Validation of Seized Drugs Fourier Transform Infrared Spectrometer (FTIR)

Spectra Format	Absorbance
Accessory	Attenuated Total Reflectance (ATR)
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Laboratory Director	Derek Sanders
Start Date	December 12, 2023
Completion Date	August 5, 2024
Instrument	FTIR-1
Method	BCCL FTIR.exp

Validation Approval

Analyst: Kayla M. Baylor Date: 11/7/2024

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Laboratory Director: [signature] Date: 7 Nov 2024

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Validation Summary

Purpose

Validation studies were performed on the FTIR in the seized drugs section to show the instrument is fit for the analysis of commonly encountered controlled and non-controlled substances and dangerous drugs. These studies were used to determine if the instrumentation and method were able to produce accurate identifications and meet criteria for selectivity, repeatability, and matrix effects. During the validation process, known standards were analyzed and used to create an in-house BCCL library for comparison.

Results

Selectivity of the FTIR instrument was assessed by running and evaluating 65 different known standards. The resulting spectra were compared to an existing FTIR library, SWGDRUG IR Spectral Library Version 2.1 released on August, 27, 2019. Of the known standards, 34 produced acceptable library matches to the SWGDRUG library demonstrating the instrumentations high degree of selectivity. The remaining 31 standards were not present within the SWGDRUG library. One of these standards, tetramisole, was added to the library after an acceptable match was found in literature and online resources. Of the 65 known standards run, the laboratory was not able to find an acceptable match utilizing external sources for 30 of these standards. Consideration will be made in the future for the addition of these standards if matches are found using external sources. Some of these known standards are old and, although prevalent at one time, they are not commonly seen in seized drugs casework today. The purchase of two new known standards, ephedrine HCl and morphine sulfate, were attempted for addition to this validation. Due to supply issues with the manufacturer, they have been on back order and were not able to be analyzed as part of this validation. Once these standards are received they will be added to the FTIR library. Known standards from reputable vendors that are analyzed and a library match not found may be deemed acceptable to be added to the in-house library upon review by the analyst. A list of the standards run is located in Table 1 and printouts of the spectra and their associated library matches will be retained. Expired standards used to create the in-house library are noted with "expired" next to the standard name in Table 1. The use of expired standards was considered acceptable since they are known and were compared to an external library prior to addition to the BCCL in-house library.

Table 1. BCCL In-House Library Entries

In-House Library Entry #	Standard Name	Inventory #	Collection Date	Library Verified
1	Methamphetamine HCl	S-09-204	March 1, 2024	✓
2	Cocaine HCl	S-09-201	December 21, 2023	✓

3	+/- Methamphetamine	S-09-208	January 8, 2024	✓
4	(-) Tetramisole HCl	S-23-018	March 1, 2024	✓
5	Acetaminophen	S-23-025	December 21, 2023	✓
6	Aspirin	S-23-023	December 21, 2023	✓
7	Benzocaine	C-10-0049	January 8, 2024	✓
8	Caffeine	S-23-022	December 21, 2023	✓
9	Clonazepam -Expired	S-09-212	January 8, 2024	✓
10	Codeine - Expired	S-09-213	December 12, 2023	✓
11	Amoxicillin trihydrate	S-23-027	December 21, 2023	✓
12	Cyclobenzaprine	C-10-0087	January 5, 2024	✓
13	d-Amphetamine Sulfate	S-09-205	January 5, 2024	✓
14	d,l-Amphetamine Sulfate - Expired	S-09-207	January 5, 2024	✓
15	Dextrose	S-23-028	December 21, 2023	✓
16	Dimethyl Sulfone	S-23-019	December 20, 2023	✓
17	Flunitrazepam	C-10-0089	January 5, 2024	✓
18	Gabapentin	S-23-021	December 20, 2023	✓
19	Guaifenesin - Expired	S-20-007	January 8, 2024	✓
20	Hydrocodone bitartrate - Expired	S-09-206	December 12, 2023	✓
21	Ibogaine HCl	C-10-0092	January 8, 2024	✓
22	Ibuprofen	S-23-020	December 20, 2023	✓
23	Inositol	C-10-0066	January 8, 2024	✓
24	Lactose (monohydrate)	S-23-029	December 21, 2023	✓
25	Levamisole HCl	S-23-026	December 21, 2023	✓
26	Lidocaine	S-09-202	January 8, 2024	✓
27	Fast Blue BB Salt	S-10-381A	March 1, 2024	✓
28	Mannitol	S-23-024	December 21, 2023	✓
29	MDA HCl - Expired	S-09-210	January 8, 2024	✓
30	Methocarbamol	S-18-033	January 8, 2024	✓
31	Procaine HCl - Expired	S-09-209	January 8, 2024	✓
32	Tropacocaine	C-10-0085	January 8, 2024	✓
33	Cocaine base	Base ext of S-09-201 w/ EtAc	March 1, 2024	✓
34	Pregabalin	S-24-003	May 24, 2024	✓
35	Trazadone	S-24-004	August 5, 2024	✓

The ability of the FTIR to distinguish the salt and base forms of a substance make it an advantageous technique. Repeatability studies for this validation were performed by analyzing ten samples of cocaine HCl and ten samples of cocaine base over two days, while demonstrating that the FTIR can differentiate between the salt and base forms of the drug. A summary of the results for the repeatability study are located in Table 2.

Table 2. Repeatability Study Summary

Standard	Inventory #		Peak 1 (cm^{-1})	Peak 2 (cm^{-1})	Peak 3 (cm^{-1})	Peak 4 (cm^{-1})	Peak 5 (cm^{-1})	Peak 6 (cm^{-1})	Peak 7 (cm^{-1})	Peak 8 (cm^{-1})
Cocaine base Day 1	Base ext of S-09-201 w/ EtAc		713.0	723.3	1037.1	1109.1	1149.0	1180.8	1228.6	1274.8
			713.4	723.5	1037.4	1109.6	1149.3	1181.3	1228.9	1275.2
			713.1	723.3	1037.1	1109.1	1149.0	1180.6	1228.6	1275.0
			713.2	723.2	1037.2	1109.2	1149.1	1180.7	1128.6	1275.1
			713.2	723.3	1037.1	1109.2	1149.1	1181.1	1228.7	1275.2
			713.1	723.4	1037.1	1109.1	1149.0	1180.7	1228.6	1274.9
			713.1	723.2	1037.1	1109.5	1149.0	1180.6	1228.7	1275.0
			713.0	723.6	1037.1	1109.2	1149.1	1180.8	1228.4	1275.0
			713.0	723.2	1037.2	1109.4	1149.1	1180.6	1228.6	1275.0
			712.9	723.1	1037.0	1108.9	1148.9	1180.7	1228.5	1274.8
Average			713.1	723.3	1037.1	1109.2	1149.1	1180.8	1218.6	1275.0
High Value			713.4	723.6	1037.4	1109.6	1149.3	1181.3	1228.9	1275.2
Low Value			712.9	723.1	1037.0	1108.9	1148.9	1180.6	1228.4	1274.8
Range (target: ≤2 cm^{-1})			**0.5**	**0.5**	**0.4**	**0.7**	**0.4**	**0.7**	**0.5**	**0.4**
Standard	**Inventory #**		**Peak 1 (cm^{-1})**	**Peak 2 (cm^{-1})**	**Peak 3 (cm^{-1})**	**Peak 4 (cm^{-1})**	**Peak 5 (cm^{-1})**	**Peak 6 (cm^{-1})**	**Peak 7 (cm^{-1})**	**Peak 8 (cm^{-1})**
Cocaine base Day 2	Base ext of S-09-201 w/ EtAc		712.9	723.2	1037.2	1109.2	1149.2	1180.7	1228.7	1275.0
			712.7	723.0	1036.8	1108.7	1148.7	1180.2	1228.1	1274.3
			713.2	723.8	1037.4	1109.6	1149.3	1181.2	1229.0	1275.4
			712.9	723.3	1037.3	1109.5	1149.1	1180.6	1228.8	1275.4
			712.9	723.3	1037.1	1109.2	1149.0	1180.6	1228.6	1274.8
			712.9	723.2	1037.1	1109.1	1149.0	1180.7	1228.5	1274.9
			713.1	723.5	1037.2	1109.2	1149.1	1181.0	1228.7	1275.1
			714.6	No peak	No peak	No peak	1148.1	1178.4	No peak	1278.5
			712.8	723.0	1036.9	1108.6	1148.7	1180.2	1228.4	1274.1
			713.3	723.4	1037.3	1109.5	1149.3	1181.2	1228.8	1275.3
Average			713.13	723.3	1037.1	1109.2	1149.0	1180.5	1228.6	1275.3
High Value			714.6	723.8	1037.4	1109.6	1149.3	1181.2	1229.0	1278.5
Low Value			712.7	723.0	1036.8	1108.6	1148.1	1178.4	1228.1	1274.1
Range with Sample 8			1.9	0.8	0.6	1.0	1.2	2.8	0.9	4.4
Range Without Sample 8 (target: ≤2 cm^{-1})			**0.6**	**0.8**	**0.6**	**1.0**	**0.6**	**1.0**	**0.9**	**1.2**

Standard	Inventory #		Peak 1 (cm^{-1})	Peak 2 (cm^{-1})	Peak 3 (cm^{-1})	Peak 4 (cm^{-1})	Peak 5 (cm^{-1})	Peak 6 (cm^{-1})	Peak 7 (cm^{-1})	Peak 8 (cm^{-1})
Cocaine HCl Day 1	S-09-201		729.1	1010.6	1025.6	1070.5	1104.3	1229.1	1247.8	1264.2
			729.1	1010.6	1025.6	1070.5	1104.4	1229.2	1247.9	1264.2
			729.2	1010.8	1025.7	1070.6	1104.6	1229.2	1248.0	1264.2
			729.1	1010.8	1025.7	1070.6	1104.5	1229.3	1248.0	1264.3
			729.1	1010.6	1025.6	1070.5	1104.3	1229.2	1247.9	1264.2
			729.2	1010.9	1025.9	1070.7	1104.7	1229.4	1248.1	1264.2
			729.1	1010.7	1025.7	1070.6	1104.5	1229.2	1247.9	1264.2
			729.0	1010.8	1025.8	1070.6	1104.4	1229.3	1248.0	1264.0
			729.2	1010.9	1025.8	1070.7	1104.7	1229.3	1248.0	1264.2
			729.0	1011.0	1025.9	1070.8	1104.7	1229.4	1248.1	1264.2
Average			729.1	1010.8	1025.7	1070.6	1104.5	1229.3	1248.0	1264.2
High Value			729.2	1011.0	1025.9	1070.8	1104.7	1229.4	1248.1	1264.3
Low Value			729.0	1010.6	1025.6	1070.5	1104.3	1229.1	1247.8	1264.0
Range (target: ≤2 cm^{-1})			**0.2**	**0.4**	**0.3**	**0.3**	**0.4**	**0.3**	**0.3**	**0.3**
Standard	**Inventory #**		**Peak 1 (cm^{-1})**	**Peak 2 (cm^{-1})**	**Peak 3 (cm^{-1})**	**Peak 4 (cm^{-1})**	**Peak 5 (cm^{-1})**	**Peak 6 (cm^{-1})**	**Peak 7 (cm^{-1})**	**Peak 8 (cm^{-1})**
Cocaine HCl Day 2	S-09-201		728.9	1010.7	1025.6	1070.5	1104.2	1229.1	1247.9	1263.8
			729.0	1010.8	1025.7	1070.6	1104.4	1229.3	1247.9	1264.0
			729.1	1010.8	1025.8	1070.7	1104.5	1229.3	1248.0	1264.2
			729.1	1010.7	1025.7	1070.6	1104.4	1229.3	1247.9	1264.2
			729.1	1010.8	1025.8	1070.6	1104.6	1229.3	1248.0	1264.1
			729.0	1010.8	1025.7	1070.6	1104.3	1229.2	1247.9	1264.0
			729.1	1010.8	1025.8	1070.7	1104.6	1229.2	1248.0	1264.1
			729.2	1010.9	1025.8	1070.7	1104.6	1229.3	1248.0	1264.2
			729.1	1010.9	1025.8	1070.8	1104.7	1229.3	1248.0	1264.1
			729.2	1010.8	1025.7	1070.6	1104.5	1229.3	1248.0	1264.2
Average			729.1	1010.8	1026.0	1070.6	1104.5	1229.3	1248.0	1264.1
High Value			729.2	1010.9	1025.8	1070.8	1104.7	1229.3	1248.0	1264.2
Low Value			728.9	1010.7	1025.6	1070.5	1104.2	1229.1	1247.7	1263.8
Range (target: ≤2 cm^{-1})			**0.3**	**0.2**	**0.2**	**0.3**	**0.5**	**0.2**	**0.3**	**0.4**

The comparison of the spectra, in addition to the resulting library match, demonstrates that the FTIR is able to distinguish between the salt and base forms. Cocaine base sample 8 on day 2 of the repeatability study did not contain peaks at four of the positions monitored during this validation study. When the cocaine base sample 8 was compared to other samples that day, it was noted that the recovery was much lower. Preparation of the cocaine base sample involved taking a cocaine HCl standard and base extracting it. The base extracted sample was then placed in KBr and dried down for analysis. The variability introduced when extracting 10 separate preparations of cocaine base is expected and will be noted as a limitation of the method. The range for the base extracted samples for day 2 were performed with and without sample 8 to show the impact of this sample on the results of this validation. Comparing the known standard of cocaine HCl to a base extracted cocaine sample, the range is much lower and consistent for the standard compared to the base extracted sample. The range being lower for the standard versus the base extracted standard is expected due to the variability introduced when extracting each of the 10 samples and placing them in KBr and drying down prior to analysis. Repeatability results from Table 2 above show that the variation in wavenumber for each of these peaks in the cocaine HCL sample over two days was less than 0.5 cm^{-1}. The variation in wavenumber for each of these peaks for the cocaine base sample over 2 days including peak 8 data is less than 2.8 cm^{-1}, while variation for these peaks excluding peak 8 data as an outlier is less than 1.2 cm^{-1}. The acceptance criteria for the variation in wavenumber is less than or equal to 2 cm^{-1}. Acceptance criteria for the repeatability study was met when excluding sample 8 on day 2 as an outlier. In instances where samples need to be placed in KBr in order to perform the analysis, it should be noted that results will be variable and repeatability of these samples is a limitation of this method. Printouts of the spectra and the resulting library match will be retained.

The ability to distinguish between mixtures is a limitation of the FTIR instrumentation which was demonstrated by analyzing common controlled substances with adulterants and diluents. FTIR is a technique that does not include a separation step causing the spectra to be a combined result of the absorptions from all substances present in a sample. Sample mixtures may contain adulterants or diluents that are high in concentration which can often mask the presence of the controlled substance. The library match produced will often give the analyst an indication as to what substances may be present within a sample. Examples of mixture samples can include caffeine in a tablet, levamisole in a cocaine sample, or dimethyl sulfone in a methamphetamine sample.

To demonstrate matrix effects on a sample, cocaine HCl, cocaine base, and methamphetamine were combined with commonly encountered cutting agents. In order to account for the variety of mixtures that may be encountered, the cocaine HCl samples were prepared with a 1:1 measured amount, the cocaine base samples were prepared with an estimated 1:1 amount, and the methamphetamine sample with a 10:1 measured amount. Clandestine samples contain unknown amounts of drugs and adulterants/diluents which do not undergo any quality controls testing. These samples, more than likely, are prepared unmeasured or inconsistent quantities of drug to adulterant/diluent; therefore, mixture

samples in this validation study were also prepared in an estimated 1:1 amount to mimic real case examples. The following mixtures and proportions were prepared and analyzed:

1 part cocaine HCl : 1 part inositol
1 part cocaine HCl : 1 part procaine
1 part cocaine HCl : 1 part benzocaine
1 part cocaine HCl : 1 part levamisole

1 part cocaine base : 1 part inositol
1 part cocaine base : 1 part procaine
1 part cocaine base : 1 part benzocaine
1 part cocaine base : 1 part levamisole

10 parts methamphetamine HCl : 1 part dimethylsulfone

Each of these mixtures were analyzed to produce a spectra with in-house library match search results. Printouts of the spectra will be retained. Comparison of the spectra from the mixture study shows that for 4 of the 9 mixtures, the library search best match was to the cutting agent present. In the mixtures where the components were measured in equal proportion using a balance, 3 of the 4 mixtures best match was to the cutting agent with a greater than 74%. In the mixtures that contained an estimated 1:1 mixture, 3 of the 4 mixtures best match was to the controlled substance as opposed to the cutting agent. Each spectra contained the presence of significant extraneous absorption peaks in the mixture spectra. These extraneous peaks would need to be considered before an identification of a controlled substance could be made.

The FTIR data processing software has the ability to perform subtractions, which may help remove part of the cutting agent from the spectra allowing for a better match to the controlled substance. The resulting spectra for the mixtures where the controlled substance was the better match were improved further through the use of the subtraction function. The subtraction function proved very useful in the mixture of cocaine base and levamisole. Initially the levamisole had a better match than the cocaine base. After subtraction was performed, the extraneous peaks from levamisole were completely removed resulting in cocaine base being the best match. A similar result was demonstrated with the cocaine HCl and inositol mixture. Through the use of subtraction, the inositol extraneous peaks were completely removed. Subtraction of the cocaine HCl and benzocaine allowed for cocaine to be the better match, although the presence of extraneous peaks from benzocaine were not removed entirely. For the cocaine HCl and procaine mixture, the subtraction function did not improve the match of the cocaine HCl, which was the better match in both instances. Using the subtraction function with the cocaine HCl and levamisole mixtures allowed cocaine HCl to have the better match removing some of the extraneous peaks from the levamisole although not entirely. Subtraction of the methamphetamine and dimethylsulfone provided a slightly increased match score for methamphetamine, although methamphetamine was the best match without subtraction due to the ratio used. For a summary of results regarding the mixture validation study see Table 3.1 and Table 3.2. Table 3.1 displays the results of the mixture study without the aid of subtraction while Table 3.2 displays the results when the subtraction tool was used to aid in the identification of a drug. When the subtraction tool was used for

the cocaine HCl and inositol mixture, there was no match data for inositol as it was fully resolved from the cocaine HCl peak. The same occurred with the cocaine base and levamisole mixture, no match data for levamisole was displayed and it was fully resolved from the cocaine base peak.

Table 3.2 Mixture Study Summary (Unsubtracted)

Sample	Match Fit Percent	
	Drug	Adulterant/Diluent
1 part cocaine HCl : 1 part inositol	**32.11%**	**93.64%**
1 part cocaine HCl : 1 part procaine	**89.56%**	**42.82%**
1 part cocaine HCl : 1 part benzocaine	**34.44%**	**92.86%**
1 part cocaine HCl : 1 part levamisole	**74.73%**	**50.03%**
1 part cocaine base : 1 part inositol	**75.19%**	**72.10%**
1 part cocaine base : 1 part procaine	**91.90%**	**37.69%**
1 part cocaine base : 1 part benzocaine	**85.06%**	**40.83%**
1 part cocaine base : 1 part levamisole	**66.54%**	**65.81%**
10 parts methamphetamine HCl : 1 part dimethylsulfone	**98.15%**	**14.13%**

Table 3.2 Mixture Study Summary (Subtracted)

Sample	Match Fit Percent	
	Drug	Adulterant/Diluent
1 part cocaine HCl : 1 part inositol	**88.13%**	**No Match**
1 part cocaine HCl : 1 part procaine	**86.05%**	**44.77%**
1 part cocaine HCl : 1 part benzocaine	**74.72%**	**58.33%**
1 part cocaine HCl : 1 part levamisole	**91.68%**	**40.90%**
1 part cocaine base : 1 part inositol	**96.36%**	**11.21%**
1 part cocaine base : 1 part procaine	**92.35%**	**27.82%**
1 part cocaine base : 1 part benzocaine	**95.60%**	**26.68%**
1 part cocaine base : 1 part levamisole	**96.09%**	**No Match**
10 parts methamphetamine HCl : 1 part dimethylsulfone	**99.62%**	**12.19%**

In instances where an analyst is unsure about the resulting match, a secondary confirmation technique such as GC-MS should be utilized.

Validation Plan Results Assessment

The ability of the FTIR to distinguish between the salt and base forms of a drug was successful as demonstrated in the repeatability study through the analysis of the cocaine base and cocaine HCl samples. However, when sample preparation is required through the use of KBr, variability is expected which may affect repeatability and is a limitation of this study. In the analysis of mixtures, careful consideration needs to be made when interpreting results as cutting agents can mask the presence of a

controlled substance resulting in extraneous peaks in a spectrum. Through the use of subtraction, extraneous peaks can be removed, which may allow the controlled substance to have a better match over the presence of cutting agents.

Conclusions

The purpose of this study was to validate the FTIR instrumentation in order to incorporate it as an analysis technique in the examination of seized drugs workflow for the analysis of commonly submitted controlled substances. Throughout this validation the operation and performance of the FTIR instrumentation was evaluated by performing a series of validation studies. After the analysis portion of the validation was complete, the results were evaluated. It was determined that the instrument method and performance is valid, reliable, and selective. This method is fit for the qualitative identification of unknown substances including the substances analyzed in this validation study. Prior to the identification and reporting of a substance, an acceptable infrared spectral comparison with a library match shall be documented.

In conclusion, the analysis and results in this validation study demonstrate that the FTIR instrumentation and the BCCL method are acceptable for use in the analysis of unknown substances in the Seized Drugs section.