FORENSIC TOXICOLOGY LABORATORY
OFFICE OF THE CHIEF MEDICAL EXAMINER
CITY OF NEW YORK

METHOD VALIDATION CRITERIA

IMMUNOASSAYS

Methods developed and implemented for immunoassay screening must be validated for accuracy, and precision.

Accuracy of candidate immunoassays is evaluated **qualitatively** against established immunoassays (drug class assays) and more specific tests whenever possible (GC, GCMS-SIM, GCMS-SCAN, LCMS, HPLC). Within the limitations of the candidate immunoassay (cross reactivity to various drugs), there should be **qualitative** agreement between the candidate immunoassay results, both negative and positive, and the results from established methods. In addition, accuracy is determined by participation in proficiency testing programs.

Precision is determined by a minimum of eight within-run replicate analyses performed to determine the coefficient of variation for the candidate assay. The coefficient of variation should not be greater than 10%.

CHROMATOGRAPHIC METHODS

Methods developed and implemented for chromatographic methods (GC, GCMS, LCMS, HPLC) must be validated for accuracy, precision, limit of detection, limit of quantitation and method linearity.

Accuracy of the developed method is determined by analysis of controls (external or internal) by analysis of casework previously analyzed by other validated methods or by reference laboratories. Measured results are evaluated against expected results. Accuracy is acceptable if results obtained by the new method are within ± 25% of expected value. In addition, accuracy is determined by participation in proficiency testing programs.

Precision of the developed GC method is determined by triplicate analysis of prepared calibrators at the low end of the calibration curve and by analysis of control material.

Limit of detection (LOD) is the lowest concentration of the analyte present in the sample matrix that is detected, although not necessarily quantitated, under the method acceptance criteria.

Limit of Quantitation (LOQ) is the lowest concentration of the analyte present in the sample matrix that is detected under the method acceptance criteria at a concentration within $\pm$ 20% of target concentration.

Upper Limit of Quantitation (ULOQ) is the highest concentration of the analyte present in the sample matrix that is detected under the method acceptance criteria at a concentration within ± 20% of target concentration.

GAS CHROMATOGRAPHY

Procedure

1. Primary standards and internal standards are checked for purity by full scan MS analysis.
2. Primary standards and internal standards are separately injected, to determine retention time.
3. Standards and internal standards are extracted and analyzed by the method to determine recovery.
4. Once extraction method and GC method are established, proceed with the following:
    a. Multi-point calibration curve

    Note: *Concentrations depend on reporting levels established for the analyte.*

    b. Establish linearity of the calibration curve by running controls.

    c. LOD and LOQ are established by analyzing, in triplicate, analyte concentrations 50%, 25% and 10% of the lowest calibrator.

    d. ULOQ is established by analyzing, in triplicate, analyte concentrations 150%, 200% and 250% of the highest calibrator.
5. After upper and lower limits of quantitation are established, samples that have been previously analyzed by other methodologies are run to establish accuracy of the method. These may include casework, proficiencies, samples sent to reference laboratories, etc.

Acceptance criteria

1. A three point (minimum) calibration curve must be established.
2. Review calibrators and negative control, checking that all analyte peaks are present and correctly integrated and that the peaks are ± 2 % of the calibrator retention times, and the peaks meet chromatography criteria. Regression coefficient ($r^{2)}$ for each analyte must be equal to or higher than 0.98.
3. Negative control must not contain detectable amounts of any analytes.
4. Reprocess the remaining samples and controls using the calibrated and checked method.
5. Review the entire batch using the same criteria as in step 2.

Note: *Make sure the signal does not saturate detector limits for largest peaks.*

6. Print all data and submit to QA/QC Supervisor for evaluation.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY (SIM)

Procedure

1. Primary standards and internal standards are checked for purity by full scan MS analysis.

2. Primary standards and internal standards are injected separately un-extracted and derivatized (if necessary for good chromatography) and analyzed by full scan MS to establish retention times and ions of interest. Data from these runs is used to create the SIM data acquisition and processing methods.

3. Derivatized standards and internal standards are analyzed under SIM conditions to establish response and required dwell times. (Standards and internal standards are combined, if needed, and run under SIM conditions)

4. Combined standards and internal standards are extracted and derivatized, then analyzed by SIM method to establish recovery.

5. Once extraction method and data acquisition/processing methods are established, proceed with the following:

   a. Multi-point calibration curve

   Note: *Concentrations depend on reporting levels established for the analyte.*

   b. Establish linearity of the calibration curve by running controls.

   c. LOD and LOQ are established by analyzing, in triplicate, analyte concentrations 50%, 25% and 10% of the lowest calibrator.

6. After upper and lower limits of quantitation are established, samples that have been previously analyzed by other methodologies are run to establish accuracy of the method. These may include casework, proficiencies, samples sent to reference laboratories, etc.

Acceptance criteria

1. A four point (minimum) calibration curve must be established.
2. Review calibrators and negative control, checking that the ion peaks are present and correctly integrated and that the ion ratios are ± 20 % of the average of all calibrators and the peaks are ± 2 % of the calibrator retention times. Peaks must meet chromatography criteria. Regression coefficient (r^2) for each analyte must be equal to or higher than 0.99.
3. Negative control must not contain detectable amounts of any analytes.
4. Reprocess the remaining samples and controls using the calibrated and checked method.

5. Review the entire batch, using the same criteria as in step 2.

Note: *Sometime ratios will be off in exceptionally low or high concentrations. The analyst must evaluate this on a sample to sample basis.*

6. Print all data and submit to QA/QC Supervisor for evaluation.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY (FULL SCAN)

Procedure

1. All full scan samples have been previously injected by GC. Full scan MS is used to confirm the identity of GC peaks.
2. A blank and a control at 0.2 mg/L are injected with each batch of full-scan GCMS, Identification of the peaks in the control by library match and comparison to known spectra is the criteria for full scan GCMS.

Acceptance criteria

1. The blank must contain only the internal standard peak, which must have a comparable mass spectrum to known internal standard mass spectrum.
2. The control peaks must contain all peaks from the GC-NPD chromatograph, and the mass spectra of those peaks must be comparable to known mass spectra of those compounds.

LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (LCMS)

Procedure

1. Primary standards and internal standards are checked for purity by full scan MS analysis when possible.
2. Primary standards and internal standards are injected separately unextracted and analyzed by LCMS to establish retention times and ions of interest. Data from these runs is used to create the LCMS data acquisition and processing methods.
3. Standards and internal standards are analyzed under LCMS conditions to establish response and required dwell times. (Standards and internal standards are combined, if needed, and run under LCMS conditions)
4. Combined standards and internal standards are extracted and analyzed by LCMS method to establish recovery.

Once extraction method and data acquisition/processing methods are established, proceed with the following:

a. Multi-point calibration curve

Note: *Concentrations depend on reporting limits established for the analyte.*

b. Establish linearity of the calibration curve by running controls.

5. After upper and lower limits of quantitation are established, samples that have been previously analyzed by other methodologies are run to establish accuracy of the method. These may include casework, proficiencies, samples sent to reference laboratories, etc.

Acceptance criteria

1. A four point (minimum) calibration curve must be established.
2. Review calibrators and negative control, checking that the ion peaks are present and correctly integrated and that the ion ratios are ± 20 % of the average of all calibrators (or of one calibrator: method specific) and the peaks are ± 2 % of the calibrator retention times. Peaks must meet chromatography criteria. Regression coefficient (r^2) for each analyte must be equal to or higher than 0.99. If non-deuterated internal standards are used, the r^2 must be greater than or equal to 0.98.
3. Negative control must not contain detectable amounts of any analytes.
4. Reprocess the remaining samples and controls using the calibrated and checked method.
5. Review the entire batch, using the same criteria as in step 2.

Note: *Sometime ratios will be off in exceptionally low or high concentrations. The analyst must evaluate this on a sample to sample basis.*

6. Print all data and submit to QA/QC Supervisor for evaluation.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Procedure

1. Primary standards and internal standards are checked for purity by full scan MS analysis or other appropriate methodology.
2. Primary standards and internal standards are separately injected, to determine retention time and UV spectra.
3. Standards and internal standards are extracted and analyzed by the method to determine recovery.

4. Once extraction method and HPLC method are established, proceed with the following:

   a. Multi-point calibration curve

   Note: *Concentrations depend on reporting levels established for the analyte.*

   b. Establish linearity of the calibration curve by running controls.

   c. LOD and LOQ are established by analyzing, in triplicate, analyte concentrations 50%, 25% and 10% of the lowest calibrator.
   d. ULOQ is established by analyzing, in triplicate analyte concentrations 150%, 200% and 250% of the highest calibrator.

2. After upper and lower limits of quantitation are established, samples which have been previously analyzed by other methodologies are run to establish accuracy of the method. These may include casework, proficiencies, samples sent to reference laboratories, etc.

Acceptance criteria

1. A four point (minimum) calibration curve must be established.

2. Review calibrators and negative control, checking that all analyte peaks are present and correctly integrated, match the UV spectra of the analyte, the peaks are ± 2 % of the calibrator retention times, and the peaks meet chromatography criteria. Regression coefficient (r^2) of each analyte must be equal to or higher than 0.98.

3. Negative control must not contain detectable amounts of any analytes.

4. Reprocess the remaining samples and controls, using the calibrated and checked method.

5. Review the entire batch, using the same criteria as in step 2.

   Note: *Make sure that the signal does not saturate detector limits for largest peaks.*

6. Print all data and submit to QA/QC Supervisor for evaluation.