Technical Procedure for Infrared Spectroscopy

1.0 Purpose - This procedure specifies the required elements for the performance verifications, quality control checks, and use of Fourier Transform Infrared Spectrophotometers (FTIR) with Universal Attenuated Total Reflectance (ATR) Sampling Accessories.

2.0 Scope - This procedure applies to all infrared spectrophotometers used in the Drug Chemistry Sections of the State Crime Laboratory.

3.0 Definitions

- **Performance verification -** The initial confirmation of the reliability of a previously or externally validated method or instrument.
- **Quality control (QC) check -** Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
- **Reference Material** – Material sufficiently homogeneous and stable, with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

4.0 Equipment, Materials, and Reagents

4.1 Equipment

- Fourier Transform Infrared Spectrophotometer (FTIR) with Universal Attenuated Total Reflectance (ATR) Sampling Accessory

4.2 Materials and Reagents

- Standard or Traceable Reference Material (SRM or TRM) Polystyrene film
- SRM or TRM Polystyrene film standard spectra (see Forensic Advantage (FA) Resource Manager)
- Print2PDF capability with instrument network
- Spatula
- Methanol or other suitable solvent

5.0 Procedure

5.1 Standards and Controls

5.1.1 Naming of Instrument Files

5.1.1.1 Instrument files used for documenting performance verifications and monthly/yearly QC checks shall be named with at least the instrument identifier, year/month/day and a description (abbreviations are acceptable).

5.1.1.2 Instrument files published to a casefile for blanks, sample scans, and spectral subtractions shall be named with at least the case number, item number and a description (abbreviations are acceptable).

5.1.1.3 If multiple spectral subtractions are performed on the same sample and published to the case record, each resulting scan shall be named as above, with a unique identifier for each subtraction.

5.1.2 Saving of Instrument Files

5.1.2.1 Instrument files described above (See **5.1.1**) shall be saved on the instrument computer hard drive, or the specific instrument folder on the instrument network.

5.1.2.2 Instrument files described above (See **5.1.1**) shall be placed in a compressed (.zip) file named with the instrument identifier, year, and month in which they were collected.

5.1.2.3 The compressed (.zip) files containing at least the instrument files described above (See **5.1.1**) shall be archived annually in the FA Resource Manager object repository ("Manage Files") associated with the FTIR instrument on which they were collected.

5.1.3 Operating parameters – The following operating parameters shall be used in the Drug Chemistry Section:

- Accumulations – four scans
- Resolution 4.00 cm^{-1}
- Range 4000.00 to 650.00 cm^{-1}
- CO_2/H_2O Correction "On"
- Diamond/Zinc Selenide crystal – one bounce

5.1.4 Negative control

5.1.4.1 Perform a background spectrum upon instrument start up, and at the beginning of each day the instrument is in use. Additional background spectrums may be obtained as needed.

5.1.4.2 Perform a blank (clean sample path). This shall be done in the same manner as the sample to be analyzed, however caution shall be taken to not overtighten the ATR pressure arm.

5.1.4.3 An acceptable blank spectrum does not exhibit extraneous peaks indicative of contamination. The blank spectrum shall be evaluated to ensure the instrument is free of any controlled substance(s), any substance(s) being identified in the sample, or any substance(s) that may interfere with the identification of the sample component(s).

5.1.4.4 If the blank is contaminated, clean the crystal again and repeat the blank until no contamination is present.

5.1.4.5 An acceptable blank (clean sample path) shall be obtained between each new sample scan obtained.

5.1.4.6 If the negative control is unacceptable, follow the steps outlined under **5.1.5.2.3.2** below.

5.1.5 Positive control - Monthly QC Check

5.1.5.1 A Forensic Scientist, or designee, shall obtain a polystyrene scan monthly for each instrument to ensure proper functioning, and record the completion of the Monthly QC Check.

5.1.5.2 The internal polystyrene or the external traceable polystyrene standard reference material (SRM or TRM) may be used for the Monthly QC Check.

5.1.5.2.1 Name the instrument file according to section **5.1.1**, and label the resulting peaks utilizing instrument software.

5.1.5.2.2 Label the scan with notations for internal or external polystyrene reference material (include serial number if the external polystyrene was used), initials, and date.

5.1.5.2.3 Evaluate the designated wave numbers from the Monthly QC check data and compare to the traceable reference material polystyrene film. Refer to the chart below for parameters.

Traceable Polystyrene Reference Material Parameters*

3082.XX cm^{-1} (+/- 1 cm^{-1})
3060.XX cm^{-1} (+/- 1 cm^{-1})
1601.XX cm^{-1} (+/- 1 cm^{-1})
1583.XX cm^{-1} (+/- 1 cm^{-1})
1028.XX cm^{-1} (+/- 1 cm^{-1})

***Refer to current certificate for Polystyrene Standard Reference Material for full peak wave numbers.**

5.1.5.2.3.1 The allowable variance from the certified values shall be within the interval parameters (+/- 1 cm^{-1}), which are determined by the manufacturer based on the set resolution.

5.1.5.2.3.2 If the results of the QC check are unacceptable, steps such as rebooting the software or changing the desiccants may be taken by the Forensic Scientist, or designee, to remediate the failed QC. If those steps are unsuccessful, the instrument shall be removed from casework immediately and the following shall be done:

- Place an "Out of service" sign on the front of the instrument and enter the Out of Service status in the logbook.
- Notify the Section IR Coordinator, or designee, so he/she can call the service engineer to schedule

an on-site assessment and document by entering an "Out of Service" Action History.
- Prior to placing an instrument back in service, the IR Coordinator or designee shall follow the procedure as outlined in section **5.2**.

5.1.5.2.4 Print the labeled scan as a .pdf file and save on the instrument hard drive (or the specific instrument folder on the instrument network).

5.1.5.2.5 Copy the .pdf file to the FA Resource Manager object repository ("Manage Files") associated with the FTIR instrument on which it was collected.

5.1.5.2.6 After evaluation of data, record completion in the instrument log and/or the FA Resource Manager for QC checks.

5.1.5.2.7 Reset the FA Resource Manager expiration date for one month to signal the next Monthly QC check.

5.1.6 Annual Internal Polystyrene QC Check

5.1.6.1 A scan of a traceable reference material polystyrene film shall be collected yearly for each instrument with the KBr accessory in place, followed by the collection of a scan of the internal polystyrene with the ATR attachment in place. This shall be performed by the IR Coordinator or designee. Both spectra must meet the criteria as established in the Positive Control - Monthly QC Check (See **5.1.5)**.

5.1.6.2 The instrument file and .pdf scans generated for the yearly QC Check shall be handled according to the specifications listed in **5.1.5** for the monthly QC check, except for resetting the instrument expiration date, which has been designated for use as a monthly reminder.

5.1.7 Initial Performance Verification for New Instrument Set Up

5.1.7.1 New FTIR instruments shall be installed by a certified engineer according to the manufacturer's guidelines, and procedure specifications (See **5.1.3**).

5.1.7.2 External and internal polystyrene scans shall be obtained according to **5.1.4** & **5.1.5**.

5.1.7.3 Scans from at least three controlled substance primary standards shall be obtained (e.g., methamphetamine, phentermine, and cocaine base). Other controlled substances may be used depending on the availability of standards. The data obtained shall be reviewed by the IR Coordinator, or designee, and shall be compared to the IR spectrum of the known reference material.

5.1.7.4 The .pdf files generated during the performance verification shall be filed in the FA Resource Manager object repository ("Manage Files") associated with

the instrument by the IR Coordinator or designee to document set up of the new instrument.

5.1.7.5 If the polystyrene checks are acceptable, and the controlled substance standard spectra match the respective library entries (as outlined by the criteria in **5.4.2.2**), the instrument shall be released for casework. A new entry for the instrument shall be made in the FA Resource Manager prior to use in casework. The new entry shall include:

- The manufacturer's serial number.
- The unique section identifier for the new instrument. Infrared instruments are numbered in numerical order with the notation "FTIR" in front of the number.
- An "Initial Validation" Action History, stating that the instrument has been released for casework.
- A notation under "Verification Date" to reflect the date the initial performance validation was completed.
- The FA Resource Manager expiration date shall be set for one month to signal the Monthly QC Check.

5.2 Maintenance Schedule

5.2.1 Yearly preventive maintenance shall be performed by an approved outside vendor.

5.2.2 Desiccants shall be changed at approximately six month intervals, or sooner when needed if external indicators (where existing) begin to change color.

5.2.3 Record completion of maintenance and repairs, the date and identity of the person performing the work in the instrument log for Maintenance/Repairs. The instrument log shall be kept in a notebook near the instrument.

5.2.4 Document the maintenance or repair by entering a "Maintenance" Action History, listing the name of the vendor/person performing the work, and the work performed on the instrument. Any documentation associated with a service call may be placed in the associated FA resource.

5.2.5 Prior to returning a piece of equipment to use (out of service for any reason – e.g. maintenance, malfunction, leaving the direct control of the laboratory) correct operation shall be demonstrated by a successful negative and positive control (as outlined in sections **5.1.4** & **5.1.5** above). Document the results by entering a "Quality Control Check" Action History in FA upon returning the instrument to use with casework.

5.3 Shutdown/Startup

5.3.1 The power switch to the infrared instrument shall be left ON at all times to ensure the optics stay warm and excess moisture does not build up in the instrument.

5.3.2 The software and computer may be shut down at the end of each business day.

5.3.3 Each time the software is restarted or following instrument shutdown, a background and an acceptable blank (clean sample path) shall be performed.

5.4 Application of Procedure on Evidence

5.4.1 Analysis of samples using the ATR Method

5.4.1.1 Clean the ATR sampling accessory crystal using a suitable solvent. Ensure the crystal is completely dry.

5.4.1.2 Perform a background scan at least daily and additional backgrounds as needed (e.g., when atmospheric conditions warrant).

5.4.1.3 Perform the negative control check as described above in **5.1.4.**

5.4.1.4 Print the results of the blank (clean sample path) for the FA case record. The blank scan may be ATR and then baseline corrected before it is printed for the FA case record.

5.4.1.5 Place approximately 1 milligram of solid sample evenly onto the ATR crystal. For liquid samples, apply enough liquid sample to cover the ATR crystal.

5.4.1.6 Apply force using the ATR force arm to ensure contact between the sample and the surface of the crystal.

5.4.1.7 Scan to acquire data.

5.4.1.8 Data from samples shall be ATR and then baseline corrected.

5.4.1.9 Data can now be processed.

5.4.2 Data Evaluation and Comparison

5.4.2.1 The spectral peaks obtained from an unknown spectrum shall be evaluated prior to comparison to ensure they are of sufficient intensity for comparison to reference material or published spectral data.

5.4.2.1.1 Any peaks generated that are below 100% transmission will be considered sufficient intensity and will be valid for further comparison.

5.4.2.2 Compare the unknown spectrum to a known reference material.

5.4.2.2.1 Six prominent and well-defined peaks in the sample spectrum between 2000 cm^{-1} to 650 cm^{-1} shall be labeled. The same six peaks shall be present within +/- 1 cm^{-1} of those in the reference spectrum during comparison for identification.

- If there are less than six prominent and well-defined peaks in the sample spectrum between 2000 cm^{-1} to 650 cm^{-1} then all peaks

shall be present within +/- 1 cm^{-1} of those in the reference spectrum during comparison.

5.4.2.2.2 The overall spectral pattern shall correspond to that of the reference material in regards to the absence or presence of major peaks and relative peak intensities.

5.4.2.2.3 No prominent unexplainable extraneous peaks shall be observed in the sample spectrum.

5.4.2.3 Print the properly labeled data generated by the FTIR instrument to a .pdf file. There shall be no more than two printed spectra per page.

5.4.2.4 If the Forensic Scientist, based on his/her training and experience, determines that a mixture is present, the controlled substance may be isolated from the mixture via chemical extraction or may be subtracted from the IR spectrum by using the "difference" function of the FTIR. Multiple spectral subtractions may be required for the isolation of a substance. A printout of the straight material before any extractions or spectral subtractions are performed shall be required for the FA case record. In addition, a printout after each spectral subtraction shall be included in the FA case record.

5.4.2.4.1 If a spectral subtraction or extraction is performed, **5.4.2.1** through **5.4.2.4** shall be followed for the resulting spectrum.

5.4.2.5 A positive identification can be made for a substance if the criteria in **5.4.2.2** is met.

5.4.2.6 The reference material(s) used for identification, and any reference material(s) used in a spectral subtraction, shall be imported into the FA case record if a positive identification is made.

5.4.2.6.1 When available, only reference materials from the in-house generated reference collection may be used in the course of casework to identify a substance. The lot number or the unique identifier for the reference material shall be included in the case file. If the reference material is unavailable from the in-house generated reference collection, a reference standard from a published source may be used. A citation for the source shall be included in the case file.

5.4.2.7 When using FTIR to differentiate cocaine base from cocaine hydrochloride or another salt form, the areas of the spectrum which are different between cocaine base and cocaine hydrochloride shall be clear. Other areas may have interfering peaks present that do not mask the "salt form" identity.

5.5 Sampling - See Drug Chemistry Section Administrative Procedure for Sampling.

5.6 Calculations - N/A

5.7 Uncertainty of Measurement - N/A

6.0 Limitations

6.1 Generally, infrared spectra cannot distinguish between optical isomers.

6.2 Compounds may exist in different crystal forms which may produce unique spectra. (Mannitol and some substituted cathinones are examples of compounds that exhibit these polymorphic characteristics.)

6.3 Due caution shall be exercised when using the similarity index generated by the library search algorithm. The Forensic Scientist shall evaluate the data and not singularly rely on the computer software index.

7.0 **Safety -** Monitor the force gauge to ensure that the ATR pressure arm is not over tightened.
Refer to Appendix 1 for Chemical Hygiene and Safety Precautions

8.0 References

Moffat, A.C., et al., ed. *Clarke's Isolation and Identification of Drugs*. 2nd Edition. London: Pharmaceutical Press, 1986.

Mills, III, Terry and J. Conrad Roberson. *Instrumental Data for Drug Analysis*. 2nd Edition. CRC Press,Inc.: Volumes 1-5, 1993.

Mills, III, Terry, et al. *Instrumental Data for Drug Analysis*. 3rd Edition. CRC Press, Inc.: Volumes 6-7, 1996.

Silverstein, Robert M., et al. *Spectrometric Identification of Organic Compounds*. 5th Edition. New York Wiley, 1991.

Keller, Roger. *The Sigma Library of FT-IR Spectra*. 1st Edition. Missouri: Sigma Chemical Company, Volumes 1 and 2, 1986.

Pouchert, Charles J. *The Aldrich Library of Infrared Spectra*. Aldrich Chemical Company: 1981.

ASTM Standard E-1252, 2002, "Standard Practice for General Techniques for Obtaining Infrared Spectra for Qualitative Analysis." ASTM International: West Conshohocken, PA, 2002, www.astm.org.

ASTM Standard E2224-18, 2018, "Standard Guide for Forensic Analysis of Fibers by Infrared Spectroscopy." ASTM International: West Conshohocken, PA, 2018, www.astm.org.

Moffat, A.C., et al., ed. *Clarke's Isolation and Identification of Drugs*. 4th Edition. London: Pharmaceutical Press, 2011.

9.0 Records

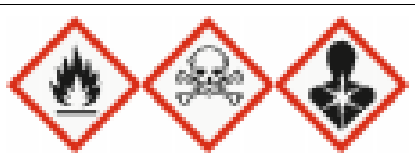
- FA Resource Manager for Initial Performance Verification
- FA Resource Manager for Traceable Polystyrene Film Infrared Spectrum
- FA Resource Manager/Instrument log for maintenance and QC Checks
- FA Resource Manager for instrument files
- FA Case Record for .pdf data imported to case records

- Laboratory Safety Manual- Chemical Hygiene Plan and Hazardous Communication Program

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/21/2020	10	Added "FTIR" abbreviation and FA "Resource Manager" throughout document for clarity. **4.2 –** Removed organic requirement and water. **5.1.1 and 5.1.2 –** Removed reference to Nicolet instruments. **5.1.2.3 –** Added "annually". **5.1.3 –** Changed range to end at 650 $cm^{-1.}$ **5.1.4.2 –** Reworded **5.1.4.3** – Added criteria to evaluate negative control **5.1.4.6** – Added procedure for unacceptable negative control **5.1.5.1** – Added "or designee" **5.1.5.2, 5.1.6, & 5.1.7.2** – Removed reference to non-Perkin Elmer instruments. **5.1.5.2.3.2** – Added corrective action steps for failed QC **5.1.5.2.7** – Relocated FA resource manager expiration update requirement (previously 5.1.5.2.5) **5.1.5.3 & 5.1.7** – Removed reference to two instrument types **5.1.6** – Added references to 5.1.5 **5.1.7.3 and 5.1.7.5 –** Added designee; updated wording and added expiration date requirement **5.2 –** Removed "Suggested" from header. **5.2.5** – Relocated requirements for returning instrument to service (relocated from former 5.3) **5.4** – Consolidated procedures for solid and liquid samples **5.4.1.8 –** Added requirement for samples to be ATR and baseline corrected, and removed macro reference **5.4.2** – Added criteria for evaluation and comparison of data **5.4.2.4** – Relocated procedure for mixtures/subtractions (former 5.4.3) **5.4.2.6** – Added criteria for reference materials **6.2** – Added examples of limitations **7.0** - Reworded **8.0 –** Added *Clarke's Isolation and Identification of Drugs 4th Edition* to references and corrected typo in author name

Appendix 1: Chemical Hygiene and Safety Precautions

Methanol **DANGER: HIGH RISK SUBSTANCE** *	
	**HEALTH 2** **FLAMMABILITY 3** **REACTIVITY 0**
Detection of Release	Colorless liquid with a sweet, pungent odor.
Signs/Symptoms of Exposure	Headache, Nausea, Dizziness, Eye damage. May cause intoxication that includes central nervous system depression, headache, dizziness, nausea, lack of coordination, and confusion.
PEL	OSHA (TWA) 200 ppm
Associated Hazards	Flammable. Acute oral, dermal, and inhalation toxin. Toxic if swallowed, comes in contact with skin, or inhaled. Specific target organ toxicity of eyes.
Controls	Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Handle with gloves. Wear lab coat. Gloves: nitrile (break through time less than 1minute), butyl-rubber (break through time greater than 8 hours)
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Use explosion-proof equipment. Keep away from sources of ignition. Take measures to prevent the build-up of electrostatic charge. Dispose in Hazardous Chemical Waste. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Emergency Procedures	**Eye Contact:** Flush eyes with water as a precaution. **Inhalation Exposure:** If inhaled, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. **Ingestion:** After swallowing: fresh air. Make victim drink ethanol (e.g. 1 drinking glass of a 40% alcoholic beverage). Call a doctor immediately (mention methanol ingestion). Only in exceptional cases, if no medical care is available within one hour, induce vomiting (only in fully conscious persons) and make victim drink ethanol again (approx. 0.3 ml of a 40% alcoholic beverage/kg body weight/hour). **Skin Contact:** Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician. **Spills:** Avoid breathing vapors, mist, or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas. Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Small spills: Contain spillage, and then collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container for disposal. Large spills: Turn off sources of heat if possible; evacuate area and call 911 (Haz Mat).