Validation for the Qualitative Analysis of Seized Drugs by GC-IRD

Introduction

This document describes the validation for the qualitative identification of seized drugs using Gas Chromatography-Infrared Detection to establish the method meets the performance specifications outlined below. An Agilent 8890 series gas chromatograph (GC), coupled with an Analytical Solutions and Providers (ASAP) Vapor Phase Infrared detector (IRD), or GC-IRD, will be validated. This method allows for analysis and identification of controlled substances.

Fit for Use Statement

The method was determined to be valid and fit for its intended use.

Technique: Strengths and Limitations

GC-MS with its high discriminating power, speed and sensitivity makes it a suitable option for the analysis and identification of many drugs; however, isomers prove challenging. Structural isomers generate similar mass spectra and even similar retention times; therefore, analysis is problematic when trying to distinguish between structural isomers of drugs. GC-IRD, a SWGDRUG category B and A technique, uses vapor-phase IR to produce spectra that allow discrimination of structural isomers making it a suitable method complementary to GC-MS.

GC coupled with IRD is a powerful technique for discriminating complex organic drug mixtures that offers advantages over other drug analysis techniques. Since GC is utilized, complex mixtures may be separated with ease and speed compared to standalone IR techniques that would otherwise be unable to easily and accurately identify individual components in mixtures even with current peak deconvolution software. GC combined with IRD allows for the analysis and identification of closely related structural isomers; IRD can unambiguously identify isomers unlike Gas Chromatography-Mass Spectrometry.

While GC-IRD is capable of identifying structural isomers of novel psychoactive substances (NPS), it is unable to differentiate between optical isomers. This limitation does not hinder its intended application in the laboratory however. Compared with GC-MS, GC-IRD is less sensitive. Similarly, the vapor phase GC-IRD, which employs light pipe technology, exhibits lower sensitivity as compared with direct deposition GC-IRD. Fortunately, drug seizures tend to encompass larger quantities or supply of sample, which in turn provides the analyst with an ample amount of sample to conduct qualitative analysis. In other words, due to the nature of drug testing employed in the laboratory, a majority of the testing will not be impacted by limitations in sensitivity.

GC-IRD requires carrier gas for operation. Helium is most often utilized in GC applications due to its speed of separation and lack of reactivity; however, as a result of a shortage in helium, alternative mobile phases were sought. ASAP does not recommend hydrogen for use. Nitrogen may be used; however, it is typically not recommended due to lengthy analysis times. Even though analysis time is expected to increase, GC-IRD may be employed with adequate peak resolution and sensitivity. Nitrogen is also readily available as a low cost alternative. Nitrogen was utilized in this method validation.

1. Purpose/Scope

This method evaluates the suitability of GC-IRD as a qualitative method for the analysis and identification of controlled substances with a specific focus on isomers of substituted cathinones, fentanyl derivatives, as well as other more traditional psychoactive substances. Due to the prevalence cathinone and fentanyl isomers in seized drugs and limitations of GC-MS, GC-IRD is a viable complimentary technique.

Thirty-two chemical standards consisting of 29 controlled substances and 3 cutting agents, as well as a drug mixture both with and without cutting agents, were prepared at concentrations of 100 µg/mL, 1 mg/mL and 2 mg/mL and run on GC-IRD to evaluate method selectivity, reproducibility, sensitivity and robustness.

2. Analytical Method

Sample Preparation:

1. Single-component Sample
   - Prepare a 2 mg/mL certified drug standard in internal standard.
     - Powder: Add 500 µL of internal standard to a pre-weighed 1 mg of powder standard or weigh 2 mg of powder and add to 1 mL of internal standard.
     - Liquid: Evaporate 1 mg/mL ampule and add 500 µL of internal standard.
   - Dilute the 2 mg/mL standard to 1 mg/mL and 100 µg/mL concentrations.
2. Multi-component Mixture
   - Prepare a 1 mL drug mixture of methamphetamine, methcathinone, cocaine and fentanyl at 2 mg/mL in internal standard (drug standard mixture).
   - Dilute the 2 mg/mL drug standard mixture to 1 mg/mL and 100 µg/mL concentrations.
   - To 500 µL of the 2 mg/mL drug standard mixture, add 2 mg/mL of cutting agents: acetaminophen, caffeine and dextrose (cut drug standard mixture).
   - Dilute the 2 mg/mL standard to 1 mg/mL and 100 µg/mL concentrations.

Instrument Parameters

Table 1. GC-IRD Instrument and Method Parameters.

Instrument and analytical conditions: GC-IRD	
Gas Chromatography	
GC Instrument Model	Agilent 8890
Column	Agilent HP-5MS UI Part #19091S-433UI; 30m x 0.250 mm i.d. x 0.25 µm film thickness [(5%-phenyl)-methylpolysiloxane]
Inlet Temperature:	280ºC
Injection Volume:	1 µL
Injection Mode:	Split
Split Ratio:	4:1
Injection Solvent:	Methanol with Internal Standard 4-Dimethylaminoantipyrine (0.375 mg/mL
Carrier Gas:	Nitrogen, 0.626 mL/min, constant flow
Temperature Program:	IRD.m - Setpoint (Initial) 160 °C, Hold Time 4 min->Ramp 10 °C/min up to 300 °C, Hold Time 8 min

Total Run Time:	26 min
Infrared Spectroscopy	
MS Model Number	ASAP IRD3
Light Pipe Temperature	285ºC
Transfer Line Temperature:	280ºC
Spectral Range	500-4000 cm^{-1}
Spectral Resolution	8 cm^{-1}

Table 2. GC-MS Instrument and Method Parameters.

Instrument and analytical conditions: GC-MS	
Gas Chromatography	
GC Instrument Model	Agilent 7890 (GC-MS 2) and Agilent 8890 (GC-MS 3 and 4)
Column	Agilent HP-5MS UI Part #19091S-433UI; 30m x 0.250 mm i.d. x 0.25 µm film thickness [(5%-phenyl)-methylpolysiloxane]
Inlet Temperature:	280ºC
Injection Volume:	1 µL
Injection Mode:	Split
Split Ratio:	75:1
Injection Solvent:	Methanol with Internal Standard 4-Dimethylaminoantipyrine (0.375 mg/mL
Carrier Gas:	Helium, 2.2176 mL/min, constant flow
Temperature Program:	LDRUG.m - Setpoint (Initial) 230 °C, Hold Time 1.7 min->Ramp 120 °C/min up to 300 °C, Hold Time 9 min
Total Run Time:	11.283
Mass Spectrometry	
MS Model Number	Agilent 5977
Mass Analyzer:	Single quadrupole
Ionization Mode:	Electron ionization (70 eV)
Transfer Line Temperature:	280ºC
Source Temperature:	230ºC
Quad Temperature:	150ºC
Solvent Delay:	1.0 min.

Chemicals and Reference Materials:

Internal standard (I/S): 4-Dimethylaminoantipyrine at 0.375 mg/mL in methanol. Prepare by adding 1.5 g of 4-dimethylaminoantipyrine to a 4000 mL volumetric flask and diluting to volume (quantities may be scaled up or down) with methanol (ACS grade, EMD Millipore MX0485-7). The same I/S lot must be used to prepare all samples. Store at room temperature.

Analytical Reference Standards: Standards were purchased from Cayman Chemical Company, Cerilliant and Sigma. All standard formulations were solid or powder except for fentanyl. Fentanyl was purchased as a liquid 1 mg/mL solution in methanol. The standard drug mix and drug mix containing cutting agents were prepared in-house using the 2 mg/mL standards.

3. Reference Materials:

- Internal standard: 4-Dimethylaminoantipyrine at 0.375 mg/mL in methanol.
- Drug Standards: See Table 3.
- Methanol: Meets ACS Specifications

Table 3. Drug standards and mixtures.

#	Item Name	Drug Class	Manufacturer
1	3,4-dichloro-N-cyclohexyl Methcathinone	Cathinone	Cayman Chemical
2	2,3-Pentylone isomer	Cathinone	Cayman Chemical
3	N-ethyl Pentylone	Cathinone	Cayman Chemical
4	Pentylone	Cathinone	Cayman Chemical
5	Dibutylone	Cathinone	Cayman Chemical
6	4-Chloroethcathinone	Cathinone	Cayman Chemical
7	3-Chloroethcathinone	Cathinone	Cayman Chemical
8	2-Chloroethcathinone	Cathinone	Cayman Chemical
9	3,4-Methylenedioxy-α-Cyclohexylaminopropiophenone	Cathinone	Cayman Chemical
10	3,4-Methylenedioxy-α-propylaminobutiophenone	Cathinone	Cayman Chemical
11	2-Methoxymethcathinone	Cathinone	Cayman Chemical
12	3-Methoxymethcathinone	Cathinone	Cayman Chemical
13	α-methyl Acetyl fentanyl	Cathinone	Cayman Chemical
14	Fluoroisobutyrfentanyl	Opioid	Cayman Chemical
15	para-Fluorofentanyl	Opioid	Cayman Chemical
16	Cyclopropyl fentanyl	Opioid	Cayman Chemical
17	Furanyl fentanyl	Opioid	Cayman Chemical
18	para-Chlorobutyryl fentanyl	Opioid	Cayman Chemical
19	meta-Fluorofentanyl	Opioid	Cayman Chemical
20	ortho-Fluorofentanyl	Opioid	Cayman Chemical
21	Valeryl fentanyl	Opioid	Cayman Chemical
22	para-Methylfentanyl	Opioid	Cayman Chemical
23	Crotonyl fentanyl	Opioid	Cayman Chemical
24	ortho-methyl Furanyl fentanyl	Opioid	Cayman Chemical
25	ortho-methyl Cyclopropyl fentanyl	Opioid	Cayman Chemical
26	Fentanyl	Opioid	Cerilliant
27	Cocaine	Stimulant	Sigma
28	Methamphetamine	Stimulant	Sigma
29	Methcathinone	Stimulant	Sigma
30	Caffeine	Cutting Agent	Sigma
31	Acetaminophen	Cutting Agent	Sigma
32	Dextrose	Cutting Agent	Sigma
33	Standard Drug Mix	N/A	In-house
34	Standard Drug Mix and Cutting Agents	N/A	In-house

4. Performance Characteristics

4.1 Selectivity

4.1.1 Single-component sample

Thirty-two chemicals were prepared which included closely related cathinones and opioids, cutting agents and mixtures (Table 4). All 32 preparations were run on GC-MS followed by GC-IRD. MS spectra were searched against eight available mass spectral libraries. IRD spectra were searched against three libraries: Project Euclid, PBSO and FIU Infrared Fentanyl Library. Due to the limited number of reference libraries for vapor phase IRD drug data, an in-house library had been generated at the method development phase. Each component that was run and where detected was library searched.

Acceptance Criteria: *Height rejection sensitivity 3:1, Match ≥0.98*

Results:

Each 1 mg/mL drug was run on GC-MS prior to GC-IRD (Table 4). Detection of all 32 compounds and mixtures was successful via GC-MS. Each component was detected with a library match score of at least 80 or higher. All peaks were symmetrical and baseline resolved with consistent pattern of ions and ratios compared to the spectral library except for dextrose. Dextrose is known not to chromatograph well and displayed poor peak quality.

The 1 mg/mL samples were run on GC-IRD following GC-MS. Detection was possible for all samples except for the 2-chloroethcathinone and dextrose. Each component successfully detected matched at least 0.98 at 1 mg/mL. Peak shape, dependent on concentration, was symmetrical and baseline resolved with matching spectra and absorption bands.

It was learned through GC-MS validation that chloroethcathinone isomers, while able to be detected, are relatively unstable and readily breakdown in methanol, especially 2-chloroethcathinone. This phenomenon helps explain why GC-IRD, a less sensitive technique, could not detect 2-chloroethcathinone even at 2 mg/mL. It is possible that time lapse and poor stability of the chloroethcathinones resulted in 2-chloroethcathinone not being detected. With respect to dextrose, like most sugars, it does not chromatograph well; therefore, it was not detected using IRD which is an expected result.

Table 4. Component Quality Match

#	Name	GC-MS Detection	GC-IRD Detection	Match Score (1 mg/mL)
1	3,4-dichloro-N-cyclohexyl Methcathinone	√	√	0.992654
2	2,3-Pentylone isomer	√	√	0.998246
3	N-ethyl Pentylone	√	√	0.996449
4	Pentylone	√	√	0.998573
5	Dibutylone	√	√	0.982580
6	4-Chloroethcathinone	√	√	0.982246
7	3-Chloroethcathinone	√	√	0.989927
8	2-Chloroethcathinone	√	Not Detected	
9	3,4-Methylenedioxy-α-Cyclohexylaminopropiophenone	√	√	0.998711
10	3,4-Methylenedioxy-α-propylaminobutiophenone	√	√	0.999856
11	2-Methoxymethcathinone	√	√	0.995518

12	3-Methoxymethcathinone	√	√	0.985346
13	α-methyl Acetyl fentanyl	√	√	0.997366
14	Fluoroisobutyrfentanyl	√	√	0.998980
15	para-Fluorofentanyl	√	√	0.998068
16	Cyclopropyl fentanyl	√	√	0.996762
17	Furanyl fentanyl	√	√	0.997197
18	para-Chlorobutyryl fentanyl	√	√	0.994390
19	meta-Fluorofentanyl	√	√	0.995975
20	ortho-Fluorofentanyl	√	√	0.994338
21	Valeryl fentanyl	√	√	0.997449
22	para-Methylfentanyl	√	√	0.994866
23	Crotonyl fentanyl	√	√	0.995939
24	ortho-methyl Furanyl fentanyl	√	√	0.990022
25	ortho-methyl Cyclopropyl fentanyl	√	√	0.989369
26	Fentanyl	√	√	0.997967
27	Cocaine	√	√	0.991980
28	Methamphetamine	√	√	0.996078
29	Methcathinone	√	√	0.980674
30	Caffeine	√	√	0.981253
31	Acetaminophen	√	√	0.981902
32	Dextrose	√	Not Detected	

4.1.2 Multi-component mixture

Interference studies were performed on three concentrations containing methamphetamine, methcathinone, cocaine and fentanyl. A 1:1 mixture of the four compounds dissolved in methanol spiked with internal standard were run; 100 µg/mL, 1 mg/mL and 2 mg/m concentrations. Peak resolution and matrix interferences were evaluated.

Acceptance Criteria: *Height rejection sensitivity at 3:1, Match ≥0.98, No interference was observed at the retention time for each compound and the internal standard (resolution ≥ 1.5)*

Results: The 100 µg/mL concentration was too low for detection and the 2 mg/mL proved that dilution was necessary in order to optimize resolution between methamphetamine and methcathinone. Using the 1 mg/mL concentration for evaluating interference and resolution, it was determined that there was no matrix interference. Each component was baseline resolved. Each component was successfully detected with a match score ≥0.98.

Resolution results are summarized in Tables 5.

Table 5. Selectivity mixture and resolution.

	Drug	Retention Time (RT)	Relative Retention Time (RRT)	Peak Width (w)	Resolution (Rs = Rs = 2(tR2-tR1)/W1 + W2)			
Standard Mixture 1 mg/mL	Methamphetamine	5.45	0.42	0.142	5.5			
	Methcathinone	6.20	0.48	0.131		68.5		
	I/S	12.91	Reference Peak	0.065			39.6	
	Cocaine	15.70	1.22	0.076				59.1
	Fentanyl	20.19	1.56	0.076				

4.2 Matrix Effects

To the drug mixture containing methamphetamine, methcathinone, cocaine and fentanyl, cutting agents caffeine, acetaminophen and dextrose were added in equal parts for the 1 mg/mL mixture. Peak resolution and matrix interferences were evaluated.

Acceptance Criteria: *Height rejection sensitivity at 3:1, Match ≥0.98, No interference was observed at the retention time for each compound and the internal standard (resolution ≥ 1.5)*

Results: There was no interference between matrix components for the 1 mg/mL preparation. Each component was baseline resolved. Dextrose was added to simulate routine case analyses; however, it was not detected, which was observed in single-component sample analysis. The presence of dextrose and other cutting agents had no effect on the chromatography nor any effect on the spectra for identification.

Resolution results are summarized in Tables 6.

Table 6. Selectivity mixture and resolution.

	Drug	Retention Time (RT)	Relative Retention Time (RRT)	Peak Width (w)	Resolution (Rs = Rs = 2(tR2-tR1)/W1 + W2)					
Standard Mixture 1 mg/mL	Methamphetamine	5.43	0.42	0.120						
	Methcathinone	6.19	0.48	0.131	6.1					
	Acetaminophen	10.45	0.81	0.240		23.0				
	Caffeine	11.95	0.92	0.098			8.9			
	I/S	12.92	Reference Peak	0.065				11.9		
	Cocaine	15.70	1.22	0.076					39.4	
	Fentanyl	20.19	1.56	0.087						55.1

4.3 Accuracy

4.3.1 Precision (repeatability)

Repeatability was evaluated by analyzing three replicates. Each standard in Table 7a and 7b was run on the same day. The maximum difference between retention times, drug retention time percent coefficient of variation (%CV) and relative retention time %CV were calculated. The peak area counts were also determined and their %CV calculated.

Cutting agents and drug mixtures were not evaluated.

Acceptance Criteria: *Each component must be within ±0.05 minutes and %CV <3%*

Results: Each drug's RTs, where detected, were within 0.05 minutes and %CV for both the drug and its RRT did not exceed 0.1% for all but one drug, methamphetamine (Table 7a). The maximum retention time difference occurred for methamphetamine (#28), which is also the earliest eluting compound. Its peak is also slightly wider than the other components evaluated. 2-chloroethcathinone or standard #8 could not be detected, which likely stems from its poor stability in methanol.

The %CV for within run peak area counts ranged from 0.6-12.5%. The method is repeatable.

Table 7a. Same day sample retention time repeatability.

	Injection #1			Injection #2			Injection #3					
#	Drug	I/S	RRT	Drug	I/S	RRT	Drug	I/S	RRT	RT Difference (Max)	Drug %CV	RRT %CV
1	15.86	12.94	1.23	15.87	12.93	1.23	15.86	12.93	1.23	0.01	0.0%	0.1%
2	11.49	12.93	0.89	11.50	12.93	0.89	11.49	12.93	0.89	0.01	0.1%	0.1%
3	12.61	12.96	0.97	12.62	12.95	0.97	12.62	12.95	0.97	0.01	0.0%	0.1%
4	12.10	12.93	0.94	12.10	12.93	0.94	12.10	12.93	0.94	0.00	0.0%	0.0%
5	11.55	12.91	0.89	11.55	12.91	0.89	11.55	12.91	0.89	0.00	0.0%	0.0%
6	8.63	12.89	0.67	8.62	12.89	0.67	8.62	12.90	0.67	0.01	0.1%	0.1%
7	8.54	12.94	0.66	8.53	12.93	0.66	8.53	12.93	0.66	0.01	0.1%	0.0%
8												
9	15.63	12.93	1.21	15.63	12.93	1.21	15.63	12.93	1.21	0.00	0.0%	0.0%
10	12.73	12.95	0.98	12.73	12.96	0.98	12.73	12.95	0.98	0.00	0.0%	0.0%
11	8.37	12.92	0.65	8.37	12.92	0.65	8.38	12.92	0.65	0.01	0.1%	0.1%
12	8.73	12.92	0.68	8.73	12.92	0.68	8.74	12.92	0.68	0.01	0.1%	0.1%
13	20.23	12.93	1.56	20.23	12.92	1.57	20.23	12.93	1.56	0.00	0.0%	0.0%
14	19.68	12.93	1.52	19.68	12.93	1.52	19.68	12.93	1.52	0.00	0.0%	0.0%
15	19.86	12.93	1.54	19.86	12.93	1.54	19.85	12.93	1.54	0.01	0.0%	0.0%
16	21.54	12.93	1.67	21.55	12.93	1.67	21.54	12.93	1.67	0.01	0.0%	0.0%
17	24.34	12.93	1.88	24.34	12.93	1.88	24.34	12.93	1.88	0.00	0.0%	0.0%
18	22.90	12.93	1.77	22.90	12.93	1.77	22.90	12.93	1.77	0.00	0.0%	0.0%
19	19.68	12.93	1.52	19.69	12.93	1.52	19.69	12.93	1.52	0.01	0.0%	0.0%
20	19.98	12.93	1.55	19.98	12.93	1.55	19.98	12.93	1.55	0.00	0.0%	0.0%
21	21.96	12.93	1.70	21.96	12.93	1.70	21.95	12.92	1.70	0.01	0.0%	0.0%

22	21.09	12.93	1.63	21.09	12.92	1.63	21.09	12.92	1.63	0.00	0.0%	0.0%
23	21.63	12.92	1.67	21.63	12.92	1.67	21.62	12.92	1.67	0.01	0.0%	0.0%
24	25.07	12.93	1.94	25.06	12.92	1.94	25.07	12.92	1.94	0.01	0.0%	0.0%
25	22.17	12.92	1.72	22.17	12.92	1.72	22.17	12.92	1.72	0.00	0.0%	0.0%
26	20.22	12.92	1.57	20.22	12.92	1.57	20.22	12.92	1.57	0.00	0.0%	0.0%
27	15.72	12.90	1.22	15.71	12.90	1.22	15.71	12.90	1.22	0.01	0.0%	0.0%
28	5.28	12.90	0.41	5.30	12.91	0.41	5.28	12.91	0.41	0.02	0.2%	0.2%
29	6.20	12.91	0.48	6.20	12.91	0.48	6.20	12.91	0.48	0.00	0.0%	0.0%

Table 7b. Same day sample peak area counts repeatability.

#	Item Name	Injection #1 (area)	Injection #2 (area)	Injection #3 (area)	%CV
1	3,4-dichloro-N-cyclohexyl Methcathinone	720	726	713	0.9%
2	2,3-Pentylone isomer	1879	1851	1858	0.8%
3	N-ethyl Pentylone	4152	4054	3662	6.6%
4	Pentylone	1598	1566	1524	2.4%
5	Dibutylone	2645	2767	2822	3.3%
6	4-Chloroethcathinone	690	630	621	5.7%
7	3-Chloroethcathinone	521	543	479	6.3%
8	2-Chloroethcathinone				
9	3,4-Methylenedioxy-α-Cyclohexylaminopropiophenone	1684	1630	1613	2.2%
10	3,4-Methylenedioxy-α-propylaminobutiophenone	2316	2258	2197	2.6%
11	2-Methoxymethcathinone	880	875	891	0.9%
12	3-Methoxymethcathinone	1244	1284	1269	1.6%
13	α-methyl Acetyl fentanyl	1121	1130	1108	1.0%
14	Fluoroisobutyrfentanyl	1724	1711	1704	0.6%
15	para-Fluorofentanyl	1859	1791	1681	5.1%
16	Cyclopropyl fentanyl	1756	1750	1625	4.3%
17	Furanyl fentanyl	792	835	774	4.0%
18	para-Chlorobutyryl fentanyl	1305	1305	1320	0.7%
19	meta-Fluorofentanyl	1258	1185	1252	3.3%
20	ortho-Fluorofentanyl	1648	1599	1597	1.8%
21	Valeryl fentanyl	1347	1320	1337	1.0%
22	para-Methylfentanyl	1500	1434	1495	2.5%
23	Crotonyl fentanyl	1039	1124	1165	5.8%
24	ortho-methyl Furanyl fentanyl	599	583	556	3.8%
25	ortho-methyl Cyclopropyl fentanyl	1247	1125	1207	5.2%
26	Fentanyl	1578	1568	1604	1.2%
27	Cocaine	2954	3098	3029	2.4%
28	Methamphetamine	648	594	566	7.0%
29	Methcathinone	1348	1381	1443	3.5%

30	Caffeine	8913	8966	7132	12.5%
31	Acetaminophen	5936	5199	5220	7.7%
32	Dextrose				

4.3.2 Precision (reproducibility)

Similar to repeatability, the reproducibility was evaluated by analyzing three replicates. Each standard in Table 8a and 8b was run in triplicate on differing days. The maximum difference between retention times, drug retention time percent coefficient of variation (%CV) and relative retention time %CV were calculated. The peak area counts were also determined and their %CV also calculated.

Cutting agents and drug mixtures were not evaluated.

Acceptance Criteria: *Each component must be within ±0.05 minutes and %CV <3%*

Results: Each drug's RTs, where detected, were within 0.05 minutes and %CV for both the drug and its RRT did not exceed 0.2% (Table 8a). 2-chloroethcathine (#8) could not be detected for days 1-3. The reproducibility of 4-chloroethcathinone was also unsuitable for validation due to ample breakdown, which resulted in match values below 0.98 for days 2 and 3. For all other compounds, this method is reproducible.

Due to randomness of vibrational spectroscopy, between-run peak area counts tend to be inconsistent. Therefore, no acceptance criteria were established for the %CV peak area counts (Table 8b).

Table 8a. 3-Day sample retention time reproducibility.

	Day 1			Day 2			Day 3					
#	Drug	I/S	RRT	Drug	I/S	RRT	Drug	I/S	RRT	RT Difference (Max)	Drug %CV	RRT %CV
1	15.86	12.94	1.23	15.84	12.92	1.23	15.83	12.92	1.23	0.03	0.1%	0.1%
2	11.49	12.92	0.89	11.48	12.91	0.89	11.48	12.91	0.89	0.01	0.1%	0.0%
3	12.60	12.96	0.97	12.60	12.96	0.97	12.59	12.94	0.97	0.01	0.0%	0.0%
4	12.11	12.93	0.94	12.09	12.91	0.94	12.08	12.90	0.94	0.03	0.1%	0.0%
5	11.55	12.91	0.89	11.55	12.93	0.89	11.55	12.91	0.89	0.00	0.0%	0.1%
6	8.63	12.89	0.67									
7	8.53	12.94	0.66	8.51	12.91	0.66	8.51	12.91	0.66	0.02	0.1%	0.0%
8												
9	15.63	12.93	1.21	15.62	12.92	1.21	15.62	12.92	1.21	0.01	0.0%	0.0%
10	12.73	12.96	0.98	12.72	12.94	0.98	12.72	12.94	0.98	0.01	0.0%	0.0%
11	8.37	12.92	0.65	8.35	12.90	0.65	8.34	12.89	0.65	0.03	0.2%	0.1%
12	8.74	12.92	0.68	8.71	12.91	0.67	8.71	12.90	0.68	0.03	0.2%	0.1%
13	20.23	12.93	1.56	20.22	12.92	1.57	20.22	12.91	1.57	0.01	0.0%	0.1%
14	19.68	12.93	1.52	19.66	12.92	1.52	19.66	12.91	1.52	0.02	0.1%	0.0%
15	19.86	12.93	1.54	19.85	12.92	1.54	19.85	12.92	1.54	0.01	0.0%	0.0%
16	21.55	12.93	1.67	21.54	12.92	1.67	21.54	12.92	1.67	0.01	0.0%	0.0%
17	24.35	12.94	1.88	24.34	12.92	1.88	24.34	12.92	1.88	0.01	0.0%	0.1%
18	22.91	12.93	1.77	22.89	12.92	1.77	22.89	12.91	1.77	0.02	0.1%	0.0%

19	19.68	12.93	1.52	19.68	12.92	1.52	19.68	12.91	1.52	0.00	0.0%	0.1%
20	19.98	12.93	1.55	19.98	12.92	1.55	19.97	12.92	1.55	0.01	0.0%	0.0%
21	21.96	12.93	1.70	21.94	12.92	1.70	21.94	12.91	1.70	0.02	0.1%	0.0%
22	21.09	12.90	1.63	21.08	12.91	1.63	21.09	12.91	1.63	0.01	0.0%	0.1%
23	21.63	12.92	1.67	21.62	12.91	1.67	21.63	12.91	1.68	0.01	0.0%	0.0%
24	25.09	12.92	1.94	25.05	12.92	1.94	25.06	12.91	1.94	0.04	0.1%	0.1%
25	22.17	12.92	1.72	22.18	12.92	1.72	22.20	12.92	1.72	0.03	0.1%	0.1%
26	20.22	12.92	1.57	20.21	12.92	1.56	20.24	12.93	1.57	0.03	0.1%	0.0%
27	15.71	12.90	1.22	15.70	12.90	1.22	15.71	12.90	1.22	0.01	0.0%	0.0%
28	5.28	12.91	0.41	5.28	12.90	0.41	5.27	12.89	0.41	0.01	0.1%	0.1%
29	6.19	12.91	0.48	6.19	12.90	0.48	6.19	12.89	0.48	0.00	0.0%	0.1%

Table 8b. 3-Day sample peak area counts reproducibility.

#	Item Name	Day 1 (area)	Day 2 (area)	Day 3 (area)	%CV
1	3,4-dichloro-N-cyclohexyl Methcathinone	718	481	448	26.8%
2	2,3-Pentylone isomer	1727	1750	1885	4.8%
3	N-ethyl Pentylone	4116	4949	3768	14.2%
4	Pentylone	1573	1613	1574	1.5%
5	Dibutylone	2606	2887	2954	6.6%
6	4-Chloroethcathinone				
7	3-Chloroethcathinone	489	399	326	20.2%
8	2-Chloroethcathinone				
9	3,4-Methylenedioxy-α-Cyclohexylaminopropiophenone	1732	1939	1954	6.6%
10	3,4-Methylenedioxy-α-propylaminobutiophenone	2326	2697	2873	10.6%
11	2-Methoxymethcathinone	883	956	990	5.8%
12	3-Methoxymethcathinone	1312	1356	1402	3.3%
13	α-methyl Acetyl fentanyl	1167	1206	1264	4.0%
14	Fluoroisobutyrfentanyl	1745	1794	1849	2.9%
15	para-Fluorofentanyl	1923	2419	2450	13.1%
16	Cyclopropyl fentanyl	1799	2191	2247	11.7%
17	Furanyl fentanyl	864	1154	1265	18.9%
18	para-Chlorobutyryl fentanyl	1323	1519	1585	9.2%
19	meta-Fluorofentanyl	1218	1318	1537	12.0%
20	ortho-Fluorofentanyl	1590	1472	1882	12.8%
21	Valeryl fentanyl	1289	1306	1604	12.6%
22	para-Methylfentanyl	1471	1478	1847	13.5%
23	Crotonyl fentanyl	1178	1263	1341	6.5%
24	ortho-methyl Furanyl fentanyl	617	593	624	2.6%
25	ortho-methyl Cyclopropyl fentanyl	1154	1733	1976	26.1%
26	Fentanyl	1555	1892	3307	41.3%
27	Cocaine	3088	2353	3826	23.8%

4.4 Sensitivity

Sensitivity was evaluated at 100 µg/mL, 1 mg/mL and 2 mg/mL for each of the compounds (Table 9). Each compound was searched against three libraries: Project Euclid, PBSO and FIU Infrared Fentanyl Library.

Acceptance Criteria: *Height rejection sensitivity at 3:1, Match ≥0.98, group frequency region (4000-1450 cm^{-1}) match and fingerprint region match (600-1450 cm^{-1}). (Figure 1)*

Results: Each compound was detected except for 2-chloroethcathinone and dextrose. Of the 30 remaining compounds, a match of 0.98 or higher was achieved for the 1000 µg/mL concentration. Three compounds, N-ethyl pentylone, dibutylone and caffeine, were detected at 100 µg/mL. It is known that vapor phase GC-IRD is less sensitive compared to solid phase GC-IRD and GC-MS; however, for the majority of drugs studied, 1 mg/mL will allow detection of drug mixtures. This is further supported by the drug standard mixture and mixture containing cutting agents, which were detected at 1 mg/mL. Since 1 mg/mL is the targeted concentration in routine drug analysis preparations, the method's sensitivity is suitable for most drugs.

Table 9. 3-Day Sample Reproducibility.

#	Item Name	Concentration (µg/mL)	Match Score
1	3,4-dichloro-N-cyclohexyl Methcathinone	1000	0.992654
2	2,3-Pentylone isomer	1000	0.998246
3	N-ethyl Pentylone	100	0.996449
4	Pentylone	1000	0.998573
5	Dibutylone	100	0.982580
6	4-Chloroethcathinone	1000	0.982246
7	3-Chloroethcathinone	1000	0.989927
8	2-Chloroethcathinone	Not Detected	
9	3,4-Methylenedioxy-α-Cyclohexylaminopropiophenone	1000	0.998711
10	3,4-Methylenedioxy-α-propylaminobutiophenone	1000	0.999856
11	2-Methoxymethcathinone	1000	0.995518
12	3-Methoxymethcathinone	1000	0.985346
13	α-methyl Acetyl fentanyl	1000	0.997366
14	Fluoroisobutyrfentanyl	1000	0.998980
15	para-Fluorofentanyl	1000	0.998068
16	Cyclopropyl fentanyl	1000	0.996762
17	Furanyl fentanyl	1000	0.997197
18	para-Chlorobutyryl fentanyl	1000	0.994390
19	meta-Fluorofentanyl	1000	0.995975
20	ortho-Fluorofentanyl	1000	0.994338
21	Valeryl fentanyl	1000	0.997449

22	para-Methylfentanyl	1000	0.994866
23	Crotonyl fentanyl	1000	0.995939
24	ortho-methyl Furanyl fentanyl	1000	0.990022
25	ortho-methyl Cyclopropyl fentanyl	1000	0.989369
26	Fentanyl	1000	0.997967
27	Cocaine	1000	0.991980
28	Methamphetamine	1000	0.996078
29	Methcathinone	1000	0.980674
30	Caffeine	100	0.981253
31	Acetaminophen	2000	0.981902
32	Dextrose	Not Detected	

4.5 Robustness

Validation studies were performed over 3 days or more.

All analysts were competency tested by analyzing 5 unknown samples by GC-IRD.

Acceptance Criteria:

- *Comparable results for GC-IRD runs over 3 days.*
- *Successful completion of competency samples by all analysts.*

Results:

- As was observed in section 4.3, the data results were repeatable.
- All analysts correctly identified 3 unknown samples in accordance to section 5, Quality Control.
    - Match ≥0.98
    - Comparison to corresponding drug standard

Table 10. Analyst Competency Results

Analyst	Date of analysis	Sample Name	Replicate #	d9-THC (area)	IS (area)	Weight (g)	THC/IS Ratio Sample	%CV	d9-THC (area)	IS (area)	d9-THC/IS Ratio Control	Dilution	Instrument
DL	5/19/21	T5	1	3805334	532381	0.0204	7.01	7.31%	951453	394653	**2.41**	20X	GCMS 1
		T5	2	3152441	519719	0.0192	6.32						
	5/19/21	T6	1	200717	518580	0.0209	0.37	6.38%				0	
		T6	2	220784	544604	0.0200	0.41						
KM	5/20/21	T5	1	4254358	434902	0.0201	9.73	5.62%	783943	340419	**2.30**	20X	GCMS 1
		T5	2	4058388	451457	0.0200	8.99						
	5/20/21	T6	1	174249	470892	0.0202	0.37	2.99%				0	
		T6	2	204223	[illegible]	[illegible]	[illegible]						
MW	5/26/21	T5	1	3821578	[illegible]	[illegible]	8.4[illegible]	[illegible]2%	671580	324092	**2.07**	20X	GCMS 2
		T5	2	3469741	406208	0.0198	8.63						
	5/26/21	T6	1	126641	451209	0.0197	0.28	0.39%				0	
		T6	2	123306	426077	0.0202	0.29						
SW	5/20/21	T5	1	4831184	525078	0.0204	9.02	4.86%	913745	393717	**2.32**	20X	GCMS 1
		T5	2	4598000	537929	0.0203	8.42						
	5/20/21	T6	1	250078	613328	0.0207	0.39	2.17%				0	
		T6	2	250496	642805	0.0204	0.38						
IKA	4/22/21	T5	1	4199430	420708	0.0206	9.68	3.23%	774972	340700	**2.27**	20X	GCMS 1
		T5	2	4283836	420817	0.0201	10.13						
	4/29/21	T6	1	229287	580584	0.0201	0.39	0.79%	1181020	463381	**2.55**	0	
		T6	2	227275	590745	0.0198	0.39						

4.6 Carryover

Sample carryover was evaluated in each of the standards (1-34) by injecting methanol blanks between the samples (Table 3).

Acceptance Criteria: *A blank sample must be free of any components when run after a sample prepared according to the validated method.*

Results: There were no instances of interfering carryover. In a couple instances peaks were observed; however, no defined, positive spectrum was observed and no match could be made. An internal standard blank must be analyzed prior to each case sample in casework to demonstrate that no carryover is present.

5 Quality Control

It was determined through validation three acceptance criteria must be met for drug confirmation.

1. Peak retention time of the unknown must match the retention time of the reference standard peak for confirmation.
2. Library spectra must match.
3. Spectra group frequency or functional group region and the fingerprint region must match using overlay in superimpose mode - .

Acceptance Criteria: *Retention Time within ±0.05 for confirmation only, Height rejection sensitivity at 3:1, Match ≥0.98, group frequency region (4000-1450 cm^{-1}) match and fingerprint region match (600-1450 cm^{-1}). (Figure 1)*

Results:

1. Drug retention time is both repeatable and reproducible. Same day retention time shits did not exceed 0.02 minutes and day-to-day retention time shifts did not exceed 0.04, respectfully. For a positive match, if making a confirmation, retention times cannot exceed +/- 0.05 minute.

2. Library matches less than 0.98 exhibit noise and poor spectral quality. Fentanyl, for example, at 2 mg/mL had a match of 0.997873. While the 100 µg/mL fentanyl was detectable, the spectral match does not exceed 0.98 (Figures 2 and 3). Positive matches, confirmatory and tentative, must exceed a minimum of 0.98 match score.

3. When reviewing data for a match, the library and/or standard spectra must be overlaid with the unknown spectrum in superimpose mode. The peak shape, intensity and wavenumbers can be evaluated and matched. The fingerprint region can be especially helpful when making a match.

   Slight variations in spectra may occur, similar to GC-MS, with respect to baseline noise, co-elution and concentration. Air moisture introduced into the GC-IRD can impact a spectrum by exhibiting broadened spectral peaks and spectral artifacts. When spectral quality is impacted, background subtraction may be adjusted to optimize the match.

   It is important to point out that while GC-IRD possesses some notable advantages over GC-MS for structurally similar compounds, the method is not intended to replace GC-MS. The method compliments GC-MS, and at times, GC-MS may be more suitable for identification. For example, pentylone and N-ethyl pentylone are synthetic cathinones that differ in structure (Figure 4). They possess unique retention times and mass spectra (Figure 5 and 6); however, they yield relatively similar GC-IRD spectra (Figure 7 and 8). There are some notable differences in the group frequency region (3000-2750 cm^{-1}) and the fingerprint region (1400-1075 cm^{-1}). When searched, both are identified correctly with matches >0.99. When the N-Ethylpentylone is overlaid with its library match, there is virtually no difference in spectra, which is also true of pentylone (Figure 9). So, while the two compounds can be correctly identified, GC-MS carries better discriminating power and this must be taken into consideration.

Figure 1. IR spectrum regions.

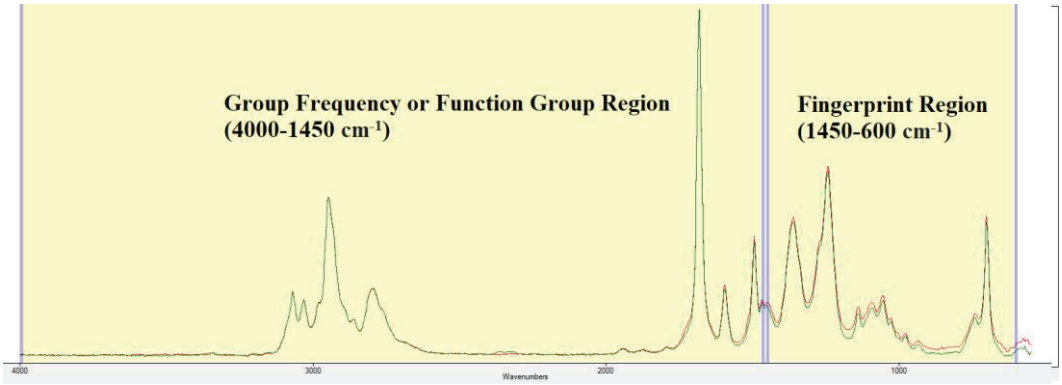


Figure 2. Library match of 2 mg/mL and 100 µg/mL fentanyl.

		Sample	Metric	Name	Library	Entry
1	⊟	IRDATA.SPC	0.997873	20.26 minutes: AVE (20.19: 20.30) Ref. (20.65: 21.16) of STD_101922MW_2022_October_20_032711_26\irdata.cgm	PBSO	14: Fentanyl.spc

		Sample	Metric	Name	Library	Entry
1	⊟	IRDATA.SPC	0.951665	20.26 minutes: AVE (20.19: 20.30) Ref. (20.65: 21.16) of STD_101922MW_2022_October_20_032711_26\irdata.cgm	PBSO	14: Fentanyl.spc

Figure 3. Overlay of 2 mg/mL fentanyl (green), 100 µg/mL (blue) fentanyl and library (red).

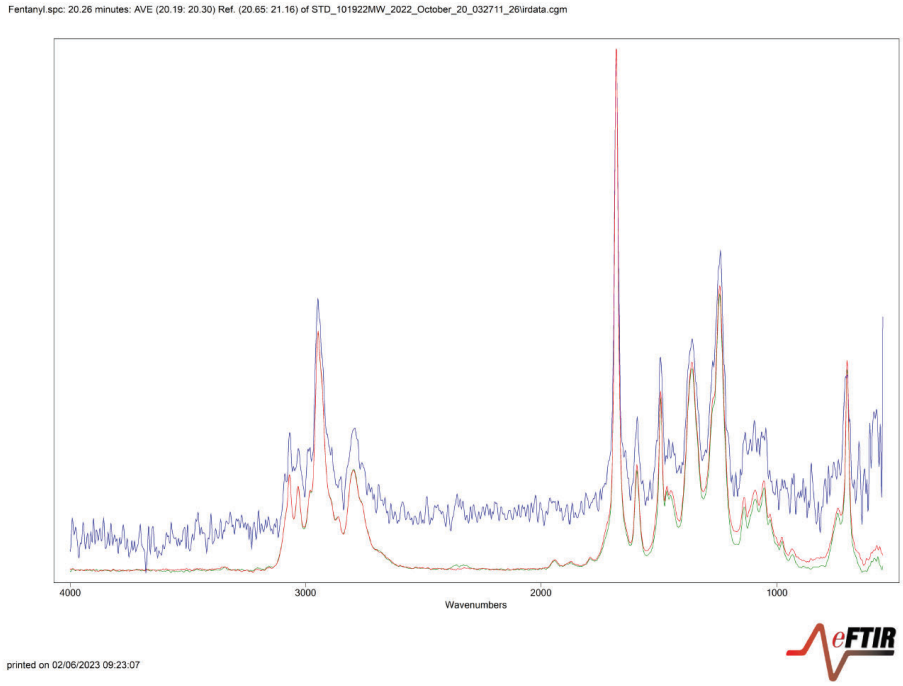


Figure 4. N-Ethyl pentylone and pentylone chemical structures.

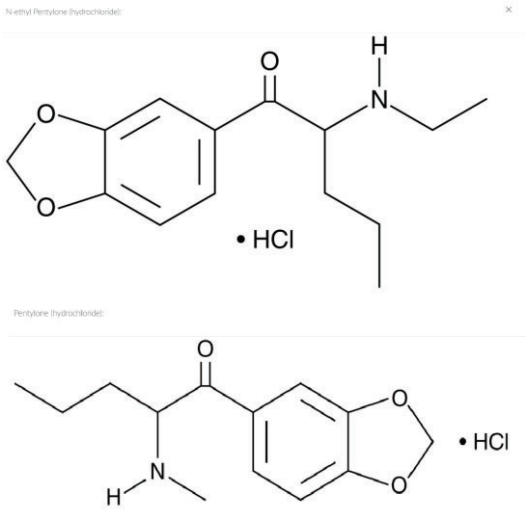


Figure 5. N-Ethyl pentylone and pentylone total ion chromatograms.

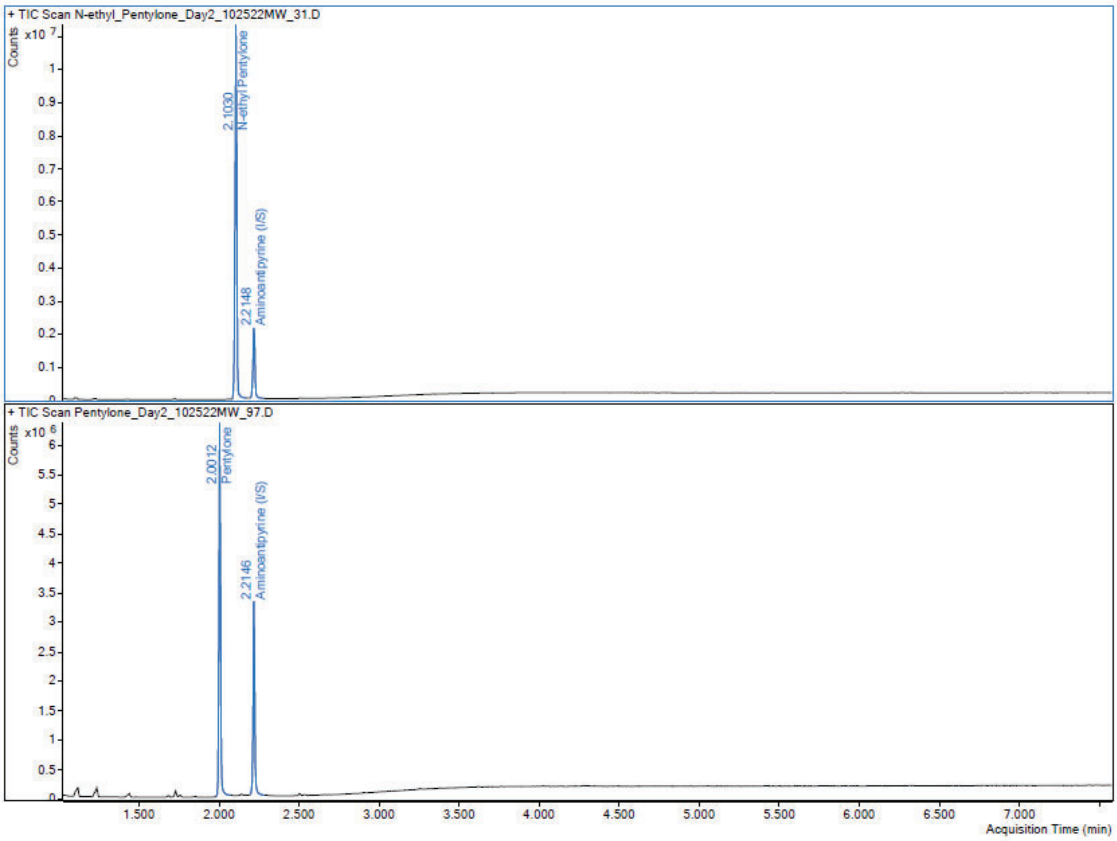
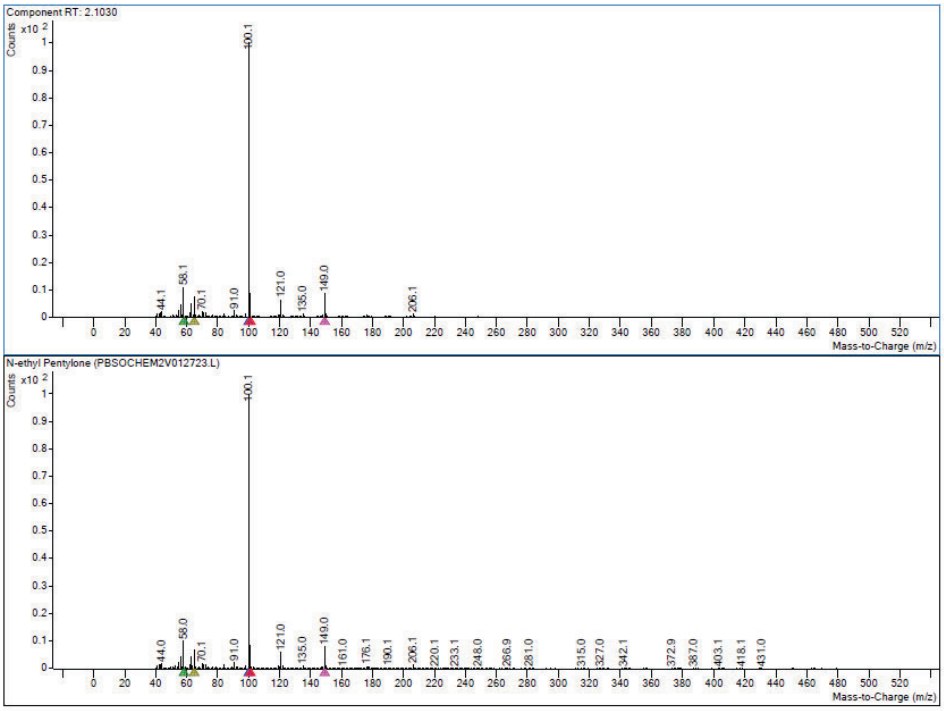


Figure 6. N-ethyl pentylone and pentylone mass spectra.



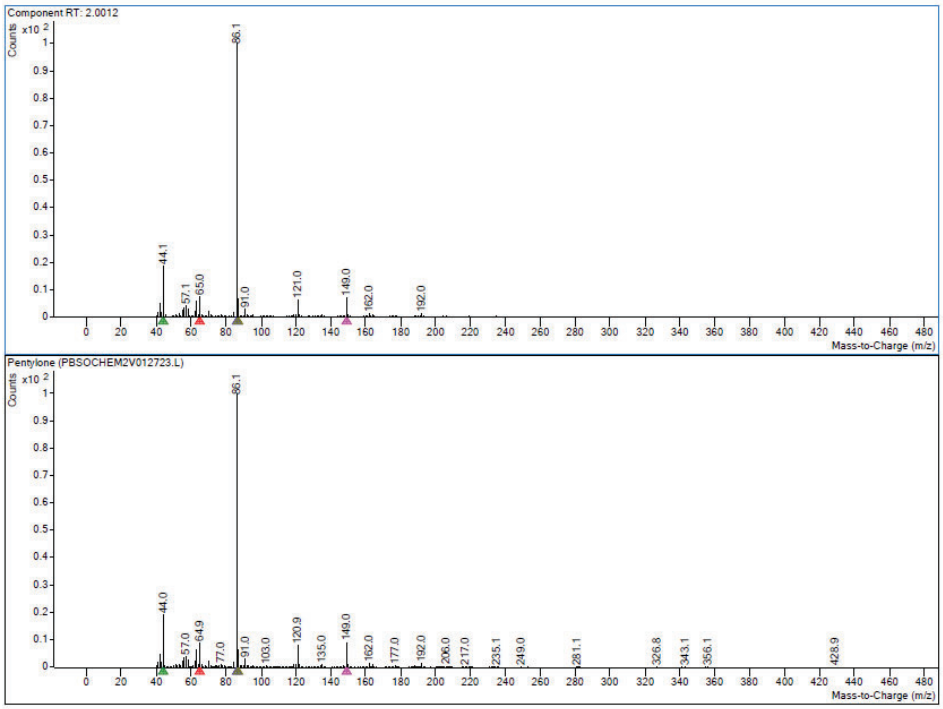
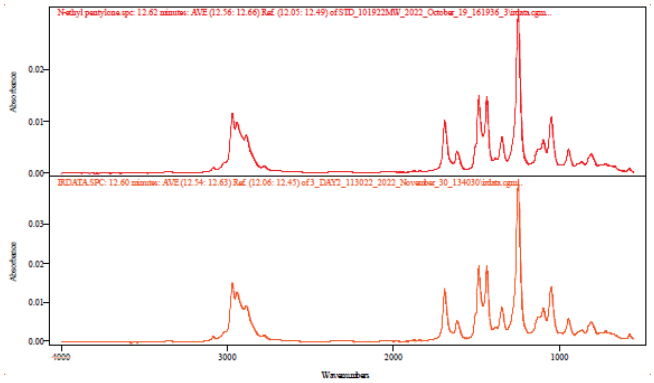


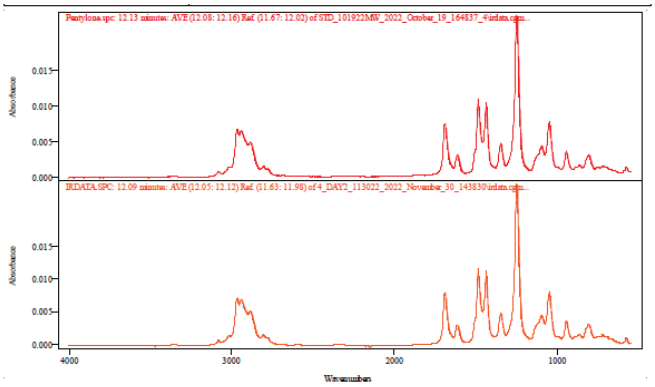
Figure 7. N-Ethyl pentylone and pentylone GC-IRD spectra and library match.



IRDATA.SPC: 12.60 minutes: AVE (12.54: 12.63) Ref. (12.06: 12.45) of 3_DAY2_113022_2022_November_30_134030.cgm

N-ethyl pentylone.spc: 12.62 minutes: AVE (12.56: 12.66) Ref. (12.05: 12.49) of STD_101922MW_2022_October_19_161936_3\irdata.cgm

Metric	Name	Library	Entry
0.999488	12.62 minutes: AVE (12.56: 12.66) Ref. (12.05: 12.49) of STD_101922MW_2022_October_19_161936_3\irdata.cgm	PBSO	25: N-ethyl pentylone.spc

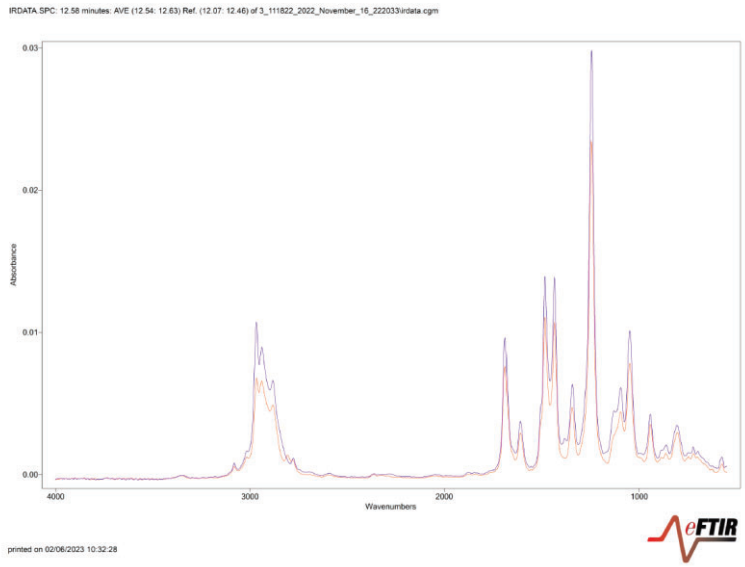


IRDATA.SPC: 12.09 minutes: AVE (12.05: 12.12) Ref. (11.63: 11.98) of 4_DAY2_113022_2022_November_30_143830.cgm

Pentylone.spc: 12.13 minutes: AVE (12.08: 12.16) Ref. (11.67: 12.02) of STD_101922MW_2022_October_19_164837_4\irdata.cgm

Metric	Name	Library	Entry
0.999399	12.13 minutes: AVE (12.08: 12.16) Ref. (11.67: 12.02) of STD_101922MW_2022_October_19_164837_4\irdata.cgm	PBSO	35: Pentylone.spc

Figure 8. N-ethyl pentylone (purple) and pentylone (orange) GC-IRD spectra overlay.



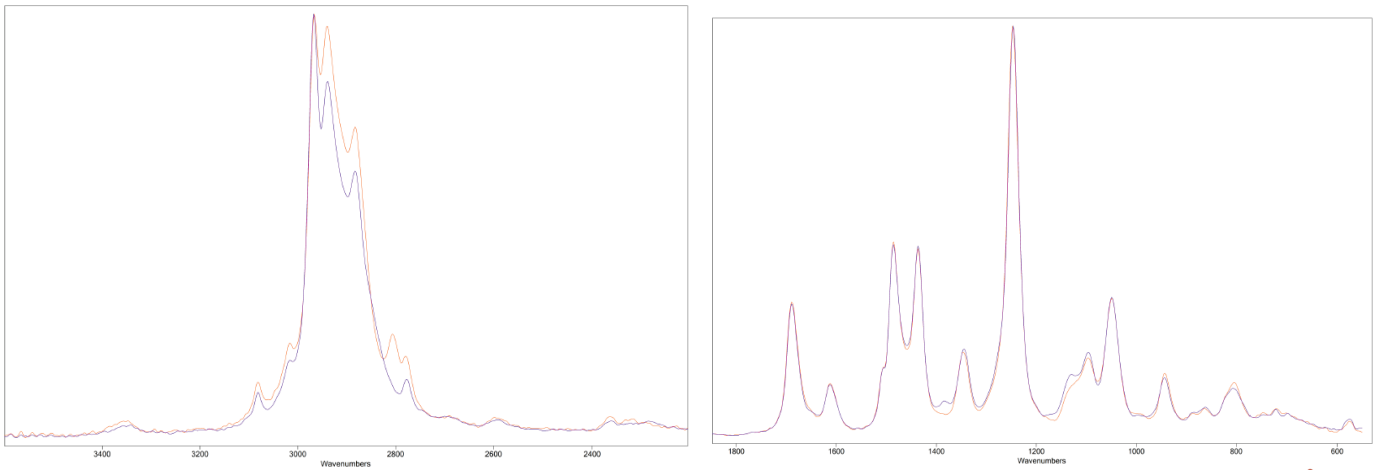
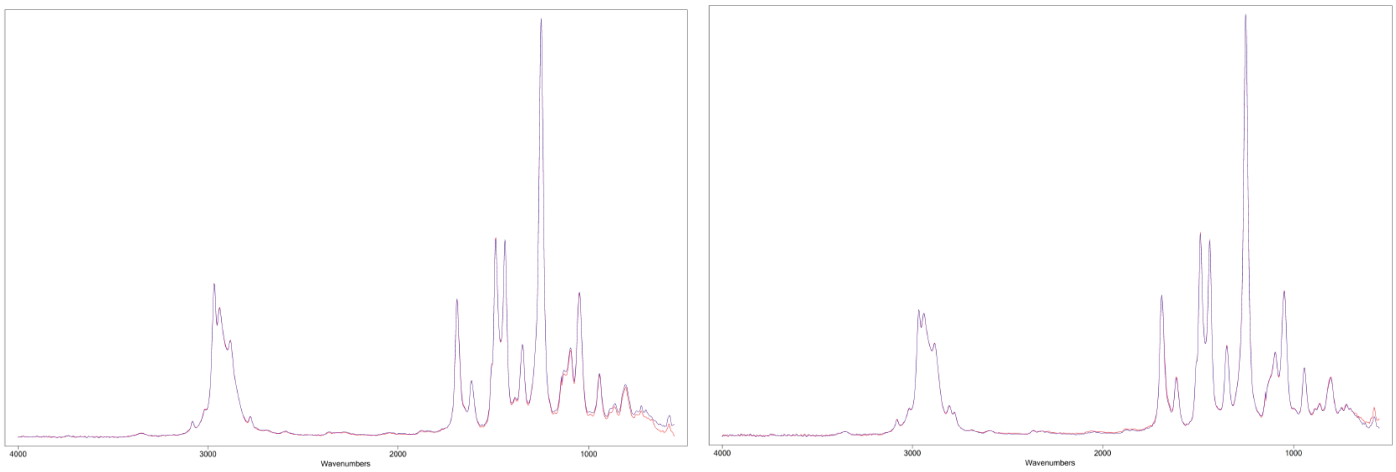


Figure 9. N-Ethylpentylone and pentylone GC-IRD library overlay.



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