Technical Procedure for Infrared Spectroscopy

1.0 Purpose - This procedure specifies the required elements for the performance check and use of the Perkin-Elmer Fourier Transform Infrared Spectrophotometers.

2.0 Scope - This procedure applies to all infrared spectrophotometers used in the Drug Chemistry Section of the Raleigh location 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 with Universal Attenuated Total Reflectance (ATR) Sampling Accessory

4.2 Materials and Reagents

- Traceable Reference Material (TRM) polystyrene film
- TRM Polystyrene film standard spectra (see Certified Polystyrene in Section Files)
- Printer/ink cartridge/paper and/or Print2PDF capability with instrument network
- Spatula
- Methanol or other suitable organic solvent
- Water

5.0 Procedure

5.1 Standards and Controls

5.1.1 Negative control

- Perform a contamination check in the same manner as the sample to be analyzed.
- If the check fails, clean the crystal again and repeat the contamination check until no contamination is present.
- A clean contamination check shall be obtained between each sample scan obtained.

5.1.2 Monthly QC Check

5.1.2.1 A Forensic Scientist shall obtain a polystyrene scan monthly for each instrument to ensure proper functioning.

5.1.2.2 The internal polystyrene or external TRM polystyrene may be used for the Monthly QC Check. (See below for instructions on both.)

5.1.2.3 Software Instructions for Obtaining Polystyrene Scans for *Perkin Elmer* FT-IR/ATR Models: *Spectrum One* and *Spectrum 100*

5.1.2.3.1 Start Software - double click on the "*Spectrum*" software icon.

- Login- click "OK" through prompts (no passwords are set).

5.1.2.3.2 Scan Background:

- Choose "Scan and Instrument Setup" hotkey.
- Choose "Scan" tab – check for Scan duration set to 4.
- Choose "Instrument" tab – check for Resolution set to 4.00 cm^{-1}
- Choose "Background" hotkey.
- Choose "Scan."

5.1.2.3.3 Perform a successful clean contamination check.

5.1.2.3.4 Obtain polystyrene scan using the instrument's internal polystyrene:

- Choose "Scan and Instrument Setup" hotkey.
- Choose "Beam" tab.
- Double click on the picture of the disk with holes in it.
- Choose "Polystyrene."
- Choose "OK."
- Choose "Apply."
- Choose the macro button used for casework samples to collect data.

5.1.2.3.5 Obtain polystyrene scan using an external TRM polystyrene:

- Remove the ATR accessory and replace with the KBr attachment.
- Ensure the instrument beam is unobstructed.
- Obtain a background scan.
- Place the Traceable Reference Material Polystyrene in the instrument beam.
- Choose the macro button used for casework samples.

5.1.2.3.6 Choose "Label Peaks" hot key to display peak data.

- Choose the textbox hotkey ("ABC" icon)

5.1.2.3.7 Label the scan with notations for internal or external (TRM) polystyrene (include serial number if TRM polystyrene was used) and analyst's initials.

- Choose "OK" to place the textbox on the screen.
- Move textbox to a position so no data is obliterated.
- Ensure time/date stamp is turned on for print options.

5.1.2.3.8 Print the data using a hard copy, or Print2PDF software. If Print2PDF option is chosen, store files with "QC" and the date as the file name in the following location:

- Save the file on the instrument hard drive in the folder designated for the instrument.
- File names shall be formatted as follows:
  FTIR identifier Year Month Date initials of Forensic Scientist
  Example: FTIR1 - 20120901XXX
- Save a backup copy on the Instrument Network (See: "processed" and "error" folders).
- Click on the "back one level" hotkey.
- Open the "Drug Chemistry" folder.
- Save the .pdf file in the instrument folder that corresponds to the FT-IR used in data collection.
- Initial the log in the instrument notebook if Print2PDF was used, or place the hardcopy in the instrument notebook.

5.1.2.3.9 Reset the beam and instrument for casework samples:

- Choose "Scan and Instrument Setup" hotkey.
- Choose "Beam" tab.
- Double click on the picture of the disk with holes in it.
- Choose "None."
- Choose "OK" (this removes the internal polystyrene from the beam).
- Choose "Apply."
- Choose "Contamination check" hotkey.
- Choose "OK" (this runs a contamination check in preparation for samples).
- A background may be printed at this point and saved with the polystyrene scan to ensure the polystyrene has been removed from the instrument beam.

5.1.2.4 Evaluate the following three wavenumbers from the Monthly QC check data:

3060 cm^{-1} (+/- 0.5 cm^{-1})
1601 cm^{-1} (+/- 0.5 cm^{-1})
1028 cm^{-1} (+/- 0.5 cm^{-1})

Refer to the Calibrated TRM values on the current Certificate of Calibration for MIR Polystyrene Traceable Reference Material for specific wavenumbers.

5.1.2.5 The allowable variance from the certified value for the peaks appearing at the listed wave numbers shall be +/- 0.5 cm^{-1}. If the results are outside these specifications, 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.
- Notify the Section IR Key Operator so he/she can call the Service Engineer to schedule an on-site assessment.

5.1.2.6 The monthly polystyrene scan shall be filed by the Forensic Scientist who collected the data. Records shall be maintained by the IR Key Operator.

- Record completion in the instrument log for QC checks. (See Monthly QC Check Log in Logbook and see Section files for printable version.)

5.1.3 Yearly Internal Polystyrene QC Check

5.1.3.1 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 Section IR Key Operator or designee.

5.1.3.2 Print both scans according to the specifications for the monthly QC check.

5.1.3.3 The Section IR Key Operator is responsible for evaluating the data according to the specifications for the monthly QC checks. The allowable variance from the certified value for the peaks appearing at the listed wave numbers shall be +/- 0.5 cm^{-1}. If the results are outside these specifications, 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.
- Notify the Section IR Key Operator so he/she can call the service engineer to schedule an on-site assessment.

5.1.3.4 This data shall be filed and maintained by the Section IR Key Operator.

- Record completion in the instrument log for QC checks. (See Instrument Log for Monthly/Yearly QC Checks in logbook, and see Section Files for printable version.)

5.1.4 Performance Verification for New Instrument Set Up

5.1.4.1 New FT-IR instruments shall be installed by a certified engineer according to the manufacturer's guidelines.

5.1.4.2 External and internal polystyrene scans shall be obtained according to the procedure for Yearly Internal Polystyrene Verification listed above.

5.1.4.3 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 Section IR Key Operator and found to be substantially the same as the library standard for that compound.

5.1.4.4 The data shall be filed and maintained by the Section IR Key Operator to document set up of the new instrument.

5.1.4.5 If the polystyrene checks are acceptable, and the controlled substance standard spectra match the respective library entries, the instrument shall be released for casework. A new entry for the instrument shall be made in the Resource Manager section of FA prior to use in casework. The new entry shall include:

- The manufacturer's serial number.
- The unique section identifier for the new instrument. Infrareds are numbered in numerical order with the notation "FT-IR" in front of the number.
- A notation under "Verification Date" to reflect the date the performance verification was completed.

5.2 Suggested Maintenance Schedule

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

5.2.2 Dessicant packs shall be changed at approximately six month intervals, or sooner when needed if external indicators begin to change color.

5.2.3 Instructions for Dessicant Changes - *Perkin-Elmer* FT-IR/ATR Models *Spectrum One* and *Spectrum 100*

5.2.3.1 Remove the ATR accessory from the sample compartment. (Pull lever underneath to release before removing.)

5.2.3.2 Loosen the two captive screws securing the dessicant cover.

5.2.3.3 Open the cover and remove all the dessicant packs, noting how they were installed.

5.2.3.4 Place the first pack of dessicant in the recess at the front of the dessicant holder.

5.2.3.5 Place the remaining packs one at a time into the holder. Ensure that when the packs have been installed they do not rise above the level of the black rubber purge seal.

5.2.3.5.1 NOTE: The number of dessicant packs installed depends on the model number of the instrument. The dessicant pack area should be as full as possible.

5.2.3.6 Close the cover and tighten the screws.

5.2.3.7 Carefully refit the ATR accessory into the sample compartment area.

5.2.3.8 Change the dessicant alert clock in the software:

- Choose "Scan and Instrument Setup" (hotkey).
- Choose "Adjustment Toolbox" ("Hammer/wrench" icon).

- Choose "Maintenance" ("Oil can" icon).
- Choose "Changed" checkbox and the date will reset to the current day.
- 180 days is the typical interval for the counter.

5.3 Record completion of maintenance and repairs, the date and identity of person performing the work in the instrument log for Maintenance/Repairs. (See FTIR Maintenance Log in Section files for printable version.) The instrument log shall be kept in a notebook near the instrument. If converted to electronic format, the maintenance/repair records shall be maintained by the IR Key Operator and stored in section files.

5.4 Shutdown/Startup

- 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.
- The software and computer may be shut down at the end of each business day.
- Each time the software is restarted, a background and clean contamination check shall be performed.
- When an IR has been placed out of service (e.g., maintenance, malfunction, leaving the direct control of the Laboratory), correct operation shall be demonstrated by a performance verification following the procedure for a Monthly QC Check outlined above.
- Laboratory personnel shall examine the effect(s), if any, of a malfunction on analysis results and implement the Procedure for Corrective Action as required.

5.5 Application of Procedure on Evidence

5.5.1 Solid samples using the ATR Method

5.5.1.1 Clean the ATR sampling accessory crystal using water or an organic solvent. Ensure the crystal is completely dry.

5.5.1.2 Perform a background scan at least daily, and additional backgrounds as needed. (For example, when instrument is restarted after power down or when atmospheric conditions warrant.)

5.5.1.3 Perform the negative control check as described above in **5.1**.

5.5.1.4 Place approximately 1 milligram of sample evenly onto the ATR crystal.

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

5.5.1.6 Scan to acquire data.

5.5.1.7 Data can now be processed. The following operating parameters shall be used in the Drug Chemistry Section:

- Resolution 4 cm^{-1}
- Range 4000.00 to 550.00 cm^{-1}
- CO_2/H_2O Correction "On"
- Diamond/Zinc Selenide crystal – one bounce

5.5.1.7.1 "ATRNoPrint" Macro:

- Delete
- Scan sample (4 times)
- ATR Correction, 0.00
- Auto baseline correction, 4000.00-550.00

5.5.1.8 Spectral subtractions may be used as a means to remove diluents from the IR of controlled substances. If this technique is used, a printout of the straight material before any spectral subtractions are performed shall be required for the FA case record.

5.5.1.9 Print the data generated by the FT-IR instrument for the FA case record. Label the generated data with the case number, item number, date, and instrument identifier, for inclusion in the FA case record. If a hard copy is obtained, it shall be labeled with initials and date before being scanned into the FA case record.

5.5.1.9.1 If more than one scan is obtained from the same sample, labels shall be affixed noting the identity of the scan, such as an extraction, solvent wash, or spectral subtraction. .

5.5.1.10 Compare the completed scan to a known reference standard.

5.5.1.11 The reference standard shall also be included in the FA case file if a positive identification of a controlled substance is made.

5.5.2 Liquid samples using the ATR Method

5.5.2.1 Clean the ATR sampling accessory crystal using water or an organic solvent. Ensure that the crystal is completely dry.

5.5.2.2 Perform the negative control check as described above in **5.1.**

5.5.2.3 Apply enough liquid sample to cover the ATR crystal.

5.5.2.4 Scan to acquire data.

5.5.2.5 Data can now be processed. The following operating parameters shall be used in the Drug Chemistry Section:

- Resolution 4 cm^{-1}
- Range 4000.00 to 550.00 cm^{-1}
- CO_2/H_2O Correction "On"
- Diamond/Zinc Selenide crystal – one bounce

5.5.2.5.1 "ATRNoPrint" Macro:

- Delete
- Scan sample (4 times)
- ATR Correction, 0.00
- Auto baseline correction, 4000.00-550.00

5.5.2.6 Print the data generated, labeled with at least the case number, item number, date, and instrument identifier, for inclusion in the FA case record.

5.5.2.6.1 If more than one scan is obtained from the same sample, labels shall be affixed noting the identity of the scan, such as an extraction or solvent wash.

5.5.2.7 Compare the completed scan to a known reference standard.

5.5.2.8 The reference standard shall also be included in the FA case file if a positive identification of a controlled substance is made.

5.5.2.9 **Identification:** If the Forensic Scientist, based on his/her training and experience, determines that the spectrum of the controlled substance is substantially altered due to the presence of other substances in the mixture, the controlled substance shall be separated from the mixture and an IR spectrum obtained of the isolated controlled substance.

5.5.2.9.1 A known impurity within a mixture containing a controlled substance can also be subtracted from the IR spectrum by using the Adifference@ function of the FT-IR.

5.5.2.9.2 An IR spectrum of a controlled substance shall be substantially comparable (i.e., equivalent) to the IR spectrum of a known reference standard before an identification is confirmed.

5.5.2.10 When using FT-IR as the primary structural elucidation technique, the sample spectrum shall compare favorably with a spectrum of a known standard in both its overall appearance and in the presence and location of the major peaks.

5.5.2.11 When using FT-IR 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.

5.6 **Sampling -** See Drug Chemistry Section Technical Procedure for Sampling.

5.7 **Calculations** - N/A

5.8 **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 is an example of one compound that exhibits 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 - Do not over tighten the force gauge.

8.0 References

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

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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.

9.0 Records

- Monthly/yearly polystyrene checks
- Traceable Polystyrene Film Infrared Spectrum
- Instrument log for monthly and yearly polystyrene checks.
- Data generated from case work – included in the FA case record.
- Maintenance Log

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document Technical Procedure F-01 converted to ISO standards. Instrument log added for monthly/yearly QC checks. Print2PDF new option for storage of polystyrene data. Instructions added for collection of polystyrene data and for dessicant changes. KBr Methods rescinded. They may be reinstated by Drug Chemistry Section FSM.