- How does your laboratory identify possible ion-suppression or enhancement?
- How does your laboratory ensure appropriate extracted calibrator(s) are analyzed?
- When are reinjection or reanalysis procedures required?

***REVISED** 08/24/2023*

FDT.24430 Instrument Calibration **Phase II**

The laboratory calibrates the mass spectrometer and reviews calibration records for acceptability.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline - Second Edition.* CLSI Document C43-A2. (ISBN 1-56238-720-0). Clinical and Laboratory Standards Institute, 940 West Valley Road, Wayne, PA 19087-1898, USA, 2010.
2) Clinical and Laboratory Standards Institute (CLSI). *Liquid Chromatography-Mass Spectrometry Methods;* 2nd ed. CLSI document C62. Clinical and Laboratory Standards Institute, Wayne, PA; 2022.

***REVISED** 08/24/2023*

FDT.24630 Mass Spectrometer Tuning **Phase II**

The mass spectrometers are tuned as defined based on the particular platform in use, assay performance requirements, and specimen types tested.

NOTE: Instruments must be tuned at least as frequently as recommended by the manufacturer. Acceptable tolerance limits for tune parameters must be defined, and tuning records retained.

Evidence of Compliance:

✓ Records of tuning

FDT.24880 Extracted Calibrators **Phase II**

Appropriate extracted calibrator(s) are analyzed with each batch of samples.

NOTE: At least one extracted calibrator at the commonly accepted cutoff for single-point calibration, or multiple calibrators above and below the commonly accepted cutoff for multipoint calibration, must be analyzed with each run.

***NEW** 08/24/2023*

FDT.24950 Validation, Monitoring, and Annual Verification of MS Data Analysis Tools **Phase II**

The laboratory validates data analysis tools used for compound identification and quantification when first installed and after any modifications, as applicable, and verifies performance at least annually.

NOTE: Data analysis tools may be used for various processes, such as integration of targeted and untargeted peaks, evaluating acceptability of calibration and control performance, stability of baseline, calculation of ion mass ratios, discrimination of positive and negative results, and assessing risk of carryover. Data analysis tools (eg, software or code-based rules, algorithms, machine learning) used for automated data analysis must be verified using defined acceptability criteria. Version control of custom data analysis tools is required. Reassessment of lower limit of quantification (LLOQ) and other decision points may be used to ensure that a shift has not occurred due to instrument performance or another factor impacting assay performance.

Customized data analysis tools, and modifications to that software, should be appropriately documented and records should allow for tracking to identify persons that have added or modified that software. The purpose of the computer program, the way it functions, and its

interaction with other programs must be clearly stated. The level of detail should be adequate to support troubleshooting, system modifications, or additional programming.

Evidence of Compliance:

- ✓ Records of validation and revalidation after modifications **AND**
- ✓ Records of monitoring for changes to software update tools and other change impacting performance

REFERENCES

1) Vincente FB, Lin DC, Haymond S. Automation of chromatographic peak review and order to result data transfer in a clinical mass spectrometry laboratory. *Clin Chim Acta.* 2019;498(11):84-9.

*****REVISED** 08/24/2023***

FDT.25130 Identification Criteria - Mass Spectrometry Phase II

The identification criteria for analytes detected by mass spectrometry methods (eg, GC/MS, LC-MS/MS) are defined.

NOTE: For single-stage mass spectrometry, one acceptable criterion for compound identification using ion ratios is that the unknown result must have ion ratios within a prescribed acceptance or tolerance limit of calibrator ratios. This limit should be supported by either literature references or through experimental means. Such ion ratio tolerance limits may differ based on the technique applied (eg, GC/MS versus LC/MS) as well as the analyte(s) being determined (eg, compounds with mainly ions of low abundance); thus, a defined limit to cover all methods and analytes cannot be given.

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

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline - Second Edition.* CLSI Document C43-A2. (ISBN 1-56238-720-0). Clinical and Laboratory Standards Institute, 940 West Valley Road, Wayne, PA 19087-1898, USA, 2010.
2) Official Journal of the European Communities. Commission Decision implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (17.8.2002)
3) Clinical and Laboratory Standards Institute (CLSI). *Liquid Chromatography-Mass Spectrometry Methods;* 2nd ed. CLSI document C62. Clinical and Laboratory Standards Institute, Wayne, PA; 2022.

*****NEW** 08/24/2023***

FDT.25150 Matrix Effect Assessment of Mass Spectrometry Assays - Validation Phase II

There is a record of assessment of matrix effects in development and validation of mass spectrometry assays.

NOTE: Matrix effects can affect analyte ionization and performance in both directions: suppression, or less frequently, enhancement. Evaluation of matrix effects must be performed during assay development and validation.

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.*
3 *Internal standard monitoring - Evaluate trends in internal standard abundance and signal to noise ratios during an analytical run that includes blank and spiked matrix specimen extracts.*

The minimum number of different matrix sources may vary based on the matrix, analytical targets, and assay design. Associated data should be used to evaluate the impact of matrix effect on results and define appropriate acceptance criteria for each reportable analyte during routine testing of donor samples.