC-06:00)
D:\MassHunter\Methods\Isocratic M90W10.m
Ultivo LC/TQ 1.2 (23)

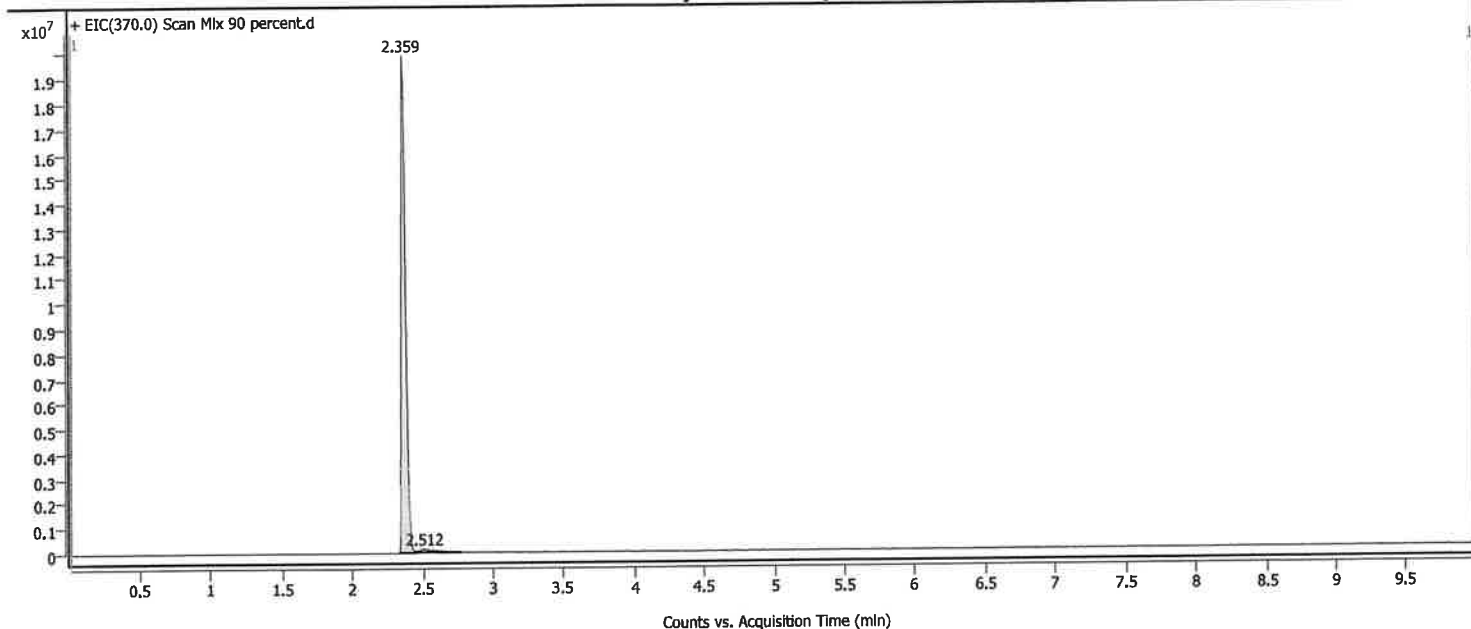
Sample Chromatograms



Analysis Report

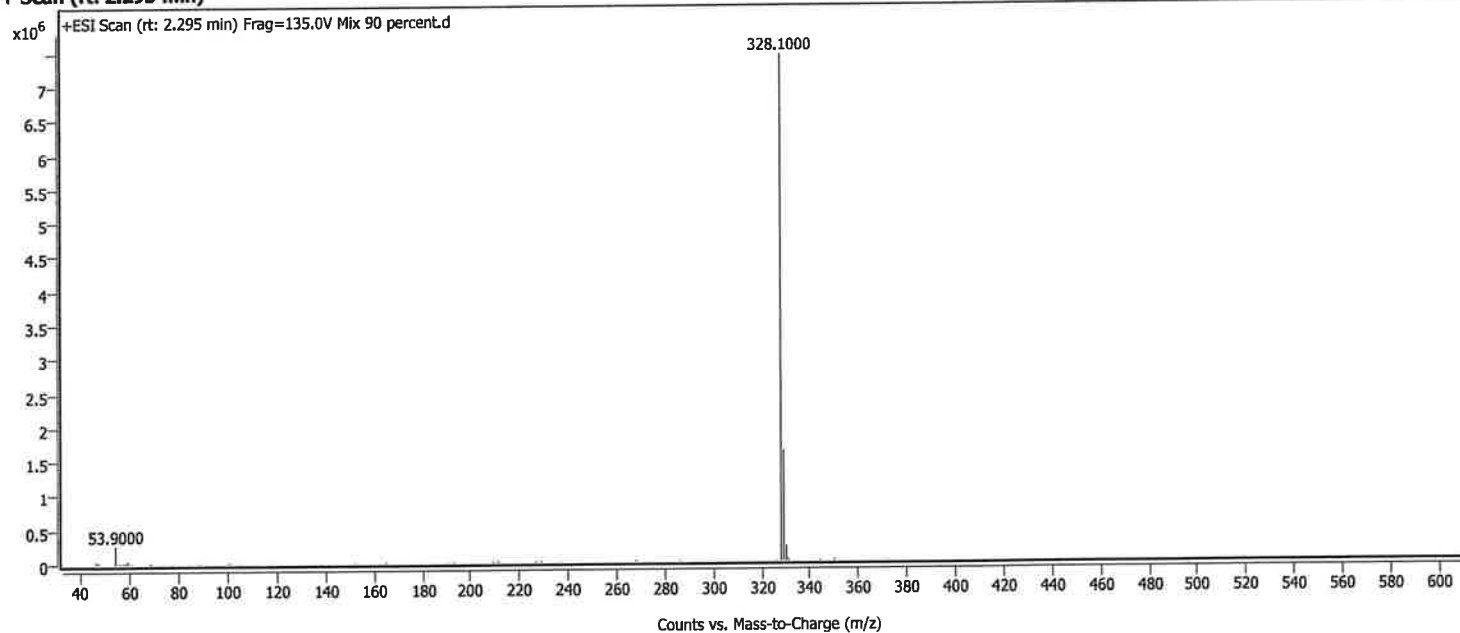


Analysis Report



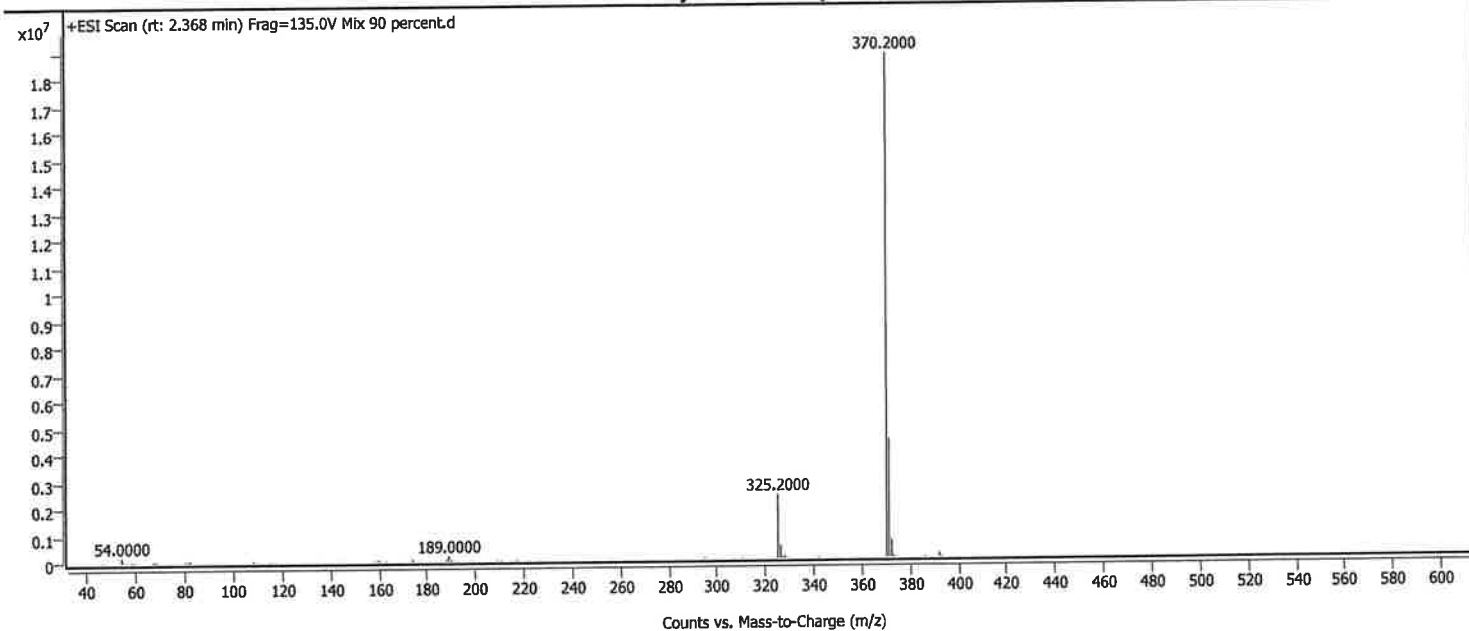
Sample Spectra

+ Scan (rt: 2.295 min)

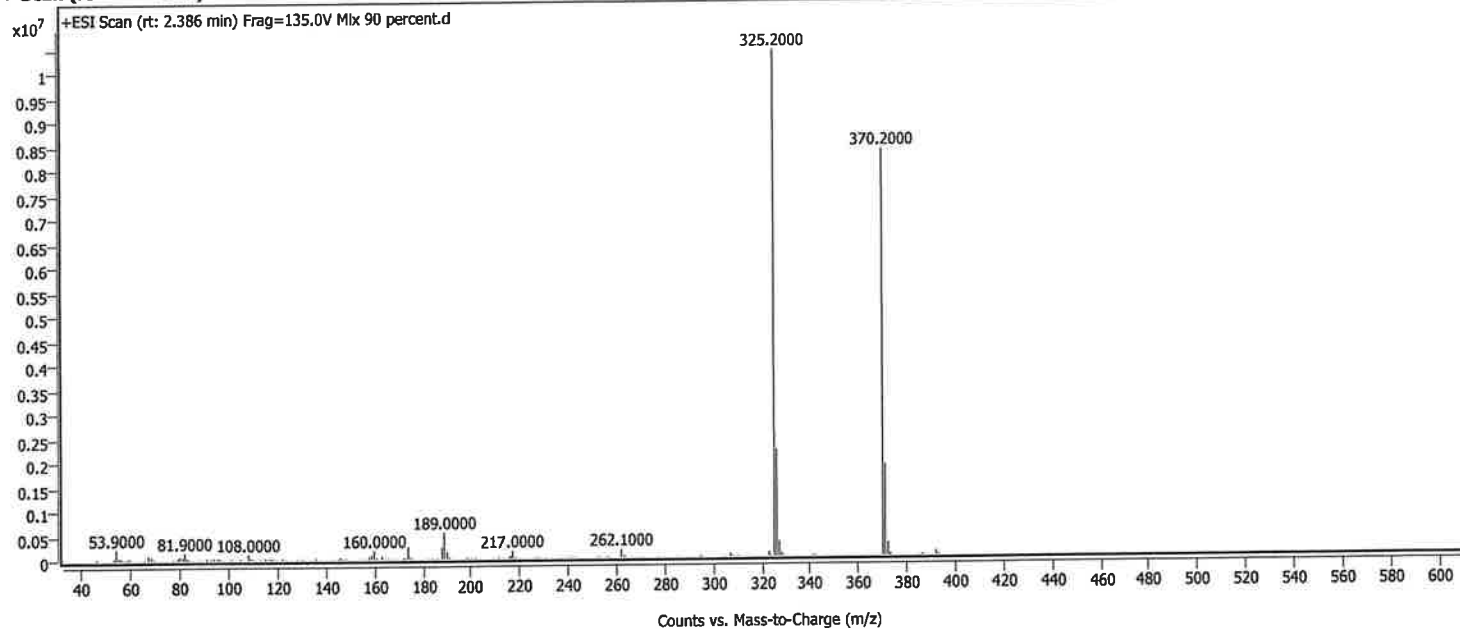


+ Scan (rt: 2.368 min)

Analysis Report

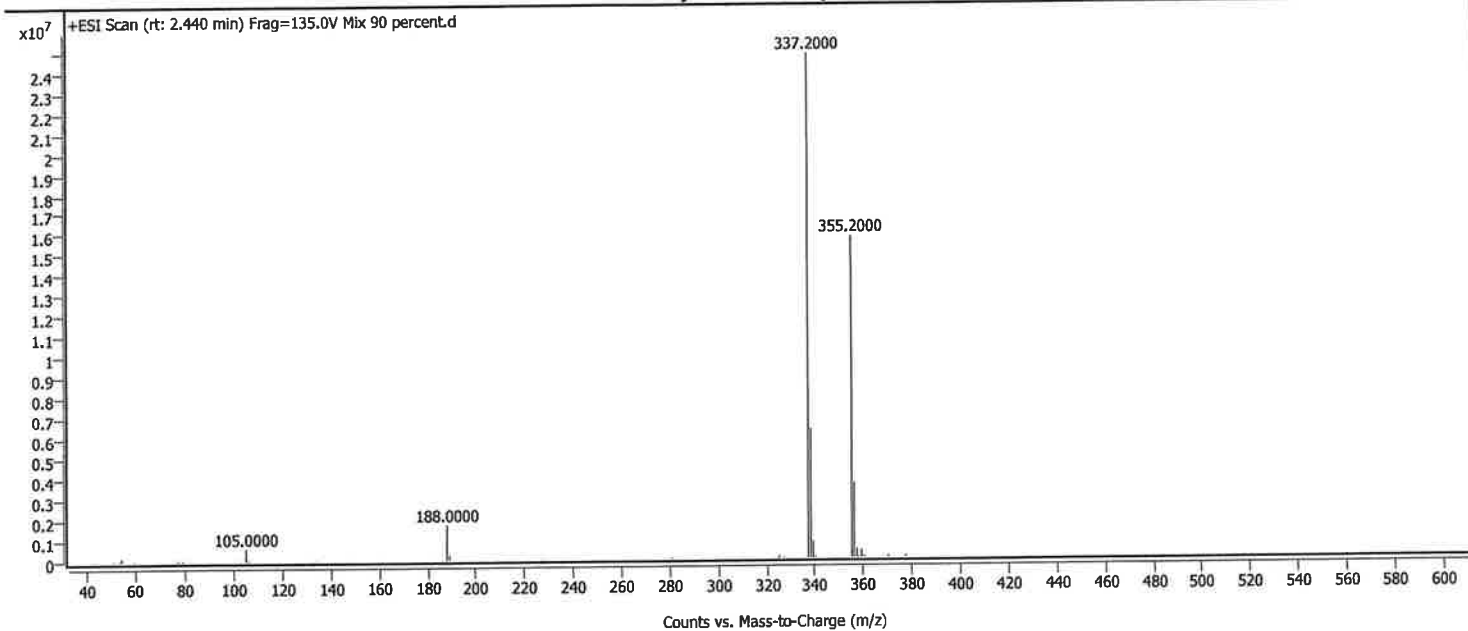


+ Scan (rt: 2.386 min)

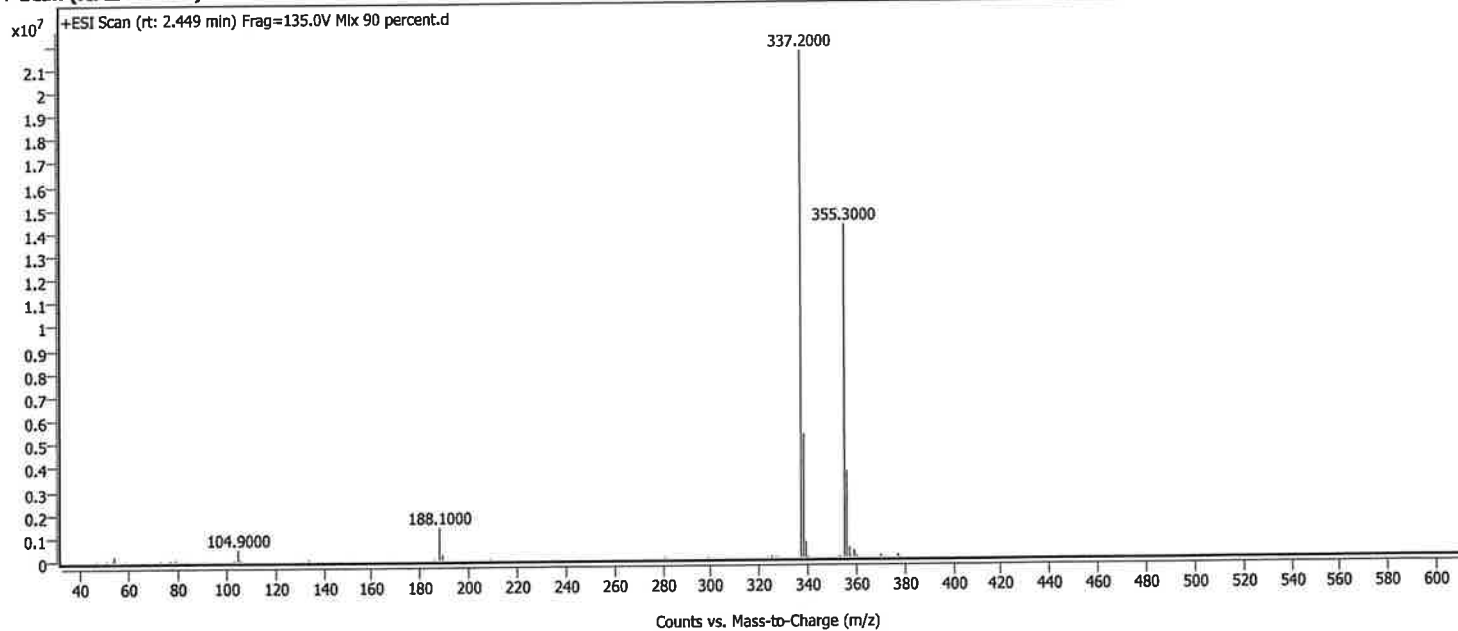


+ Scan (rt: 2.440 min)

Analysis Report

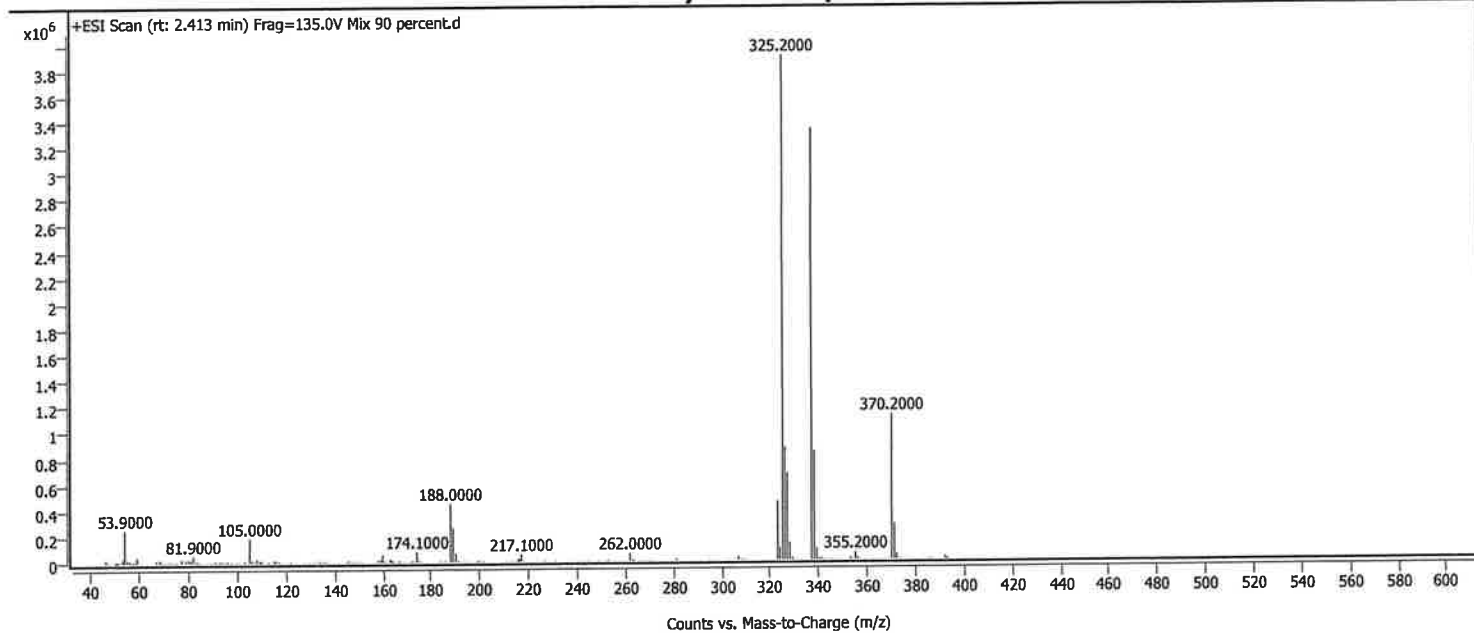


+ Scan (rt: 2.449 min)



+ Scan (rt: 2.413 min)

Analysis Report



MassHunter Qual 10.0
(End of Report)