Unknown Substance Analysis and Comparison

1. Scope

The purpose of this procedure is to analyze for an unknown substance that is to be identified and/or compared to a standard known substance that does not fit into a specific sub-discipline of Trace Evidence.

2. Examination Procedures

2.1. Evidence Types

Any item, material, or substance that might contain a substance which could be identified and/or compared to a known substance. These known substances are typically commercially available products such as cosmetics, personal care products, household cleansers, detergents, automotive care products, and other commercial products and chemicals. Known substances may be provided by the submitting agency, available in a laboratory reference collection, or obtained by laboratory personnel.

2.2. Reagents and Chemicals

Strontium Nitrate
Permount
Xylenes
Plastic embedding media
ACS grade or better solvents
ACS grade or better chemicals

2.3. Instruments and Equipment

Standard laboratory supplies
Zoom Stereomicroscope
Video Microscope (Keyence Scope)
Polarized light microscope
Polarized light comparison microscope
Microtome
Fourier Transform Infrared Microspectrometer (FTIR)
Fourier Transform Infrared Spectrometer with ATR
Scanning Electron Microscope/Energy Dispersion Spectrometer
Gas Chromatograph/Mass Spectrometer (GC/MS)
Sample press apparatus
NaCl, KBr or Csl salt plate

Diamond cell press
FTIR sample holders
Gold and silver coated slides
Vacuum carbon coater and accessories
Aluminum stubs
Carbon conductive tape and/or tabs
Conductive carbon liquid
Tungsten needles
Mounting media
Embedding media
Embedding molds
Photographic equipment with accessories

2.4. Procedural and Chemical Precautions

Refer to the TBI Safety Manual for general safety requirements and hazard information regarding the use of reagents and solvents, the handling of gas cylinders and overall safety guidelines.

Protective attire, including laboratory coat, mask, gloves and eye protection should be used when working with clothing and/or bloodstained items.

Decontamination of a scientist's work area should be performed after each use, but shall be done after analyzing bloodstained items.

Hazardous chemicals shall be used in a chemical fume hood.

When handling concentrated acids, eye protection and laboratory coats shall be worn.

When diluting acids, always add acid to water.

When necessary, consult section and laboratory Material Safety Data Sheets (MSDS) regarding any chemical used in the Microanalysis section.

Label all generated solutions and reagents with appropriate warning stickers.

When carbon coating samples, the carbon rods should only be observed using goggles rated for welders and cutters. The goggles conform to ANSIZ87.1.

When filling a dewer flask with liquid nitrogen to be used in the Fourier Transform Infrared Microspectrometer (FTIR) protective clothing shall be worn. This includes cryogenic gloves, full-face shield and laboratory coat.

2.5. Limitations

Determination and comparison is limited to the available instrumentation, information provided, and the submission/availability of a known standard.

2.6. Procedure

Document submitted samples according to *Microanalysis Quality Assurance Policy*.

Evidence may be photographed for case file documentation.

Due to the varied nature of these products and their chemical formulation, every procedure listed here will not be amenable to every sample. Therefore, it will be necessary for the analyst to choose the most appropriate techniques to provide the most discriminating information. As many different tests should be performed as possible, if the tests will give different information about the samples. All testing that does not destroy or consume the evidence shall be performed first if applicable.

2.6.1. Visual Examination

Each piece of evidence shall be thoroughly examined visually. Look for any similarities or differences between each piece of evidence.

Documentation of visual examination may include, but not limited to, the following:

- Size
- Shape
- Color
- Texture and appearance
- Physical structure
- Foreign matter
- Reaction to pH paper. See *Microanalysis Standard Operating Procedure Acid and Base Analysis.*

2.6.2. Microscopic Examination

Microscopic examination of unknown and known substances should be performed with a stereomicroscope or a video microscope (Keyence). The specimens shall be placed in a clean petri dish and labeled, or on a clean piece of butcher paper for examination. Separation shall be maintained between unknown and known samples to avoid cross-contamination.

Microscopic examination of each specimen should include documentation of the following, where applicable:

Color – attempt to describe the color of the sample. Color should be noted using reflected light.

Appearance and Texture – describe the physical appearance of the sample. Surface textures noted include features such as gloss, coarseness, and roughness.

Physical structure – characteristics as to the condition that the specimen exhibits when probed. These include pliability, brittleness, and resiliency.

Foreign matter – note if any foreign matter is embedded in the unknown substance.

Similarities or differences between unknown and known samples shall be noted, if applicable. If meaningful differences are noted in the visual and microscopic examination, report that the unknown and known are not the same.

Photomicrographs of samples may be obtained. Retain photomicrographs as case notes.

2.6.3. Transmitted and Polarized Light Microscopy Analysis

Obtain Kohler illumination on transmitted and polarized light microscopes prior to each use. Note in case file that this has been performed.

If microscopic analysis is probative, unknown and known samples may be dry mounted or mounted in an appropriate mounting media for the sample using glass slides and cover slips.

Observe samples using transmitted light with uncrossed and crossed polarized filters and document particle features observed including but not limited to: color, size, shape, extinction positions, birefringence colors, and refractive index.

Photomicrographs of samples may be obtained. Retain photomicrographs as case notes.

In some cases, it may be possible to identify certain particles in the sample by comparing the particles with micrographs published in "The Particle Atlas", a laboratory reference collection, or by microchemical testing. See *Microanalysis Standard Operating Procedure Polarized Microscopy Analysis.*

2.6.4. Instrumental Analysis

Instrumental analysis offers a method to characterize solids and liquids both organically and inorganically. The initial step of the instrumental analysis shall follow a set protocol using instrument parameters that are the same or similar to one used in another sub-discipline test method. The instrument parameters shall be noted in the case record.

2.6.4.1. Fourier Transform Infrared Spectrometer (FTIR) Microscope Analysis

Specimens of unknown and known substances shall each be prepared for analysis by one of the following techniques:

Press the sample with a sample press and attach the pressed sample to tape adhering to a FTIR sample holder, or lay the pressed sample on a salt plate.

Spread a thin layer of sample on a salt plate.

Press sample in a diamond cell press.

Spread sample on a gold or silver reflective surface (such as a coated slide or a metal press) for reflectance analysis.

Embed the unknown and known samples in an embedding media. After drying, microtome the sample in thin increments. Press, if necessary, in a diamond cell or sample press. Attach the samples to tape adhering to a FTIR sample holder or lay the pressed sample on a salt plate.

Acquire a minimum of 50 scans for each sample analyzed and a minimum of 50 background scans. Other instrument parameters are printed on the spectrum printout. Acquire a spectrum of a polystyrene standard for each day of case analysis and retain in the case file. Observe the following peak wavenumbers: 3082, 3060, 2849, 1943, 1601, 1028 and 906. The bands will not vary more than +/- 2 wavenumbers.

Acquire spectra of the unknown and known substances. If possible, acquire multiple spectra of each substance to assess within sample variation. Compare generated spectra for the unknown and known substances.

If there are meaningful differences between the unknown and known, report that the two substances are not the same.

If there are no meaningful differences between the unknown and known, report that the two substances are consistent or the unknown is identified as the known. Further analysis may be performed.

If appropriate differences or similarities cannot be determined, report as inconclusive. Further analysis may be performed.

If no known sample is available, the unknown spectra should be searched against the FTIR spectral libraries. Comparison samples shall be obtained of spectral matches and analyzed if this is the only instrument analysis suitable for identification.

Retain these spectra as part of the case file.

2.6.4.2. Fourier Transform Infrared Spectrometer with ATR (FTIR-ATR)

Specimens of unknown and known substances shall each be prepared for analysis.

Grind powders in a mortar and pedestal. Place a small amount of ground powder on the ATR crystal. Screw down the sample press to create good contact with the crystal.

Place a drop of liquid on crystal.

Place thin films on the crystal. Screw down the sample press to create good contact with the crystal.

Acquire a minimum of 50 scans for each sample analyzed and a minimum of 50 background scans. Other instrument parameters are printed on the spectrum printout. Analyze and acquire a spectrum of the performance standard, Strontium Nitrate, for each day of case analysis. Compare the spectrum to a known ATR spectrum and place a copy in the case file.

Clean crystal thoroughly with wipe and ethanol.

Analyze and acquire a spectrum of the unknown and known samples. If possible, acquire multiple spectra to assess within sample variation.

Follow procedure for FTIR Microscope for comparison of spectra.

If no known sample is available, the unknown spectra should be searched against the FTIR-ATR spectral libraries. Comparison samples shall be obtained of spectral matches and analyzed if this is the only instrument analysis suitable for identification.

Retain these spectra as part of the case file.

2.6.4.3. Scanning Electron Microscope/Energy Dispersion Spectrometer (SEM/EDS) Analysis

Place samples of unknown and known substances on carbon conductive tape or tabs that have been placed on an aluminum SEM stub.

The sample stubs are placed into the carbon coater chamber, placed under vacuum and coated with a thin film of carbon to increase electrical conductivity. This process is outlined in the carbon coater instructions and is also available in step-by step instructions next to the coater. It may be necessary to apply 2 coats of carbon to fully cover the samples. Some samples may also require the addition of conductive carbon liquid to provide adequate grounding.

Using specially designed forceps, place aluminum stubs with samples in the scanning electron microscope chamber and evacuate the chamber.

Perform an optimization according to the SEM-EDS QA/QC policy. These results are printed and stored in each case file. This printout also includes the instrument parameters. The SEM-EDS system will be operated at the following parameters:

Livetime (sec)	50-100
Accelerating Volt.	20 - 25 KeV
Process Time	2-5
Working distance	8.5mm (EVO10 and EVO50) or 15 mm (LEO 1450)

Using the secondary electron detector and/or the backscatter detector, or both, locate samples and analyze with the energy dispersion x-ray detector. If possible, acquire multiple spectra to assess within sample variation. Retain spectra in case file.

Compare the acquired spectra of the unknown substance to the known substance.

If there are meaningful differences between the unknown and known, report that the two substances are not the same or do not have the same elemental composition.

If there are no meaningful differences between the unknown and known, report that the two substances are consistent or the unknown has a similar elemental

composition as the known. Further analysis may be performed.

If appropriate differences or similarities cannot be determined, report as inconclusive. Further analysis may be performed.

If no known sample is available, elemental data may be reported or combined with other data to characterize the sample.

2.6.4.4. Inductively Coupled Plasma-Mass Spectrometer Analysis

Elemental analysis of the unknown and known substances may be performed using inductively coupled plasma-mass spectrometry. See the *Microanalysis Standard Operating Procedure Aqueous Solutions Analysis using ICP-MS*.

2.6.4.5. Gas Chromatograph-Mass Spectrometer (GC/MS) Analysis

Analyze blanks between samples.

Analyze the ASTM 1618 Column Resolution Test Mixture with each set of samples.

Extract, dissolve, or dilute unknown and known samples in an appropriate solvent.

Samples may be placed in autosampler vials and injected via the autosampler or the samples may be hand injected.

Analyze the samples using the GC/MS. Parameters used in the analysis shall be printed out and placed in case file. Common methods used for GC/MS analysis include, ARSON.M, HI-TEMP.M, FAME.M, and HEADSPACE.M. Other parameters may be used if necessary for the analysis of a particular compound or class of products. These parameters must be documented in the case file.

Obtain chromatograms and mass spectra.

Compare the chromatograms and mass spectra of the unknown to the chromatograms and mass spectra of the known.

If the retention times of the unknown and known compounds are consistent and the mass spectra show no meaningful differences, report that the unknown has been identified as the known.

If the retention times of the unknown and known compounds are inconsistent and/or there are meaningful differences between the mass spectra, report that the unknown is not identified as the known. Further analysis may be performed.

Sometimes pattern matching the peak groupings in a chromatogram may be helpful in classifying a complex mixture. See the *Microanalysis Standard Operating Procedure Ignitable Liquid Analysis* for proper procedures.

If no known sample is available, mass spectra may be searched against known mass spectral libraries. Comparison samples shall be obtained of spectral matches and analyzed if this is the only instrument/analysis suitable for identification.

3. Measurement Traceability

These examinations and comparisons are qualitative techniques and as such do not utilize measurements that will have a significant effect on the outcome of the analysis.

4. Reference Materials

Any of the reference materials used to ensure that the instrument that is used is working properly, which could include:

Strontium Nitrate
Polystyrene
Cobalt Standard

Any of the known libraries on the instruments that are used in the analysis.

5. Reports

The unknown and known samples should be fully described.

The identity of the chemical components of the sample or a characterization of the make-up of the sample may be described.

Comparison of unknown and known samples may be consistent or inconsistent with respect to the following:

- Color
- Texture
- Type
- Organic composition
- Inorganic composition
- Formulation