



TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division

Forensic Chemistry Standard Operating Procedure Manual

Liquid Chromatography/Mass Spectroscopy

23.0 LIQUID CHROMATOGRAPHY AND TRIPLE QUADRUPOLE MASS SPECTROMETRY

23.1 *Liquid Chromatography*

23.1.1 Application

Although Liquid Chromatography Mass Spectrometry (LC/MS) is primarily used as a confirmatory test for legally significant substances, the liquid chromatograph (LC) may also be used as a presumptive test for comparing retention times (RT) for unknown samples versus known working standards.

This aspect of LC/MS is particularly useful as a second test for analytes that can degrade under GC conditions as well as samples with weak concentrations that may not be adequately detected by the GC's FID.

The sections listed under header 23.1 will mainly discuss LC methodology; however, the information provided is relevant to the mass spectrometer component as well.

23.1.2 Equipment

The TBI FCU utilizes Agilent Technologies liquid chromatograph triple quadrupole mass spectrometers. Compounds of interest are separated using a variety of mobile phases and columns. These compounds are detected using a mass spectrometer. All systems are equipped with proprietary software for instrument control and data analysis.

23.1.3 Reagents and Standards

The mobile phase should be made with LC/MS grade or better solvents.

A check solution that consists of a combination of cocaine, pethidine, and hydrocodone working standards will be used for performance verification.

Working standards of legally significant substances will be available for RT comparisons.

23.1.4 Method

Only samples that have been analyzed with other instrument techniques and/or where the analyst has a reasonable idea of what compounds might be present will be analyzed on the LC/MS. Highly concentrated samples will overload the system and could result in excessive maintenance and repairs.

Samples will be extracted into the same solvent system as the starting mobile phase mixture when possible. The prepared sample will be filtered to remove any particulate matter that could clog or damage the column. Samples prepared for analysis on GC/MS can be diluted using 10 μ L of sample into 5mL of high purity water for analysis on LC/MS.

Retention time standards should be diluted using 10 μ L of sample into 2mL of high purity water for retention time comparisons.



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Samples are introduced to the mobile phase using an auto-sampler with a sample loop. The sample loop is cleaned internally by the instrument. The instrument's method will return the instrument conditions to the starting conditions.

The TBI FCU has several LC/MS methods to produce quality results for a wide variety of analytes.

23.1.5 Quality Assurance

A procedural blank using the same extraction and method as the sample(s) will be performed immediately before each casework sample and reviewed by the analyst. An acceptable procedural blank must be free of peaks resulting from over-the-counter, legend, or legally significant substances.

For RT analysis, a working standard will be run in addition to the sample for comparison. This standard will be valid for any additional samples analyzed within 24 hours of its original run time. A working standard will be run at the end of any sequence that may exceed 24 hours. A solvent blank may be run in lieu of a procedural blank before running a working standard.

Multiple instrument washes, procedural blanks, and/or high polarity or flush methods may be necessary to clear the column when samples have high concentrations or complex matrices. The analyst will review these data files, but they will not be considered part of the case file.

The sample loop flush solution and the mobile phase solvent bottles will be checked before running worklists or samples. Serious damage to the column and instrument could result if these solvents run out or go dry during a run.

Auto-sample vials will be verified against the worklist before and after a sample run.

23.1.6 Performance Verification and Acceptance Criteria

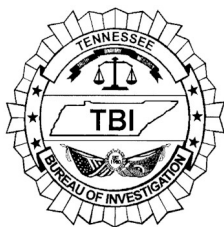
The instrument is turned on and the column is flushed with the starting mobile phase for the appropriate method that will be used. The software will indicate when the instrument is ready for use.

Maintenance will be performed if the standard does not give a satisfactory result. The instrument will be removed from service, and the unit supervisor will be notified if it continues to give unsatisfactory results for the check standard.

Refer to the manufacturer's manual for maintenance guidelines.

23.1.7 Interpretation

The sample's RT must fall within +/- 2% of the working standard's RT for the analyst to determine that it is "consistent with" the compound of interest.



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23.2 Liquid Chromatography-Mass Spectrometry

23.2.1 Application

Liquid Chromatography-Mass Spectrometry (LC-MS) is an instrumentation technique that combines the separation capabilities of LC with the compound selectivity of mass spectrometer (MS). This instrument is used in the unit for its confirmatory role in the analysis of legally significant substances.

23.2.2 Equipment

The liquid chromatograph described in 23.1.2 is combined with triple quadrupole mass spectrometers as the analyzer. This system is equipped with proprietary software for instrument control and data analysis.

23.2.3 Standards

The same check solution used in LC will be used for separation and MS performance verification.

ESI-L Low Concentration Tuning Mix from Agilent Technologies or a comparable tuning solution is the tuning standard used in the TBI FCU. It is added directly to the appropriate storage vial on the instrument as needed.

23.2.4 Method

Methodology for the LC-MS follows the same procedure as outlined in section 23.1.4. The TBI FCU has several LC-MS methods to produce quality results for a wide variety of analytes.

The data obtained from this instrument will include a total ion chromatogram (TIC) as well as a spectrum containing a distribution of ions by mass-to-charge ratio(s) unique to analyte(s) of interest.

23.2.5 Quality Assurance

Quality assurance for the LC-MS follows the same procedures as outlined in section 23.1.5.

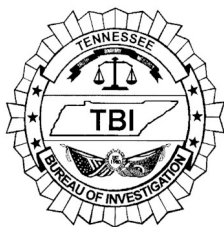
Running daily primary standards for making spectral comparisons to unknown samples is not required since all primary standards and samples are run using the same mass spectrometer parameters. Consult the instrument logbook for these parameters.

23.2.6 Performance Verification and Acceptance Criteria

Performance Verification

The MS will be tuned with the check tune program. If the check tune does not pass, then a full auto tune will be used to tune the instrument. Once the program has finished, the results need to be evaluated. The instrument must pass the instrument's tuning criteria before casework samples can be analyzed.

Any tune results that fall outside of the instrument's criteria may indicate additional maintenance is required. The analyst should follow guidelines provided by the manufacturer or as



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recommended by qualified technical support personnel when possible. If the analyst is unable to correct the problem, then a service call should be scheduled.

The LC check standard will be run on the LC-MS to complete the performance verification process. Consult the instrument log for maintenance schedules.

Acceptance Criteria

For the MS tune:

- The Overall Result for the tune must pass.
- The Measured Gain must pass for both positive and negative scan modes.
- The peak widths at the positive and negative setting must pass for normal scan speed and fast scan speed.

A valid LC component performance verification will consist of three separate and symmetrical peaks that are resolved to the baseline on the resulting TIC. The mass spectrum for each of these peaks must match to the corresponding primary standard spectrum.

If any of the above criteria are not met, the instrument will be removed from service, and the unit supervisor will be notified.

If the instrument does not pass the check tuning criteria then the analyst will perform a full auto tune of the instrument. Fresh mobile phase solvents and cleaning the ESI source might be necessary if the instrument continues to fail the tuning criteria. If the instrument cannot be cleaned or maintenance performed in a manner that leads to a successful tune, then the instrument will be removed from service and the unit supervisor will be notified.

Refer to Appendix K for maintenance requirements and intervals.

23.2.7 Criteria for Initial Evaluation

Any peak that is discernable from the baseline on the TIC will be considered valid for further evaluation and/or comparison.

23.2.8 Interpretation

The analyst will review all peaks in the TIC to ensure all legally significant compounds are identified within the sample.

The analyst will review the compound of interest's spectrum to determine if it matches the primary reference standard. The lot number and the run date of the primary standard will be noted in either the electronic library or in the primary standard spectra notebook to ensure casework traceability.