Method Development and Validation for Certification in Accordance with CLSI C62-A and CAP Guidelines

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Outline

- Regulatory guidance documents
- CLSI C62-A: assay development
- CLSI C62-A: assay verification
- Mass spec in the CAP checklist

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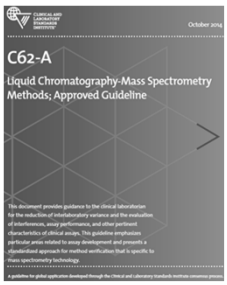
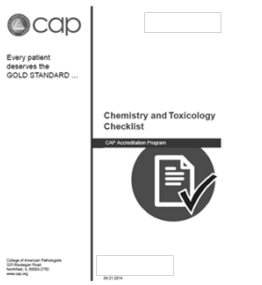
Guidance and Regulatory Documents





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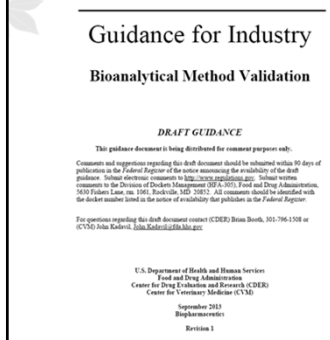
Guidance and Regulatory Documents



Guidance for Industry

Bioanalytical Method Validation

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document contact (CDER) Brian Booth, 301-796-1508 or (CVM) John Kadavil, John.Kadavil@fda.hhs.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)

September 2013
Biopharmaceutics

Revision 1

FDA Draft Guidance*
New Drug Applications
Clinical Trials
Pharmacokinetic Studies

*2013 is draft only
*not explicitly for clinical labs

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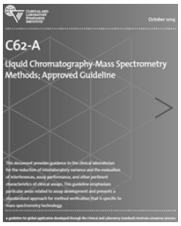
Scope of the CLSI C62-A Document



C62-A

Liquid Chromatography-Mass Spectrometry Methods; Approved Guideline

- Important features of LC-MS instrumentation
- Pre-examination factors that can impact assay performance
- Assay calibration
- Analytical variables important in method development
- Assay verification
- Quality assurance and quality control
- Post-implementation monitoring of clinical methods

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CLSI C62-A: Cross Referenced Documents

Document	Title
CLSI 50-A	Mass Spectrometry in the Clinical Lab, General Principles and Guidance-2007
CLSI C24	Statistical Quality Control For Quantitative Measurement Procedures-2006
CLSI EP06	Evaluation of Linearity for Quantitative Measurement Procedures-2003
CLSI EP07	Interference Testing in the Clinical Laboratory-2005
CLSI EP14	Evaluation of Commutability of Processed Samples-2014
CLSI EP15	User Verification of Precision and Estimation of Bias-2014
CLSI EP17	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures-2012
CLSI EP23	Laboratory Quality Control based on Risk Management-2011
CLSI GP27	Using Proficiency Testing to Improve the Clinical Laboratory-2007
CLSI GP31	Laboratory Implementation, Verification, and Maintenance-2009

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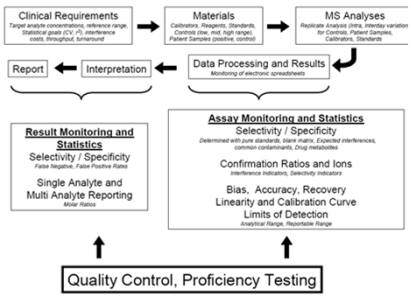
Total process of assay development



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Internal Standards

- I.S. used to account for recovery variance and matrix effects
- All quantitative methods should use I.S.
- $Signal_{analyte}/Signal_{I.S.}$ = [analyte]

My I.S. Should……	My I.S. Should Not….....
Unique to the test population	Co-elute with interferences
Be a physiochemical mimic	Share same mass transitions with other compounds
Have similar retention time	Contribute more than 20% of the signal for the test analyte at the LLMI
Be added at a concentration that does not interfere with the LLMI (10-50X LLMI)	

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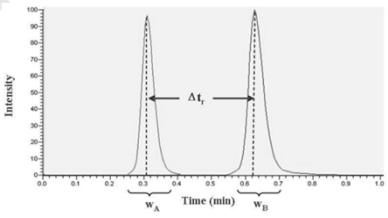
I.S.: Specificity and Selectivity

- Initial assessment of background noise is important to confirm in the testing system
- Signal from a matrix matched double blank sample (no analyte/ no internal standard) reflects the total background in the LC/MS system.
- To avoid problems with assay sensitivity, background peaks should be absent or <20% of the peak area for the analyte at the LLMI or 5% of the IS at the expected retention time.
- Quantitation of a double blank reflects the background in the whole testing system including sample processing

Columns and Chromatography

- Proper operation requires controlling factors such as temperature, mobile composition, pressure, pH, ,solvent quality, specimen clean up.
- Variables include
  - Retention time
  - Chromatographic resolution
  - “Dead volume”
  - Column efficiency
- Retention time for analyte quantitation should remain within +/-2.5% between runs.

Chromatographic Resolution



$2*([Tr_B-Tr_A] / W_A + W_B)$

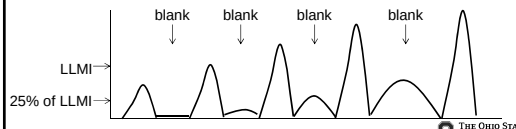
- Rs = distance between the two peak centers divided by the average peak width
- Complete baseline resolution is achieved with an Rs of at least 1.25
- Minimization of dead volume improves method performance

Carryover

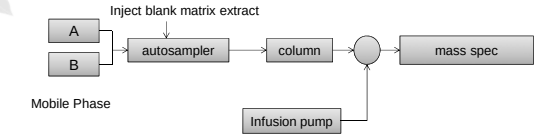
- LC/MS can be considered continuous flow measurement instead of random access
- Subject to carryover
- No generally accepted limit, must not impact bias or precision
- Signal from blank samples must well below LLMI independent of the concentrations of previous samples
- Although CLSI EP10 addresses carryover it is limited in 2 ways
  - Only addresses carryover for samples within MI
  - Does not assess the magnitude of carryover into blank samples

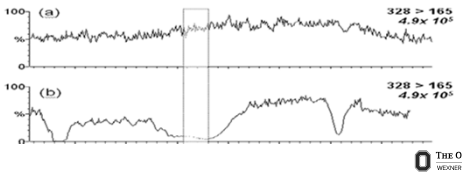
Carryover: FDA Guidance document

- Bioanalytical Method Validation (FDA) indicates one or more blank samples should be injected immediately after a high concentration
- Testing an sample well above the MI is more sensitive
- Inject an extracted negative blank after increasing concentration of analyte.
- Recommended that the carryover limit is the concentration that does not create a signal 25% of LLMI

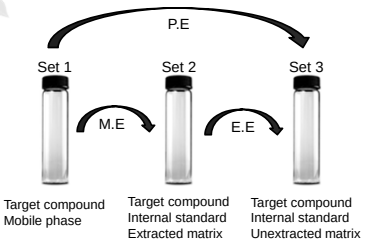


Ion Suppression Post Column Infusion





Matrix Effects: Extraction Spiking Studies



Matrix Effects
Set 2/Set 1

Extraction Efficiency
Set 3/Set 2

Processing Efficiency
Set 3/Set 1

Matuszewski BK. Anal Chem. 2003 Jul 1;75(13):3019-30.

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Calculating Matrix Effects

- Prepare 5 different calibrations curve
  - Analyte in mobile phase
  - Analyte spiked into post-extract
- Compare the signal obtained with each matrix
- Calculate %Matrix Effect
- %Matrix Bias = 100-%Matrix Effect
- %CV of peak areas should also be evaluated with a target <15%

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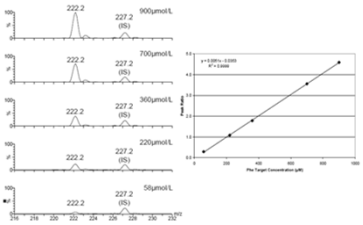
Assay Verification

- Linearity
- LOD
- LOQ
- Precision
- Bias/Trueness
- Measuring Interval
- Dilutions



Assay Verification: Linearity and Measuring Interval

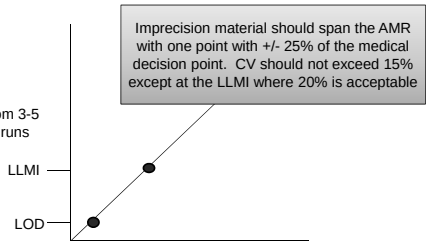
- Recommended:
  - 9-11 points with 2-4 replicates
  - Validated for each specimen type
  - Serial dilutions should be avoided for making linearity material due to propagation of pipette bias
- Measuring interval is defined by both linearity and imprecision
  - Does the assay stay linear at high concentrations
  - Do the measurements meet the acceptable criteria for reproducibility

Defining the lower limit of the measuring interval (LLMI)

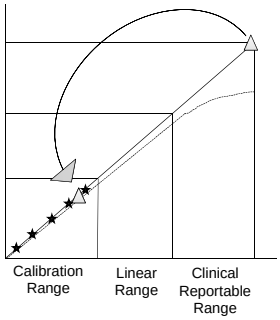
Imprecision material should span the AMR with one point with +/- 25% of the medical decision point. CV should not exceed 15% except at the LLMI where 20% is acceptable

Recommended:
40 replicates from 3-5 samples over 5 runs

CV <20%
Bias <15%



Dilutions Within and Outside the M.I.



- Dilutions within the R.I.
  - Final concentrations should be >3* LLMI
- Dilutions above the R.I.
  - Specimen extracts (not neat specimens) should be diluted
  - Diluent should contain the same amount of IS as the extract sample before dilution

Quality Control

- Quality Control has two elements
  - Composition
  - Stability
- QC material should be matrix appropriate and performance parameters related to comparability must be defined and documented
- If the lab prepares QC it must perform a study to determine storage conditions: Free thaw cycles, short-term ambient stability, thawing procedures
- Should be near medical decision points

Quality Assurance

- System Suitability checks
- Interference Monitoring
- Calibration Monitoring
- Ongoing system evaluation
  - Calibration frequency
  - Column Changes
  - Instrument Correlations
  - Reagent lot changes

System Suitability Checks

- Basic chromatography parameters are evaluated with a non-extracted sample
- Criteria are defined for examination of
  - Correct peaks
  - Intensity of background
  - Analyte signal
  - Peak resolution
  - Retention time
  - Peak symmetry
- If the system has two injectors or two LC/HPLC systems they should be considered separate with separate calibrations and QC

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Interference and Calibration Monitoring

- Active monitoring for any potential interferences
- Mean ratios should be calculated each day from standards used to make construct the calibration curve (CV < 6%)
- The slope of the calibration curve should also be documented and criteria established for acceptability
- For patient samples:
  - If the qualifier ion is >50% of the quantifier ion, the ion ratio should be +/- 20% of the mean ratios of the calibration standards.
  - If the ion ratio is >20% from the mean this suggests an interference in the patient sample

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Quality Assurance: Routine Evaluation

- Calibration and tuning should be performed every 6 months or “according to manufacturer” recommendations. The lab must establish minimum frequency allowable criteria for total ion count, ion intensity, peak resolution, and mass shift.
- Instrument correlations must be performed every 6 months
- Validation of a new column should be considered a new lot change, regardless if it is the same manufacturer's lot.
  - Recommended 5 patient samples that span the AMI
  - Defining a new lot of reagent (mobile phase) is up to the discretion of the laboratory

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CAP Checklist items

- CAP Common Checklist
  - Test Method Validation/Verification
  - Laboratory Developed Tests
  - Reference Intervals
- CAP Chemistry and Toxicology
  - Chromatography
  - Mass Spectrometry
    - Operation
    - Analyte Identification
    - Matrix Effects (including ion suppression)

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CAP: Chromatography

***REVISED** 07/29/2013

CHM.16550 Calibrator/Standard Material **Phase II**

Appropriate calibration or calibration verification is performed on each day of patient testing or following the manufacturer's instructions.

NOTE: For qualitative assays, an appropriate calibrator should be run at normal and abnormal levels. For quantitative assays, a multipoint calibration may be required if the measurement has a non-linear response. For some assays, a level near the assay's limit of detection (LOD) or at critical decision point(s) is needed. For measurement systems that have a linear response verified by periodic multipoint calibration verification and AMR verification protocols, a calibration procedure that uses a single calibrator at an appropriate concentration is acceptable. Analyses based on a single point calibration must be controlled by appropriate quality control samples. In addition, inclusion of a negative control (reagent blank) is good laboratory practice.

Evidence of Compliance:

- ✓ Written procedure defining calibrators/standards appropriate for the test system used **AND**
- ✓ Records of calibration/calibration verification

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CAP: Chromatography

CHM.16650 Quality Control **Phase II**

Appropriate controls are extracted and run through the entire procedure on each day of patient testing.

NOTE: Controls used in chromatographic procedures must evaluate as much of the complete testing process as is technically feasible. The control process includes any pre-treatment, pre-purification or extraction steps, unless non-pretreated control material is inappropriate. For qualitative assays, the negative and positive controls should be at concentrations that meaningfully confirm performance below and above the decision threshold for the analyte. For quantitative assays, appropriate controls must include at least one normal sample, and at least one sample reflecting a disease range. For some assays, an additional control concentration may be useful to confirm performance near the assay's LOD, LOQ** or cut-off, if appropriate, or at a concentration consistent with highly abnormal levels that test the AMR.*

**LOD - limit of detection*

***LOQ - limit of quantitation*

If a hydrolysis step is required in the assay, the laboratory includes a control (when available) with each batch to evaluate the effectiveness of hydrolysis.

Evidence of Compliance:
- ✓ Written procedure defining QC requirements for each test system **AND**
- ✓ QC records documenting controls at defined frequency

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CAP: Mass Spec Operation

CHM.18400 Instrument Operation **Phase II**

Procedures are documented for operation and calibration of the mass spectrometer.

CHM.18600 Mass Spectrometer Tuning **Phase II**

The mass spectrometers are tuned each day of patient/client testing, or according to manufacturer's recommendations and tune records are maintained.

NOTE: Acceptable tolerance limits for tune parameters must be defined, and tune records maintained.

CHM.18700 Identification Criteria **Phase II**

The identification criteria for single stage mass spectrometry (*i.e.* GC/MS, LC/MS) are in compliance with recommendations.

CHM.18800 Identification Criteria **Phase II**

The identification criterion for tandem mass spectrometry (MS/MS) are validated and documented.

NOTE: In tandem mass spectrometry using multiple reaction monitoring (MRM) there is at least one transition monitored for the internal standard and another for the analyte.

Evidence of Compliance:
- ✓ QC and test records

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CAP: Matrix Effects (During Validation)

***REVISED** 04/21/2014*
CHM.18825 Matrix Effect Assessment **Phase II**

There is documentation of assessment of matrix effects in LC-MS test development.

NOTE: Matrix effect on analyte ionization can be in both directions: suppression or, less frequently seen, enhancement of ionization. Evaluation of matrix effect on ionization must be performed during assay development and validation, and repeated during the periodic revalidation of the assay.

Examples of evaluation protocols may include:
1. *Post Column Infusion - Constant infusion of analyte followed by injection of blank matrix specimen extracts to measure ionization response*
2. *Mobile Phase/Post Extractions Spiking - Compare response of analyte spiked into mobile phase to that of analyte spiked into blank matrix specimen extracts*

A minimum of 10 different sources of the matrix of interest should be used during ion suppression/enhancement evaluation. If the average suppression/enhancement exceeds ±25% or the %CV of the ion suppression/enhancement exceeds ±15%, the validation studies must include data to demonstrate that matrix effects do not affect assay accuracy. One approach to validating accuracy is to assess calibration curve (slope) variation with calibrators in mobile phase and different matrices.

While the above represents recognized approaches to ion suppression/enhancement, other referenced approaches may also suffice.

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CAP: Matrix Effects (Patient Samples)

***REVISED** 04/21/2014*
CHM.18850 Matrix Effect Evaluation - Patient Samples **Phase II**

The laboratory LC-MS assay procedure includes an evaluation for possible ion-suppression or enhancement in patient samples during routine testing.

NOTE: Ion suppression (or less frequently, ion enhancement) is a recognized analytical anomaly in LC-MS assays. Such suppression can lead to false negative results or poor quantitative analyses (especially near assay limit of quantitation). While difficult to predict and observe from specimen to specimen, certain precautions should be used to try to recognize when ion suppression or enhancement occurs.

Evidence of Compliance:
✓ Written procedure requiring monitoring of internal standards **OR** records of alternative methods used

- Monitor signal intensity of I.S.
- Criteria can be based on S/N ratio or abundance in QC material
- S/N ratio of 3:1 may be used as criteria for acceptability when I.S. recovery is low

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Questions?