SUPPLEMENTAL DOCUMENT SD-2
FOR PART IVB

QUALITY ASSURANCE/VALIDATION OF ANALYTICAL METHODS

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PREPARING VALIDATION PLANS

Preface

This supplemental document is designed to assist laboratories develop a general validation plan which meets their individual requirements. The document is intended to be used in conjunction with SWGDRUG Recommendations, Section 2, Part IVB.

The supplemental document consists of two sections. Section I provides guidance on the issues to consider when using various analytical techniques. Section II is an example of a completed validation plan.

Note In completing the validation process all sections of SWGDRUG Recommendations, Part IVB need to be considered.

Section I: Analytical Techniques – Elements to Consider

Introduction

This section details technique specific properties including *technique strengths* and *technique limitations* that may affect the design of a validation plan. The analytical techniques described correspond to those in categories A, B and C of the Part III B of SWGDRUG Recommendations as provided in Table 1. Laboratories need to consider these properties with respect to their individual needs.

Table 1: Categories of Analytical Techniques

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

Reference:

- EAL-P11 European Cooperation for Accreditation of Laboratories
- ILAC Guidelines for Forensic Laboratories Feb 2001, 5.4.5.1
- Eurachem, *The Fitness for Purpose of Analytical Methods*, 1998
- Federal Register Vol. 60 no. 40 pg 11259, March 1, 1995

1 INFRARED SPECTROSCOPY (IR)

1.1 Technique Strengths

- Samples can be recovered for additional tests.
- IR generates the highest discriminating capability. It may discriminate between diastereomers (pseudoephedrine/ephedrine) and free base/acid and salt forms.

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.

1.3 Purpose/Scope

- IR yields structural information that will provide sufficient selectivity that generates the highest discriminating capability (category A).

1.4 Analytical Method

1.4.1 Sample preparation

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

1.4.2 Instrumental parameters

- Identify the instrument and equipment utilized.
- List the instrument conditions

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.

1.6 Performance Characteristics

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.

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.

1.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

1.6.4 Accuracy

1.6.4.1 Precision (Repeatability/Reproducibility)

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times.

1.6.4.2 Trueness

- Trueness must be determined for quantitative methods.

1.6.5 Range

- Limit of Detection (LOD), a peak-to-peak 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.

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).

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.

1.7 Uncertainty

- The uncertainty of the method must be assessed for quantitative methods.

1.8 Quality control

1.9 Reference

1.9.1 EAL-P11 European Cooperation for Accreditation of Laboratories

1.9.2 ILAC Guidelines for Forensic Laboratories, Feb 2001, 5.4.5.1

1.9.3 Eurachem, *The Fitness for Purpose of Analytical Methods*, 1998

2 MASS SPECTROMETRY (MS)

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 stabile/labile and polar/non-polar compounds.

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).

2.3 Purpose/Scope

- A mass spectrum yields structural information which may provide sufficient selectivity to allow for the highest discriminating capability (category A).
- 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.

2.4 Analytical Method

2.4.1 Sample preparation

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

2.4.2 Instrumental parameters

- Identify the instrument and equipment utilized.
- List the instrument conditions.

2.5 Reference Materials

- Utilize a standard calibration compound such as PFTB
    - 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.

2.6 Performance Characteristics

2.6.1 Selectivity

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

2.6.2 Matrix effects

- Co-elution and a high concentration of substance can cause a matrix effect.

2.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

2.6.4 Accuracy

2.6.4.1 Precision (Repeatability/Reproducibility):

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times.

2.6.4.2 Trueness:

- Must be determined for quantitative methods.

2.6.5 Range

2.6.5.1 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.

2.6.5.2 Limit of quantitation (LOQ):

- 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.

2.6.6 Robustness

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

2.6.7 Ruggedness

- Ruggedness may be determined for qualitative and quantitative methods.

2.7 Uncertainty

- Uncertainty should be evaluated for quantitative methods.

2.8 Quality Control

2.9 Reference

3 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR)

3.1 Technique Strengths

- Methods developed on one NMR are conveyable to any other NMR so long as the following items are the same: magnetic field strength (the same or greater), and probe linearity and uniform response are checked on one drug. This is due to the inherent stability of the instrument and the manner in which it detects compounds.
- Samples may be recovered for further analysis.
- Multiple experiments can be run on one sample depending on the capabilities of the specific instrument.
- Specific structural information may be obtained from spectra of several nuclei (i.e., hydrogen-1 or protium, carbon-13, nitrogen-15, etc.).
- Enhanced selectivity may be achieved by using various one or multi-dimensional analysis techniques, chemical exchange, or adding a shift reagent.
- Enantiomers can be differentiated.
- Thermally unstable drugs can be analyzed without decomposition.
- Target compounds can be analyzed without derivatization.
- Multiple solvents are available to enhance selectivity and/or solubility.
- Unlike UV, FID, or MS detectors, NMR response is based on the molar quantity of nuclei at a given frequency and is the same for all compounds.
- Quantitation is performed without the use of a reference drug standard of the target compound.
- The technique can enable simultaneous identification and quantitation.
- Sample run times, sample to sample, are short.

3.2 Technique Limitations

- Concentration Ranges: Can vary widely depending on probe, nuclei being observed, number of scans obtained, magnetic field strength, and other factors. This technique may not be suitable for residue analysis.
- Solvents can interfere with the peaks of the sample being analyzed. Solvents usually used for proton NMR have deuterium (^{2}H) substituted for protons (^{1}H).
- Very complex mixtures can lead to the absence of “clean” well resolved signals to integrate making integration and quantitation difficult.

3.3 Purpose/Scope

The NMR instrument can be used for both qualitative and quantitative analyses. It allows identification and structure elucidation of an analyte that will provide sufficient selectivity to generate the highest discriminating capability (category A).

3.4 Analytical Method

3.4.1 Sample preparation

List the required sample preparation schemes, including the solvent appropriate for the nucleus being monitored.

3.4.2 Instrumental parameters

- Identify the instrument and equipment utilized.
- List instrument conditions.

3.5 Reference Materials

- The referencing of the chemical shift requires either the use of tetramethylsilane (TMS) or a similar compound, or in some instruments locking of the NMR on a deuterium signal.
- Ethylbenzene can be utilized to check the calibration of the chemical shift and to demonstrate appropriate resolution.
- For quantitation, internal standards must be of high purity, non-reactive, soluble in the solvent and have chemical shifts that do not interfere with compounds that will be encountered in the sample (e.g., benzoic acid).

3.6 Performance Characteristics

3.6.1 Selectivity

- For determination of closely related compounds, standards of each should be tested on the system to show selectivity.
- In cases of signal overlap, the interfering compound's contribution can be determined and subtracted from the mixed integral to obtain the target compound's integral value.

3.6.2 Matrix Effects

The internal standard and the analyte should be stable and fully soluble in the selected NMR solvent. The NMR sample should be free of particulate matter.

3.6.3 Recovery

Sample recovery may be determined for quantitative analysis.

3.6.4 Accuracy

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

3.6.5 Range

- Limit of Detection (LOD): Select the criteria for the NMR spectrum below which no identification will be reported. Determine the LOD by measuring the response of different amounts of an appropriate analyte. For most instruments the LOD is in the microgram to 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 of the probe based on concentration must be determined for quantitation. A probe that is linear in one method will be linear in all methods.

3.6.6 Robustness

It is not applicable for this technique. The method establishes the instrument's parameters; they are not allowed to be changed.

3.6.7 Ruggedness

Not applicable for this instrument.

3.7 Uncertainty

Uncertainty should be evaluated for quantitative methods.

3.8 Quality control

3.9 Reference

4 RAMAN SPECTROSCOPY

4.1 Technique Strengths

- Raman 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.

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).

4.3 Purpose/Scope

- Raman spectroscopy yields structural information that will provide sufficient selectivity that generates the highest discriminating capability (category 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.

4.4.1 Sample preparation

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

4.4.2 Instrumental parameters

- Identify the instrument and equipment utilized.
- List the instrument conditions.

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, and Polystyrene.
- Repeat this process utilizing a commonly encountered drug suitable for this method.

4.6 Performance Characteristics

4.6.1 Selectivity

- Raman 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.

4.6.2 Matrix Effects

- Fluorescence can swamp the Raman signal.
- Compounds in aqueous solution are easily measured.

4.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

4.6.4 Accuracy

4.6.4.1 Precision

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times

4.6.4.2 Trueness

- Trueness must be determined for quantitative methods.

4.6.5 Range

4.6.5.1 Limit of Detection

- A peak-to-peak 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.

4.6.5.2 Limit of Quantitation (LOQ)

4.6.5.3 Linearity

4.6.6 Robustness

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

4.6.7 Ruggedness

4.7 Uncertainty

- Uncertainty should be evaluated for quantitative methods.

4.8 Quality Control

4.9 Reference

4.9.1 American Society for Testing and Materials, Standard Guide for Raman Shift Standards for Spectrometer Calibration, Standard E-1840-96, 1998.

4.9.2 R.L. McCreery, Raman Spectroscopy for Chemical Analysis, vol. 157 of Chemical Analysis, J.D. Winefordner, ed., Wiley-Interscience, New York, 2000.

5 CAPILLARY ELECTROPHORESIS (CE)

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.

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

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.

5.4 Analytical Method

5.4.1 Sample preparation

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

5.4.2 Instrumental parameters

- Identify the instrument and equipment utilized.
- List the instrumental conditions such as capillary temperature and specifications, voltage ramp, injection times, and buffer.

5.5 Reference Materials

- A reference material or mixtures of reference materials 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.

5.6 Performance Characteristics

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.

5.6.2 Matrix Effects

- Samples must be carefully filtered.

5.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

5.6.4 Accuracy

5.6.4.1 Precision (Repeatability/Reproducibility)

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times.

5.6.4.2 Trueness

- Trueness must be determined for quantitative methods.

5.6.5 Range

5.6.5.1 Limit of Detection (LOD)

- CE can be a very sensitive technique. Determine the LOD by measuring the response of a range of different amounts of the analyte; this may be in the picogram to nanogram range.

5.6.5.2 Limit of Quantitation (LOQ)

5.6.5.3 Linearity

5.6.6 Robustness

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

5.6.7 Ruggedness

5.7 Uncertainty

- Uncertainty should be evaluated for quantitative methods.

5.8 Quality Control

5.9 Reference

6 GAS CHROMATOGRAPHY (GC)

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.

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.

6.3 Purpose/Scope

- Gas Chromatography is 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.

6.4 Analytical Method

6.4.1 Sample preparation

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

6.4.2 Instrumental parameters

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

6.5 Reference Materials

- A reference material or mixtures of reference materials 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.

6.6 Performance Characteristics

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.

6.6.2 Matrix Effects

- Determine the common excipients, additives or solvents that may react with the analyte in the GC.

6.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

6.6.4 Accuracy

6.6.4.1 Precision (Repeatability/Reproducibility)

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times.

6.6.4.2 Trueness

- Trueness must be determined for quantitative methods.

6.6.5 Range

6.6.5.1 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.

6.6.5.2 Limit of Quantitation (LOQ)

6.6.5.3 Linearity

6.6.6 Robustness

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

6.6.7 Ruggedness

6.7 Uncertainty

- Uncertainty should be evaluated for quantitative methods.

6.8 Quality Control

6.9 Reference

7 ION MOBILITY SPECTROMETRY (IMS)

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.

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.

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.

7.4 Analytical Method

7.4.1 Sample preparation

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

7.4.2 Instrumental parameters

- Identify the instrument and equipment utilized, to include the sample collection equipment and sample collection method.
- List the instrumental conditions.

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.

7.6 Performance Characteristics

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.

7.6.2 Matrix Effects

- Dirt, hair, or fibers collected in the sampling device may prevent the desorption of the analyte.

7.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

7.6.4 Accuracy

7.6.4.1 Precision (Repeatability/Reproducibility)

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times.

7.6.4.2 Trueness

- Trueness must be determined for quantitative methods.

7.6.5 Range

7.6.5.1 Limit of Detection

- Instruments may have different detection limits.
- Determine the limit of detection by measuring the response of different amounts of target analytes.

7.6.5.2 Limit of Quantitation

7.6.5.3 Linearity

7.6.6 Robustness

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

7.6.7 Ruggedness

7.7 Uncertainty

- Uncertainty should be evaluated for quantitative methods.

7.8 Quality Control

7.9 Reference

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

8 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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.

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.

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.

8.4 Analytical Method

8.4.1 Sample preparation

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

8.4.2 Instrumental parameters

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

8.5 Reference Materials

- Reference materials or a mixture of reference materials 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.

8.6 Performance Characteristics

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.

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.

8.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

8.6.4 Accuracy

8.6.4.1 Precision (Repeatability/Reproducibility)

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times.

8.6.4.2 Trueness

- Trueness must be determined for quantitative methods.

8.6.5 Range

8.6.5.1 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.

8.6.5.2 Limit of quantitation

8.6.5.3 Linearity

8.6.6 Robustness

8.6.7 Ruggedness

8.7 Uncertainty

- Uncertainty should be evaluated for quantitative methods.

8.8 Quality Control

8.9 Reference

8.9.1 *The United States Pharmacopoeia (USP 23) / The National Formulary (NF 18).* United States Pharmacopoeial Convention, Inc. 1995. Rockville, Maryland. pp. 1774-1776.

9 MICROCRYSTALLINE TESTS

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.

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.

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.

9.4 Analytical Method

9.4.1 Sample preparation

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

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 the instrumental conditions.

9.5 Reference Materials

- Standard reference material samples of the compounds to be validated as well as closely related structures should be examined.

9.6 Performance Characteristics

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.

9.6.2 Matrix Effects

- Either the solvent or other compounds may limit crystal formation.
- High ambient temperatures may reduce crystallization.

9.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

9.6.4 Accuracy

9.6.4.1 Precision (Repeatability/Reproducibility)

- A series of samples should be examined under differing temperature and humidity conditions.

9.6.4.2 Trueness

- Trueness must be determined for quantitative methods.

9.6.5 Range

9.6.5.1 Limit of Detection

- Known solution strengths should be tested with each of the common diluents to establish the limit of detection.

9.6.5.2 Limit of Quantitation

9.6.5.3 Linearity

9.6.6 Robustness

9.6.7 Ruggedness

9.7 Uncertainty

9.8 Quality Control

9.9 Reference

9.9.1 Clarke, E.G.C., Isolation and Identification of Drugs, Volume 1, London: The Pharmaceutical Press, 1969, pp 135 – 141.

9.9.2 Fulton, Charles C., Modern Microcrystal Tests for Drugs, New York: Wiley – Interscience, 1969.

10 THIN LAYER CHROMATOGRAPHY (TLC)

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 developments 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.

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

10.3 Purpose/Scope

- Thin-layer chromatography is 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.

10.4 Analytical Method

10.4.1 Sample preparation

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

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.

10.5 Reference Materials

- A reference material or mixtures of reference materials 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.

10.6 Performance Characteristics

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.

10.6.2 Matrix Effects

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

10.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

10.6.4 Accuracy

10.6.4.1 Precision (Repeatability/Reproducibility)

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times.

10.6.4.2 Trueness

- Trueness must be determined for quantitative methods.

10.6.5 Range

10.6.5.1 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.

10.6.5.2 Limit of quantitation

10.6.5.3 Linearity

10.6.6 Robustness

10.6.7 Ruggedness

10.7 Uncertainty

10.8 Quality Control

10.9 Reference

11 COLOR TESTS

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 within a characteristic short time (usually less than a minute).
- Multitasking: A large number of samples may be tested simultaneously.

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.

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.

11.4 Analytical Method

11.4.1 Sample preparation

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

11.4.2 Instrumental parameters

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.

11.6 Performance Characteristics

11.6.1 Selectivity

- For analysis of closely related compounds, standards of each should be tested using the color test reagent to show selectivity.

11.6.2 Matrix Effects

11.6.3 Recovery

11.6.4 Accuracy

11.6.4.1 Precision (Repeatability/Reproducibility)

- Demonstrate the reproducibility by running a reference material a minimum of 10 times.

11.6.4.2 Trueness

11.6.5 Range

11.6.5.1 Limit of Detection

- Determine the limit of detection by measuring the response of different amounts of analyte.

11.6.5.2 Limit of quantitation

11.6.5.3 Linearity

11.6.6 Robustness
11.6.7 Ruggedness

11.7 Uncertainty

11.8 Quality Control

11.9 Reference

12 FLUORESCENCE SPECTROPHOTOMETRY

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 chromophores 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.

12.2 Technique Limitations

- Concentration ranges should be sufficient for the intended use.
- Although selective, many compounds contain the same chromophores 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.

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.

12.4 Analytical Method

12.4.1 Sample preparation

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

12.4.2 Instrumental parameters

- Identify the instrument and equipment utilized.
- List instrument conditions to include: excitation wavelength, absorbance wavelength and solvents.

12.5 Reference Materials

- Reference materials 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

12.6 Performance Characteristics

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 chromophore 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.

12.6.2 Matrix effects

12.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

12.6.4 Accuracy

12.6.4.1 Precision (Repeatability/Reproducibility)

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times.

12.6.4.2 Trueness

- Trueness must be determined for quantitative methods.

12.6.5 Range

12.6.5.1 Limit of detection

- Fluorescence spectrophotometry is a sensitive technique with selective compounds. It is dependent upon the compound of interest.

12.6.5.2 Limit of quantification

12.6.5.3 Linearity

12.6.6 Robustness

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

12.6.7 Ruggedness

12.7 Uncertainty

12.8 Quality Control

12.9 Reference

13 IMMUNOASSAY

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.

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. The shape of the antigenic site on the antibody controls this.
- 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.

13.3 Purpose/Scope

- Immunoassays can determine the probable identity of several different drug classes and are suitable for semi-quantitative analysis for compounds included within the tested class.

13.4 Analytical Method

13.4.1 Sample preparation

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

13.4.2 Instrumental parameters

- Identify the instrument and equipment utilized.
- List instrumental conditions

13.5 Reference Materials

- Reference materials 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.

13.6 Performance Characteristics

13.6.1 Selectivity

- Immunoassays possess moderate discriminatory ability.
- Commonly encountered drugs should be tested prior to using the assays to test the reactivity with the assay.

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

13.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

13.6.4 Accuracy

13.6.4.1 Precision (Repeatability/Reproducibility)

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times.

13.6.4.2 Trueness

- Trueness must be determined for quantitative methods.

13.6.5 Range

13.6.5.1 Limit of detection (LOD)

- Immunoassays 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.

13.6.5.2 Limit of quantitation (LOQ)

13.6.5.3 Linearity

13.6.6 Robustness

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

13.6.7 Ruggedness

13.7 Uncertainty

13.8 Quality Control

13.9 Reference

14 MELTING POINT

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.

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.

14.3 Purpose/Scope

- Melting point determination is 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.

14.4 Analytical Method

14.4.1 Sample preparation

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

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.

14.5 Reference Materials

- Reference materials of the drugs to be analyzed are suitable for method validation.

14.6 Performance Characteristics

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.

14.6.2 Matrix effects

14.6.3 Recovery

14.6.4 Accuracy

14.6.4.1 Precision (Repeatability/Reproducibility)

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times.

14.6.4.2 Trueness

14.6.5 Range

14.6.5.1 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.

14.6.5.2 Limit of quantitation (LOQ)

14.6.5.3 Linearity

14.6.6 Robustness

14.6.7 Ruggedness

14.7 Uncertainty

14.8 Quality Control

14.9 Reference

14.9.1 The United States Pharmacopoeia (USP 22) / The National Formulary (NF 17). United States Pharmacopoeial Convention, Inc. 1990. Rockville, Maryland., pp. 1588-1589.

15 ULTRAVIOLET SPECTROPHOTOMETRY (UV)

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.

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.

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.

15.4 Analytical Method

15.4.1 Sample preparation

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

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.

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.

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.

15.6 Performance Characteristics

15.6.1 Selectivity

- A 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.

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.

15.6.3 Recovery

- Sample recovery may be determined for quantitative analysis.

15.6.4 Accuracy

15.6.4.1 Precision (Repeatability/Reproducibility)

- Demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times.

15.6.4.2 Trueness

- Trueness must be determined for quantitative methods.

15.6.5 Range

15.6.5.1 Limit of detection (LOD)

- Determine the LOD below which no data will be accepted.

15.6.5.2 Limit of quantitation (LOQ)

- Determine the lowest concentration that has an acceptable level of uncertainty.
- The LOQ is the lower end of the linear determination.

15.6.5.3 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.

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.).

15.6.7 Ruggedness

15.7 Uncertainty

- Uncertainty should be evaluated for quantitative methods.

15.8 Quality Control

15.9 Reference

15.9.1 "The Fitness for Purpose of Analytical Methods", Eurachem Guide, Dec 1998, p43 and 49.

Section II: A Completed Validation Plan

Introduction

The following demonstrates a purpose-defined validation plan for a particular method within an individual laboratory. The aim is to show how a complete validation plan may appear.

The following example should not be directly applied to methodology used by any laboratory without first considering the specific purpose of a method and its relevant operational environment.

Example

Scenario: Laboratory x is tasked with validating a qualitative and quantitative method for the analysis of heroin. The laboratory has defined the performance specifications necessary to achieve the laboratory standards and meet the customer requirements. The method to be validated utilizes GC/MS.

Validation plan for GC/MS identification and quantitation of heroin

1 Purpose/Scope

Establish if the GC/MS method for heroin identification and quantitation meets the laboratory and customer specifications (performance specifications) by examination and review of objective evidence.

Performance specifications

- Selectivity: Sufficient to enable full separation of heroin from other opiates
- Matrix effects: Sufficient to enable full separation of heroin from diluents and cutting agents typically met in street samples
- Recovery: > 95%
- Precision (Repeatability): < 5%
- Precision (Reproducibility): < 8%
- Trueness: < 8%
- Limit of detection (LOD): 0.1 %
- Limit of quantitation (LOQ): 1.0 %
- Linearity: correlation coefficient > 0.99
- Robustness: sufficient for routine work
- Ruggedness: not significant as the method will be applied only in one laboratory
- Uncertainty: expanded uncertainty < 10%

Process review

The results of the validation experiments will be reviewed against the performance specifications:

A. If the experimental results achieve the performance specifications, the method is validated (fit for purpose).
B. If the experimental results do not achieve the performance specifications, the following options will be considered:

1. The performance specifications may reviewed, amended if appropriate and validation accepted based on redefined specifications.
2. The method will be redeveloped and revalidated.

2 Analytical Method

Sample preparation

Approximately 20mg (15 – 60 mg) of homogenized sample material is accurately weighed in a test tube and the weight recorded. The powder is dissolved in 5.0 mL of methylene chloride containing the internal standard (5α-cholestane, 0.5 mg/mL). The test tubes are capped, shaken for 15min and centrifuged at 2500 rpm for 5min. The mixture is filtered if turbid. Approximately 1mL of the methylene chloride solution is transferred into a GC vial for the GC/MS analysis. The vial is capped and its tightness checked.

Stability of analyte

Stability of heroin in methylene chloride will be evaluated during the course of the validation. Additionally, shelf life of the heroin standard solutions will be investigated by storing the solutions for three weeks and by controlling their concentrations against the calibration curve prepared on day one.

Instrumental parameters

Instrument:	HP 6890 gas chromatograph with autosampler, HP 5973 mass selective detector, Agilent MS Chemstation rev. B.01.00 (Agilent technologies).
Column:	A 5% phenyl methyl silicone capillary columns (HP-5MS), 30 m (L) x 0.25 mm (i.d.), d_f 0.25 μm (Agilent technologies).
Carrier gas:	Helium, 25 cm/s at 150 °C, constant flow
Sample introduction:	1 μL split, 60 mL/min total flow, 1:148 split ratio (gas saver 20mL/min after 1.5 min), glass wool packed liner with a volume of 990 μl.
Temperatures:	
Injector:	250 °C
Oven T-program:	150 °C, 10 °C/min, 300 °C (10 min)
GC/MS interface:	310 °C

MS information:

Solvent delay:	2.5 min
Mass range:	30 - 550 a.m.u.
Sample rate #:	2, A/D samples 4
MS quad temp:	150 °C
MS source:	230 °C

The total ion chromatogram is used for quantitation.

Calculations
A single-point calibration based on the internal standard method is utilized.

3 Reference Materials

MS reference material - PFTBA is used to calibrate the MS in accordance with the procedures recommended by the instrument manufacturer.

Drug reference material - Certified heroin hydrochloride monohydrate, 98.83 %, M-29-HC-500 purchased from Lipomed, Switzerland. This certified material has been verified according to laboratory practices due to limitations in the manufacturer's documentation.

Internal standard reference material - 5α-cholestane, p.a., purchased from Merck, Darmstadt, Germany.

4 Performance Characteristics

4.1 Selectivity

Selectivity of the GC/MS screening method will be investigated by adding known substances, i.e. other opiates and commonly encountered street drugs. Difference in retention time will be used as the first criterion and the mass spectrum as the second criterion to estimate selectivity.

4.2 Matrix effects

The influence of cutting agents and adulterants commonly encountered in street drugs will be investigated. Paracetamol (acetaminophen), a known interfering substance, will be added to 10 different known heroin samples (n=10) and the analyses carried out. Other commonly encountered cutting agents and adulterants will be evaluated in the same manner.

Matrix effects may also have great impact on repeatability. This will be investigated by analyzing six different heroin street samples. Ten replicates of each sample will be prepared and analyzed and their concentrations calculated. Repeatability in terms of RSD will be derived from the concentrations.

This data will be used to evaluate the magnitude of the effect, if any, on trueness and precision.

4.3 Recovery

As the method is based on dissolution, recovery can be evaluated using only typical cutting agents, i.e. lactose. Recovery of the method will be investigated by standard addition technique. Heroin standard will be mixed with lactose in different ratios (n=10) covering a concentration range of 5 – 100%. The sample will be prepared and analyzed as described above. Heroin concentration will be measured against the calibration curve and recovery established.

4.4 Accuracy

4.4.1 Precision (repeatability)

Qualitative analysis

Ten preparations of one heroin street sample will be analyzed in random order. Correct identification of heroin will be evaluated against laboratory requirements.

Quantitative analysis

Within day repeatability will be investigated by analyzing ten replicates of one heroin street sample. Repeatability in terms of RSD will be derived from the quantitative results.

4.4.2 Precision (reproducibility)

Qualitative analysis

Reproducibility will be investigated by analyzing a sample ten times by two different operators and on two different instruments and the results compared.

Quantitative analysis

Reproducibility will be investigated by two operators independently analyzing one sample ten times. Student t-test of means will be applied to the data with a 95% confidence interval.

4.4.3 Trueness

Trueness will be investigated by comparing values obtained by the current method with those obtained by a validated reference method (HPLC). Trueness will be investigated by analyzing a heroin street sample ten times. Trueness will be estimated through the Student t-test of means. Statistical difference between the results of the GC/MS and the reference method will be considered systematic error.

4.5 Range

4.5.1 Limit of detection (LOD)

LOD will be investigated through serial dilution of a known heroin solution (low end concentrations). LOD will be defined as the lowest concentration, which produces identifiable heroin spectrum and meets laboratory qualitative requirements.

4.5.2 Limit of quantitation (LOQ)
LOQ will be investigated through the analysis of ten replicates. The concentration of the replicates is determined by a serial dilution of a known heroin solution (low end concentrations). LOQ will be defined as the lowest concentration in which relative standard deviation (RSD) is not higher than the repeatability of the method.

4.5.3 Linearity
Linearity will be investigated using a minimum of eight different concentrations (0.1, 0.5, 1.0, 5.0, 10, 50, 80 and 100 % of heroin). The peak areas will be plotted against concentrations, a calibration curve drawn and correlation coefficient calculated.

4.6 Robustness
Robustness will be investigated by varying analytical procedure, i.e. the volume of the methylene chloride and the weighed amount of heroin. Evaluation of other parameters is not considered relevant for this application.

4.7 Ruggedness
Ruggedness is not relevant in this study as the method will be utilized in one laboratory only and the reproducibility study includes evaluation of intra-laboratory variation.

4.8 Uncertainty
Uncertainty will be calculated taking into consideration both systematic error (trueness) and the random error (repeatability/reproducibility). The expanded uncertainty will be established using a 95% confidence level.

5 Quality Control
Acceptance criteria for quality control parameters will be established on the basis of the validation results prior to implementation of the method.

6 References
The testing laboratory will provide the selected supporting reference literature relating to the validated method.

End of Document

