Appendix A: Properties for the Validation Process

Categories of Analytical Techniques:
The following listings of methods or instrumental techniques are from category A, B or C of the “Recommended Minimum Standards for Forensic Drug Identification” as provided in Table 1. For each instrument or method, specific properties are detailed that could have an effect on how the validation process is formulated and executed. Sections titled *technique strengths* and *technique limitations* have been added for additional guidance. The techniques selected for an analytical scheme must meet the SWGDRUG minimum recommendations for the forensic identification of seized drugs and must not be correlated.

Table 1: Categories of Analytical Techniques

<table>
<thead>
<tr>
<th>Category A</th>
<th>Category B</th>
<th>Category C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infrared Spectroscopy</td>
<td>Capillary Electrophoresis</td>
<td>Color Tests</td>
</tr>
<tr>
<td>Mass Spectrometry</td>
<td>Gas Chromatography</td>
<td>Fluorescence Spectroscopy</td>
</tr>
<tr>
<td>Nuclear Magnetic Resonance Spectroscopy</td>
<td>Ion Mobility Spectrometry</td>
<td>Immunoassay</td>
</tr>
<tr>
<td>Raman Spectroscopy</td>
<td>Liquid Chromatography</td>
<td>Melting Point</td>
</tr>
<tr>
<td></td>
<td>Microcrystalline tests</td>
<td>Ultraviolet Spectroscopy</td>
</tr>
<tr>
<td></td>
<td>Pharmaceutical Identifiers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thin Layer Chromatography</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cannabis only:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macroscopic Examination</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microscopic Examination</td>
<td></td>
</tr>
</tbody>
</table>

References:
1. EAL-P11 European Cooperation for Accreditation of Laboratories
2. ILAC Guidelines for Forensic Laboratories Feb 2001, 5.4.5.1
3. Eurachem, The Fitness for Purpose of Analytical Methods, 1998
4. Federal Register Vol 60 no. 40 pg 11259, March 1, 1995
A.1 Infrared Spectroscopy (IR)

A.1.1 Technique Strengths
• Samples can be recovered for additional tests.
• Generates the highest discriminating capability. IR may discriminate between diastereomers (pseudoephedrine/ephedrine) and free base/acid and salt forms.

A.1.2 Technique Limitations
• Pure samples may give different spectra due to polymorphism.
• Chemical composition should not change during the analysis. For example, care must be taken to address volatility, heat, and pressure effects.

A.1.3 Purpose/Scope
IR yields structural information that will provide sufficient selectivity that generates the highest discriminating capability.

A.1.4 Analytical Method

A.1.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment.

A.1.4.2 Instrumental parameters
• Identify the instrument and equipment utilized.
• List instrument conditions

A.1.5 Reference Materials
Utilize a polystyrene film and compare to a polystyrene standard spectrum. Repeat this process utilizing a commonly encountered drug standard suitable for this method.

A.1.6 Performance Characteristics

A.1.6.1 Selectivity
For determination of closely related compounds, standards of each should be tested on the system to show selectivity. IR may discriminate between diastereoisomers (pseudoephedrine/ephedrine) and free base/acid and salt forms. However, IR cannot distinguish enantiomers.

A.1.6.2 Matrix Effects
• Samples need to be dry to minimize water interferences.
• Address the possibility of ion exchange (alkali halides such as KCl and KBr) during sample preparation.
• Analytes commonly require purification sufficient for their intended purpose.

A.1.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.1.6.4 Accuracy
• Precision (Repeatability/Reproducibility): Demonstrate the reproducibility of the instrument
by running a reference standard a minimum of 10 times.

- Trueness must be determined for quantitative methods.

### A.1.6.5 Range
- Limit of Detection (LOD), a peak-to-noise ratio in either absorbance or transmittance should be determined, below which no identification will be made. Determine this through measuring the response of different amounts of analyte. For most instruments this is in the microgram range.
- Limit of quantitation (LOQ) should be determined.
- Linearity must be determined for all quantitative methods.

### A.1.6.6 Robustness
Determine the amount of change to instrumental parameters that will still allow for the identification. (e.g., wavenumber resolution, concentration, humidity, temperature).

### A.1.6.7 Ruggedness
Ruggedness may be determined for qualitative or quantitative methods. Alter the analysts, instrumentation and environment and assess the changes in accuracy.

### A.1.7 Uncertainty
The uncertainty of the method must be assessed for quantitative methods.

### A.1.8 Quality control

### A.1.9 References

a) EAL-P11  European Cooperation for Accreditation of Laboratories
b) ILAC Guidelines for Forensic Laboratories  Feb 2001, 5.4.5.1
c) Eurachem, The Fitness for Purpose of Analytical Methods, 1998

### A.2 Mass Spectrometry (MS)

#### A.2.1 Technique Strengths
- The technique may discriminate between diastereomers.
- Mass spectra can be interpreted to aid in characterizing an unknown drug through structural elucidation.
- Techniques to interface MS with GC, LC and CE are readily available.
- Different ionization techniques enable MS analysis of stable/labile and polar/apolar compounds.

#### A.2.2 Technique Limitations
- Mass Spectrometry cannot discriminate enantiomers.
- Mass Spectrometry cannot be used to identify salt forms nor determine if a salt or free drug is present.
- Stability of measured compounds: Fragmentation of some drugs may occur leaving no molecular ion (certain barbiturates), or similar patterns (e.g., Bufotenine, Psilocyn, Psilocybin).
A.2.3 Purpose/Scope
A mass spectrum yields structural information which may provide sufficient selectivity to allow for the highest discriminating capability. When used in combination with gas or liquid chromatography several compounds present in the same sample can be identified and quantified. The same applies to the multidimensional MS techniques.

A.2.4 Analytical Method

A.2.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment.

A.2.4.2 Instrumental parameters
- Identify the instrument and equipment utilized.
- List instrument conditions.

A.2.5 Reference Materials
Utilize a standard calibration compound such as PFTBA. Acquire a mass spectrum of this compound and compare it to a standard spectrum. Repeat this process utilizing a commonly encountered drug standard suitable for this method.

A.2.6 Performance Characteristics

A.2.6.1 Selectivity
For determination of closely related compounds, standards of each should be tested on the system to show selectivity.

A.2.6.2 Matrix effects
Co-elution and a high concentration of substance can cause a matrix effect.

A.2.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.2.6.4 Accuracy
- Precision (Repeatability/Reproducibility): Demonstrate the reproducibility of the instrument by running a reference standard a minimum of 10 times.
- Trueness: Must be determined for quantitative methods.

A.2.6.5 Range
- Limit of Detection (LOD), select the criteria for the mass spectrum below which no identification will be made. Determine the limit of detection by measuring the response of different amounts of analyte. For most instruments the limit of detection is in the picogram to nanogram range.
- Limit of quantitation (LOQ) must be determined for all quantitative methods. Determine the lowest concentration that has an acceptable level of uncertainty.
- Concentration ranges should be in the order of published spectra to avoid difficulties with comparison. For example, high analyte concentration in the sample preparation may cause variations in m/e ratios. The response will differ between instruments and analytes.
- Linearity must be determined for all quantitative methods.
A.2.6.6 Robustness
Determine the amount of change to instrumental parameters that will still allow for the identification (e.g., concentration, humidity, temperature).

A.2.6.7 Ruggedness
May be determined for qualitative and quantitative methods.

A.2.7 Uncertainty
Should be evaluated for quantitative methods.

A.2.8 Quality Control

A.2.9 References

A.3 Nuclear Magnetic Resonance Spectroscopy (NMR)

A.3.1 Technique Strengths
- Samples may be recovered for further analysis.
- Multiple experiments can be run on one sample depending on the capabilities of the specific instrument.
- Additional structural information may be obtained by obtaining spectra of additional nuclei. Performing decoupling or two-dimensional analysis techniques, chemical exchange, or adding a shift reagent can also enhance selectivity.
- Thermally unstable drugs can be analyzed without decomposition.
- Non-volatile drugs can be analyzed without derivatization.

A.3.2 Technique Limitations
- Concentration Ranges: Need a fairly concentrated sample and may not be suitable for residue analysis.
- Solvents should not interfere with the peaks of the sample being analyzed. Deuterated solvents are normally used for proton NMR.
- Quantitative methods must allow for full relaxation of all nuclei, which can lessen resolution. Therefore, quantitative and qualitative information are not usually gathered simultaneously.

A.3.3 Purpose/Scope
NMR is an instrumental identification technique that provides a high degree of selectivity with the ability for structural elucidation of an analyte. NMR can be used for qualitative analyses including the differentiation of isomers as well as quantitative analyses.

A.3.4 Analytical Method

A.3.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment, including the solvent appropriate for the nucleus being monitored.

A.3.4.2 Instrumental parameters
- Identify the instrument and equipment utilized.
A.3.5 Reference Materials
TMS should be used with samples as a calibrator. Acquire a mass spectrum of ethylbenzene and compare it to a standard spectrum. Repeat this process utilizing a commonly encountered drug standard suitable for this method.

A.3.6 Performance Characteristics

A.3.6.1 Selectivity
NMR offers high selectivity. For determination of closely related compounds, standards of each should be tested on the system to show selectivity.

A.3.6.2 Matrix Effects
Chemical composition should not change during the analysis. For example, it is known that some solvents (e.g., D$_2$O) exchange protons with a few structural groups like carboxylic acids, which can lessen or eliminate their signal.

A.3.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.3.6.4 Accuracy
- Precision (Repeatability/Reproducibility): Demonstrate the reliability of the technique through a predetermined number of analyses of a reference standard.
- Trueness: Must be determined for quantitative methods.

A.3.6.5 Range
- Limit of Detection (LOD): Select the criteria for the NMR spectrum below which no identification will be made. Determine the limit of detection by measuring the response of different amounts of analyte. For most instruments the limit of detection is in the milligram range.
- Limit of quantitation (LOQ) must be determined for all quantitative methods. Determine the lowest concentration that has an acceptable level of uncertainty.
- Linearity must be determined for all quantitative methods.

A.3.6.6 Robustness
Determine the amount of change to instrumental parameters that will still allow for the identification (e.g., concentration, humidity, temperature).

A.3.6.7 Ruggedness
May be determined for qualitative or quantitative methods.

A.3.7 Uncertainty
Should be evaluated for quantitative methods.

A.3.8 Quality control

A.3.9 Reference
A.4 Raman Spectroscopy

A.4.1 Technique Strengths
- Generates a very high discriminating capability unaffected by glass or plastic containers.
- Little to no sample preparation is required. It is compatible with remote sampling and fiber optics.

A.4.2 Technique Limitations
- Raman needs a fairly concentrated sample and may not be suitable for residue analysis.
- Instrumental effects can be subtle and difficult to understand and control (for example, wavelength-dependent changes in the solid angle of the collected Raman light arising from changing indices of refraction).

A.4.3 Purpose/Scope
Raman spectroscopy is most readily used for qualitative analyses with a high degree of selectivity.

A.4.4 Analytical Method
Identify the procedures to be utilized in the validation process. Verification of correct x- and y-axis calibration is required.

A.4.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment.

A.4.4.2 Instrumental parameters
- Identify the instrument and equipment utilized.
- List instrument conditions.

A.4.5. Reference Materials
- A compound must have a Raman-active vibrational mode. A series of neat organic liquids with published peak positions of the Raman spectra can be used for x-axis calibration validation. ASTM E 1848 includes Naphthalene, Sulfur, Polystyrene.
- Repeat this process utilizing a commonly encountered drug suitable for this method.

A.4.6. Performance Characteristics

A.4.6.1 Selectivity
Generates a very high discriminating capability unaffected by glass or plastic containers. This technique is highly selective, for example isomers may be detected from the changes in the molecular vibrational frequencies. For determination of closely related compounds, standards of each should be tested on the system to show selectivity.

A.4.6.2 Matrix Effects
- Fluorescence can swamp the Raman signal.
- Compounds in aqueous solution are easily measured.
A.4.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.4.6.4 Accuracy
- Demonstrate the reproducibility of the instrument by running a reference standard a minimum of 10 times.
- Trueness: Must be determined for quantitative methods.

A.4.6.5 Range
- Limit of Detection: A peak-to-noise ratio should be determined below which no identification will be made. Determine the limit of detection by measuring the response of different amounts of analyte.
- Limit of Quantitation (LOQ)
- Linearity

A.4.6.6 Robustness
Determine the amount of change to instrumental parameters that will still allow for the identification. (e.g., scan time, resolution)

A.4.6.7 Ruggedness

A.4.7 Uncertainty
Should be evaluated for quantitative methods.

A.4.8 Quality Control

A.4.9 Reference

A.5 Capillary Electrophoresis (CE)

A.5.1 Technique Strengths
- CE provides high speed, high-resolution separations on small sample volumes (0.1nL to 10mL).
- A variety of detection methods can be used, to include fluorescence, absorbance, electrochemical, and mass spectrometry detectors.
- Potentials up to 60,000V can be safely applied, allowing increases in CE’s speed and resolution.
- CE employs electro-osmotic flow. Electro-osmotic flow creates solution flow with a flat profile, as opposed to the parabolic profile created by liquid chromatography. The flat solution profile doesn’t contribute significantly to band broadening.
- CE allows the user to reverse the direction of normal electro-osmotic flow, which speeds up the separation of anions.
• CE works quite well with compounds that will not separate by gas chromatography because they are too polar, thermally labile, or nonvolatile. A chiral buffer allows for the separation of optical isomers.

A.5.2 Technique Limitations
• Long migration times may have greater variability within the peak area.
• Reproducibility of migration times is less reproducible than in GC

A.5.3 Purpose/Scope
Capillary electrophoresis is a high-speed, high-resolution separation process that can be used for qualitative and quantitative analysis and for separation of chiral pairs of drugs.

A.5.4 Analytical Method

A.5.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment.

A.5.4.2 Instrumental parameters
• Identify the instrument and equipment utilized.
• List instrumental conditions such as capillary temperature and specifications, voltage ramp, injection times, and buffer.

A.5.5 Reference Materials
A reference standard or mixtures of reference standards of the drugs to be analyzed are suitable for method validation. Mixtures may include a standard of the drug, common additives, and drugs similar to the analyte.

A.5.6 Performance Characteristics

A.5.6.1 Selectivity
During separation, CE provides various means to adjust the α values thus giving good resolution for the target compounds in most applications. Evaluate the selectivity by using a representative number of drugs and potential adulterants/diluents.

A.5.6.2 Matrix Effects
Samples must be carefully filtered.

A.5.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.5.6.4 Accuracy
• Precision (Repeatability/Reproducibility); Demonstrate the reproducibility of the instrument by running a reference standard a minimum of 10 times.
• Trueness; Must be determined for quantitative methods.

A.5.6.5 Range
Limit of Detection (LOD): CE can be a very sensitive technique. Determine the sensitivity by measuring the response of a range of different amounts of the analyte, this may be in the picogram to nanogram range.

Limit of Quantitation (LOQ)

Linearity

A.5.6.6 Robustness
Determine the amount of change to instrumental parameters that will still allow for a suitable comparison.

A.5.6.7 Ruggedness

A.5.7 Uncertainty
Should be evaluated for quantitative methods.

A.5.8 Quality Control

A.5.9 Reference

A.6 Gas Chromatography (GC)

A.6.1 Technique Strengths
- Capillary columns provide many theoretical plates.
- Detector response is proportional to sample concentration.
- GC demonstrates a high degree of selectivity
- Enantiomers can be determined using properly validated chiral columns or derivatization techniques.

A.6.2 Technique Limitations
- Although highly selective, the possibility exists that another compound will elute at the same retention time.
- Salts are usually dissociated during the injection process and cannot be identified. Some salt forms will cause excessive tailing and should be extracted prior to injection.
- Chemical decomposition can occur in the injector port or during the analysis.
- Samples must be capable of volatilization.

A.6.3 Purpose/Scope
A separation and comparison technique that will provide data that can indicate the probable identity of the analyte and the possible presence of additional sample components. It can be used as a quantitative method.

A.6.4 Analytical Method

A.6.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment.

A.6.4.2 Instrumental parameters
Identify the instrument and equipment utilized.
- List instrumental conditions, to include, injector and detector temperature, column temperature and ramp (if appropriate), mobile phase.

A.6.5 Reference Materials
A reference standard or mixtures of reference standards of the drugs to be analyzed are suitable for method validations. Mixtures may include a standard of the drug, common additives, and drugs similar to the analyte.

A.6.6 Performance Characteristics

A.6.6.1 Selectivity
Gas Chromatography possesses moderate discriminatory power. For determination of closely related compounds, standards of each should be tested on the system to show selectivity.

A.6.6.2 Matrix Effects
Determine the common excipients, additives or solvents that may react with the analyte in the GC.

A.6.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.6.6.4 Accuracy
- Precision (Repeatability/Reproducibility): Demonstrate the reproducibility of the instrument by running a reference standard a minimum of 10 times.
- Trueness: Must be determined for quantitative methods.

A.6.6.5 Range
- Limit of Detection: Gas chromatography is a sensitive technique and is dependent upon the chromatographic system used and the analyte present. Determine the sensitivity by measuring the response of different amounts of analyte. Typical sensitivity is on the order of picogram to nanogram range.
- Limit of Quantitation (LOQ)
- Linearity

A.6.6.6 Robustness
Determine the amount of change to instrumental parameters that will still allow for a suitable comparison.

A.6.6.7 Ruggedness

A.6.7 Uncertainty
Should be evaluated for quantitative methods.

A.6.8 Quality Control

A.6.9 Reference
A.7 Ion Mobility Spectrometry (IMS)

A.7.1 Technique Strengths
• IMS instruments are relatively small and may be utilized in the field to presumptively screen drugs.
• Generally, results can be obtained in less than one minute.
• Analytes are detectable in the nanogram range.
• A properly obtained IMS plasmagram provides the presumptive identification of drugs.

A.7.2 Technique Limitations
• IMS is not a specific identification technique.
• Drugs may have similar drift times
• IMS is a destructive technique
• Concentration of reference material and unknowns should not be so high as to saturate the instrument. For example, high analyte concentration may change the drift time. The concentration ranges should be determined by experiment to identify the effective range.
• Instrument is sensitive to temperature fluctuation and changes in atmospheric pressure.

A.7.3 Purpose/Scope
Ion Mobility Spectrometry refers to the principles, practice, and instrumentation for characterizing chemical substances by measurement of their gas-phase ion mobilities. This analytical technique may provide presumptive identification of drugs.

A.7.4 Analytical Method

A.7.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment.

A.7.4.2 Instrumental parameters
• Identify the instrument and equipment utilized, to include the sample collection equipment and sample collection method.
• List instrumental conditions.

A.7.5 Reference Materials
Utilize the internal calibrant recommended by the instrument manufacturer. Utilize standard external calibrants as references such as cocaine or methamphetamine for the target compounds.

A.7.6 Performance Characteristics

A.7.6.1 Selectivity
For determination of closely related compounds, standards of each as well as a mixture should be tested on the system to show selectivity.

A.7.6.2 Matrix Effects
Dirt, hair, or fibers collected in the sampling device may prevent the desorption of the analyte.

A.7.6.3 Recovery
Sample recovery may be determined for quantitative analysis.
A.7.6.4 Accuracy

- Precision (Repeatability/Reproducibility): Demonstrate the reproducibility of the instrument by running a reference standard a minimum of 10 times.
- Trueness: Must be determined for quantitative methods.

A.7.6.5 Range

- Limit of Detection: Instruments may have different detection limits. Determine the limit of detection by measuring the response of different amounts of target analytes.
- Limit of Quantitation
- Linearity

A.7.6.6 Robustness

Determine the amount of change to instrumental parameters that will still allow for a suitable comparison.

A.7.6.7 Ruggedness

A.7.7 Uncertainty

Should be evaluated for quantitative methods.

A.7.8 Quality Control

A.7.9 Reference

Eiceman, Karpas, Ion Mobility Spectrometry, CRC Press, 1993, p.2

A.8 High Performance Liquid Chromatography (HPLC)

A.8.1 Technique Strengths

- Non-destructive, samples can be recovered for additional tests.
- Thermally labile drugs can be analyzed without decomposition.
- Mobile and stationary phase composition have a large effect on the resolution between peaks.
- Non-volatile drugs can be analyzed without derivatization. HPLC can be a screening tool for certain groups or compounds.

A.8.2 Technique Limitations

- The highest purity solvents available should be used.
- As peak symmetry decreases, integration becomes less reliable.
- System should be allowed to equilibrate before samples are run in order to assure reproducible conditions.
- An appropriate standard must be included with each set of samples.
- Potential carryover must be taken into consideration.

A.8.3 Purpose/Scope

HPLC is a separation and comparison technique that provides data that can indicate the probable identity of the analyte and the possible presence of additional sample components. It can be used as a quantitative method, combined with various detectors for greater selectivity, used for preparative purposes or used to separate enantiomers by utilizing chiral columns.
A.8.4  Analytical Method

A.8.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment.

A.8.4.2 Instrumental parameters
- Identify the instrument and equipment utilized.
- List instrumental conditions, such as elution time, temperature, flow rates and detector settings.

A.8.5  Reference Materials
Reference standards or a mixture of reference standards of the drugs to be analyzed are suitable for method validation. Mixtures may include a standard of the drug, internal standards, common additives, and drugs similar to the analyte.

A.8.6  Performance Characteristics

A.8.6.1 Selectivity
HPLC possesses moderate discriminatory power. Selectivity can be enhanced with the use of different detectors. For determination of closely related compounds, standards of each should be tested on the system to show selectivity.

A.8.6.2 Matrix Effects
Compounds other than the analyte may impede the progress of the analyte through the system. The solvent containing the analyte may require a solvent system of a similar strength.

A.8.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.8.6.4 Accuracy
- Precision (Repeatability/Reproducibility): Demonstrate the reproducibility of the instrument by running a reference standard a minimum of 10 times.
- Trueness: Must be determined for quantitative methods.

A.8.6.5 Range
- Limit of detection: HPLC can be a sensitive technique. Determine the sensitivity by measuring the response of different amounts of analyte. For most systems this is in the microgram or submicrogram range.
- Limit of quantitation
- Linearity

A.8.6.6 Robustness

A.8.6.7 Ruggedness

A.8.7 Uncertainty
Should be evaluated for quantitative methods.
A.8.8 Quality Control

A.8.9 References

A.9 Microcrystalline Tests

A.9.1 Technique Strengths
• Most crystals formed are temporary complexes and the test compounds are recoverable from the test slide.
• Individual tests require only simple glass slides and one or two drops of the crystal reagent.
• Crystal tests adopted by laboratories form quickly and are easily read or they are not incorporated into the analytical scheme.
• Many habits of crystals differ significantly from each other and are easily described. Closely related analogs may be readily differentiated.

A.9.2 Technique Limitations
• Slower forming crystals may be due to the reagent drying and will form on the edges of the solution.
• Habits often change with continued crystal growth.
• Relatively large sample amounts are required to obtain crystals [usually several milligrams].
• Reviewable data must be produced through observation by an additional analyst or the crystals must be photographed/imaged before overgrowth occurs.

A.9.3 Purpose/Scope
Microcrystalline tests can be used for determining the presence of many chemicals including both controlled substances and other related compounds. Microcrystals form readily from the combination of many controlled substances and specific reagents and are recognized by their visual characteristics (habits) to the trained analyst.

A.9.4 Analytical Method

A.9.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment.

A.9.4.2 Instrumental parameters
• Identify the instrument and equipment utilized.
• Identify the procedures to be utilized. Provide the necessary documentation such as the techniques used to induce microcrystal growth with the substance.
• List instrumental conditions.

A.9.5 Reference Materials
Standard reference material samples of the compounds to be validated as well as closely related structures should be examined.

A.9.6 Performance Characteristics
A.9.6.1 Selectivity
Known analogs of the desired compound as well as common diluents should be examined to verify that the selectivity of the reagent is adequate.

A.9.6.2 Matrix Effects
Either the solvent or other compounds may limit crystal formation. High ambient temperatures may reduce crystallization.

A.9.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.9.6.4 Accuracy
- Precision (Repeatability/Reproducibility): A series of samples should be examined under differing temperature and humidity conditions.
- Trueness: Must be determined for quantitative methods.

A.9.6.5 Range
- Limit of Detection: Known solution strengths should be tested with each of the common diluents to establish the limit of detection.
- Limit of Quantitation
- Linearity

A.9.6.6 Robustness

A.9.6.7 Ruggedness

A.9.7 Uncertainty

A.9.8 Quality Control

A.9.9 References

A.10 Thin Layer Chromatography (TLC)

A.10.1 Technique Strengths
- Samples can be recovered for additional tests if non-destructive visualization techniques are employed.
- Multiple samples can be spotted on the same plate.
- Exposure of an eluted and dried plate to iodine vapor will, in general, visualize the drug of interest.
- Selection of a specific eluent can increase the selectivity of the system for isolation of the targeted compound. Selectivity can also be increased by multiple development on a single plate.
- An appropriate standard must be included with each analysis. If a mixture of standards
displaying adequate separation is included, self-verification is provided.

A.10.2 Technique Limitations

- The amount of analyte spotted on a TLC plate should be sufficient for the intended use. If comparison to a standard is being made, the amounts of sample and standard spotted should be similar.

- Edge effects result from the eluent evaporation off of the edges of the plate and inequalities in thickness and density of the stationary phase at the edge of the plate. Edge effects may result in analyte migration toward the edge of the plate and non-circular spot shape.

- Salt forms can also affect spot shape and Rf values. For example, cocaine hydrochloride usually tails more than cocaine base.

- Chemical composition of the analyte should not change during TLC. The analyte should be stable in the eluent.

A.10.3 Purpose/Scope

A quick separation and comparison technique that will provide data that can indicate the probable identity of the analyte and the possible presence of additional sample components. It can be used as a semi-quantitative method, be combined with degradation methods for greater selectivity, or be used as a preparative method.

A.10.4 Analytical Method

A.10.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected equipment.

A.10.4.2 Instrumental parameters

- Identify the equipment utilized.
- Identify the procedures to be utilized. Provide the necessary documentation regarding solvent systems, and visualization techniques.
- Provide the necessary documentation such as Rf values and description, such as the color and shape of visualized analytes.

A.10.5 Reference Materials

A reference standard or mixtures of reference standards of the drugs to be analyzed are suitable for method validation. Mixtures may include a standard of the drug, common additives, and drugs similar to the analyte.

A.10.6 Performance Characteristics

A.10.6.1 Selectivity
TLC possesses moderate selectivity. A match of Rf between two spots only means that the two compounds have some probability of being identical in composition. Selectivity may be enhanced by the use of different visualization techniques.

A.10.6.2 Matrix Effects
Oils and very concentrated co-eluting compounds can affect the Rf of the drug of interest.

A.10.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.10.6.4 Accuracy
- Precision (Repeatability/Reproducibility): Demonstrate the reproducibility of the instrument by running a reference standard a minimum of 10 times.
- Trueness: Must be determined for quantitative methods.

A.10.6.5 Range
- Limit of detection: The limit of detection of TLC is very dependent on the nature of the analyte and the selected detection method. Determine the sensitivity by measuring the response of different amounts of analyte.
- Limit of quantitation
- Linearity

A.10.6.6 Robustness

A.10.6.7 Ruggedness

A.10.7 Uncertainty

A.10.8 Quality Control

A.10.9 References

A.11 Color Tests

A.11.1 Technique Strengths
- Inexpensive: The equipment needed, a test tube or multi-well porcelain spot plate and a dropping bottle, is not expensive.
- Speed: If a color is to be developed by the sample and reagent, it will happen (in a minute or two) or (within 15 seconds).
- Multitasking: A large number of samples may be tested simultaneously.

A.11.2 Technique Limitations
- Drugs with similar structure may give the same colors. For example, dextropropoxyphene can give the same color changes as cocaine in the Scott test.
- Some color test reagents consist of chemicals that are inherently dangerous. For example, the Marquis reagent contains concentrated sulfuric acid. This makes wearing of eye protection very important while using the Marquis reagent.
- Colors developed after a lengthy exposure of the sample to the reagent are not reliable. For example, the sulfuric acid in the Marquis reagent will decompose almost any drug over time. Therefore, the brown color developed by the sample in Marquis solution over ten or twenty minutes cannot be taken as an indication of the presence of methamphetamine in the sample.

A.11.3 Purpose/Scope
Color tests are used as preliminary tests to indicate that a certain drug may or may not be present in an unextracted sample. A positive result does not indicate that a specific drug is present, but it does indicate that a certain class of drug is present. The result of the color test depends on the
reaction of a certain moiety of the drug molecule with the color test reagent which is characteristic of the sample and causes a color change. Since the results are detected visually, care must be taken that the analyst be thoroughly tested for the visual ability to detect very slight color changes.

A.11.4 Analytical Method

A.11.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected equipment.

A.11.4.2 Instrumental parameters

A.11.5 Reference Materials
Reaction of a standard compound with a color reagent to give the expected color will serve as a validation test of that color reagent.

A.11.6 Performance Characteristics
A.11.6.1 Selectivity
For analysis of closely related compounds, standards of each should be tested using the color test reagent to show selectivity.

A.11.6.2 Matrix Effects

A.11.6.3 Recovery

A.11.6.4 Accuracy
- Precision (Repeatability/Reproducibility): Demonstrate the reproducibility by running a reference standard a minimum of 10 times.
- Trueness

A.11.6.5 Range
- Limit of Detection: Determine the limit of detection by measuring the response of different amounts of analyte.
- Limit of quantitation
- Linearity

A.11.6.6 Robustness

A.11.6.7 Ruggedness

A.11.7 Uncertainty

A.11.8 Quality Control

A.11.9 References

A.12. Fluorescence Spectrophotometry
A.12.1 Technique Strengths
- Samples can be recovered for additional tests.
- Identification: The fluorescence spectrum of an unknown pure analyte when compared to a known standard can provide preliminary identification of the compound. The fluorescence spectrum of an unknown pure analyte can provide information concerning chromaphores present in the analyte.
- Can be used as a screening method for unknown drug compounds.
- Pure analytes or analytes showing no interference, are suitable for quantitative analysis.
- Appropriate standards should be run to demonstrate reproducibility of the procedure.

A.12.2 Technique Limitations
- Concentration ranges should be sufficient for the intended use. Although selective, many compounds contain the same chromaphores that contribute to the data received. Under normal conditions, these appear identical.
- The presence of the analyte as a salt will effect its solubility in a given solvent, however, the type of salt cannot be determined, and once dissolved, the analyte and its salt will give the same response.
- Under normal conditions the compounds are stable.
- Not all compounds show characteristic fluorescence spectra.
- Phosphorescence, solvent fluorescence, matrix fluorescence, and light scattering can affect results.

A.12.3 Purpose/Scope
A fluorescence spectrum of an unknown analyte can give a preliminary identification as to what compound may be present by comparison of the data received to that of a standard run under the same conditions. It can be used as a quantitative method.

A.12.4 Analytical Method

A.12.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment

A.12.4.2 Instrumental parameters
- Identify the instrument and equipment utilized.
- List instrument conditions to include, excitation wavelength, absorbance wavelength and solvents.

A.12.5 Reference Materials
Reference standards of the drugs to be analyzed are suitable for method validation. Mixtures may include a standard of the drug, common additives, and drugs similar to the analyte may also be used

A.12.6 Performance Characteristics

A.12.6.1 Selectivity
Fluorescence spectra possess limited discriminatory power. A match of the emission spectra between a sample analyte and a known standard may mean that the two are identical. However, in reality, it means that the two may possess the same types of chromaphores and respond the
same under the conditions used. Standards must be run frequently to insure method and instrument stability. It provides a useful screening method, or if used in a proper scheme, a confirmation of previously identified substances.

A.12.6.2 Matrix effects

A.12.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.12.6.4 Accuracy
• Precision (Repeatability/Reproducibility): Demonstrate the reproducibility of the instrument by running a reference standard a minimum of 10 times.
• Trueness: Must be determined for quantitative methods.

A.12.6.5 Range
• Limit of detection: Fluorescence spectrophotometry is a sensitive technique with selective compounds. It is dependent upon the compound of interest.
• Limit of quantification
• Linearity

A.12.6.6 Robustness
Determine the amount of change to instrumental parameters that will still allow for a suitable comparison.

A.12.6.7 Ruggedness

A.12.7 Uncertainty

A.12.8 Quality Control

A.12.9 References

A.13. Immunoassay

A.13.1 Technique Strengths
• Many labels available for detection: radionucleotides, enzymes, fluorescence
• Need to establish the limits of detection for the controlled substance and cross-reacting compounds.
• Suitable for manual or automated batch analyses: Procedure should be designed with included controls to demonstrate that the method is free of carryover.

A.13.2 Technique Limitations
• Majority of reagents have cross reactivities. Some stereo specific reagents exist with stereoisomers exhibiting less activity than compound that is being sought. This is controlled by the shape of the antigenic site on the antibody.
• Concentration ranges should be sufficient for the intended use. Commercial kits are designed for concentrations appropriate for toxicology samples. To be used as semi-quantitative analyses, appropriate range standards must be included.
A.13.3 Purpose/Scope
Imunoassays can determine the probable identity of several different drug classes and are suitable for semi-quantitative analysis for compounds included within the tested class.

A.13.4 Analytical Method
A.13.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment

A.13.4.2 Instrumental parameters
- Identify the instrument and equipment utilized.
- List instrumental conditions

A.13.5 Reference Materials
Reference standards of the drugs to be analyzed are suitable for method validation. Study should also include any compounds reported by the manufacturer as cross-reacting species. Compounds, which are structurally similar, should also be examined even if no previous cross-reactions have been reported.

A.13.6 Performance Characteristics
A.13.6.1 Selectivity
Imunoassays possess moderate discriminatory ability. Commonly encountered drugs should be tested prior to using the assays to test the reactivity with the assay.

A.13.6.2 Matrix Effects
Solvents, pH, light, and temperature can interfere with the reaction. A study should document controls placed in procedure to reduce or eliminate adverse effects.

A.13.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.13.6.4 Accuracy
- Precision (Repeatability/Reproducibility): Demonstrate the reproducibility of the instrument by running a reference standard a minimum of 10 times.
- Trueness: Must be determined for quantitative methods.

A.13.6.5 Range
- Limit of detection (LOD); Imunoassays have sufficient sensitivity to detect drugs in the nanogram level. Using various concentrations of known standards and measuring the response should determine the limit of detection.
- Limit of quantitation (LOQ);
- Linearity

A.13.6.6 Robustness
Determine the amount of change to instrumental parameters that will still allow for a suitable comparison.

A.13.6.7 Ruggedness
A.14 Melting Point

A.14.1 Technique Strengths
Selectivity: Mixed-melting determination adds selectivity by first running the sample alone, then mixing a standard of the suspected compound with the sample and checking for agreement.

A.14.2 Technique Limitations
- The temperature should rise at a constant, slow rate to allow for accurate observation.
- Samples should be dry and free from diluents or other adulterants. Re-crystallization of street samples may be necessary.
- Availability may be limited.

A.14.3 Purpose/Scope
The determination of a physical property of a compound that may be compared to literature values or a standard. This also can be used to aid in the identification of a compound when a mixed melting point determination is performed.

A.14.4 Analytical Method

A.14.4.1 Sample preparation
List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment

A.14.4.2 Instrumental parameters
- Identify the instrument and equipment utilized.
- Identify the procedures to be utilized.
- List instrumental conditions such as temperature rate increase and melting range.

A.14.5 Reference Materials
Reference standards of the drugs to be analyzed are suitable for method validation.

A.14.6 Performance Characteristics

A.14.6.1 Selectivity
Melting point ranges provide physical information about the analyte. Selectivity is greatly increased by utilizing the mixed-melting point technique. Standards should be run with each set of samples. For determination of closely related compounds, standards of each should be tested on the system to show selectivity.

A.14.6.2 Matrix effects

A.14.6.3 Recovery
A.14.6.4 Accuracy

- Precision (Repeatability/Reproducibility): Demonstrate the reproducibility of the instrument by running a reference standard a minimum of 10 times.
- Trueness

A.14.6.5 Range

- Limit of detection (LOD): Melting point determination requires sufficient sample for the apparatus being employed. Determine the sensitivity by measuring the response of different amounts of analyte. For most systems this is in the milligram range.
- Limit of quantitation (LOQ)
- Linearity

A.14.6.6 Robustness

A.14.6.7 Ruggedness

A.14.7 Uncertainty

A.14.8 Quality Control

A.14.9 References


A.15 Ultraviolet (UV) Spectrophotometry

A.15.1 Technique Strengths

- Samples can be recovered for additional tests.
- UV can easily be combined with HPLC for greater selectivity and specificity. Hyphenation with chromatography also makes automation of the technique easy.

A.15.2 Technique Limitations

- Compounds lacking suitable chromophore provide no signal.
- High analyte concentration in the sample may cause full absorption at all wavelengths yielding saturated spectra.
- UV spectrum often varies depending upon the pH of the sample solution.
- Chemical composition may change during the analysis.

A.15.3 Purpose/Scope

UV yields rough structural information providing modest selectivity to allow for some discriminating capability. It can be used as a quantitative method. Moreover, it is more commonly used in combination with liquid chromatography for greater selectivity.

A.15.4 Analytical Method

A.15.4.1 Sample preparation

List the required sample preparation schemes and the introduction techniques for the selected instrument and equipment

A.15.4.2 Instrumental parameters
• Identify the instrument and equipment utilized, to include the UV spectrophotometer (or detector) used in the present laboratory.
• List instrumental conditions.

A.15.4.3 Calculations
The equations and calculations used in quantitation must be delineated to include unit specifications, number of repeated measurements, significant figures, conditions for data rejection, reference values and uncertainty determination.

A.15.5 Reference Materials
• Utilize a Holmium Oxide filter for conducting a validation run on the UV and compare to the reference spectrum provided.
• Repeat this process utilizing commonly encountered drugs suitable for this method.

A.15.6 Performance Characteristics
A.15.6.1 Selectivity
An Ultra-Violet Spectrum yields limited structural information and providing modest selectivity to allow for some discriminating capability. Validation data will show the ability of the method to discriminate between different compounds. Standard spectra collection (library) shall be used as reference in the identification of the active compound.

A.15.6.2 Matrix effects
Organic solvents have varying UV absorbance and this may interfere with the absorbance of the analytes. Influence of sample preparation on the results (direct dissolving of the sample in buffer/solvents, liquid-liquid extraction) should be investigated with extreme care, taking in the consideration the pH, type of buffer and solvent, and the matrix.

A.15.6.3 Recovery
Sample recovery may be determined for quantitative analysis.

A.15.6.4 Accuracy
• Precision (Repeatability/Reproducibility): Demonstrate the reproducibility of the instrument by running a reference standard a minimum of 10 times.
• Trueness: Must be determined for quantitative methods.

A.15.6.5 Range
• Limit of detection (LOD); determine the LOD below which no data will be accepted.
• Limit of quantitation (LOQ); determine the lowest concentration that has an acceptable level of uncertainty. The lower end of the linear determination.
• Linearity; determine the mathematical relationship (calibration curve) that exists between concentration and response over a selected range of concentration. For purposes of UV this is normally a straight line. The highest linear concentration serves as the upper limit for quantitative purposes.

A.15.6.6 Robustness
Determine the amount of change in instrumental parameters that will still allow for the level of acceptance required. (e.g. vary solvent, pH, scan time, analysts, etc.)

A.15.6.7 Ruggedness
A.15.7. Uncertainty
Should be evaluated for quantitative methods.

A.15.8. Quality Control

A.15.9 Reference