



<b>ATF-LS-E23</b> <b>Liquid Chromatography/Tandem Mass Spectrometry</b> <b>(LC-MS/MS)</b>	Published Online: <b>March 2018</b>
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## I. SCOPE

This document provides guidelines establishing a common basis for identification of analytes using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). MS/MS methods, provide enhanced fragmentation due to collisions of analyte ions with a gas target yielding more than one step of mass separation/filtration. They offer an additional level of confirmation when very specific results are required. LC-MS/MS is suitable for a broad spectrum of compounds with different polarities. For simplicity, the term liquid chromatography is used for both HPLC and UHPLC methods. Tandem instruments may also be run using single MS detection. This protocol provides the general procedures when using tandem LC-MS/MS technologies for the identification of compounds in explosives casework. Specific instrumentation, procedures, validation and identification information (including masses of relevant ions) are provided in separate method documents. In addition, detailed analytical conditions and instrumental settings for these methods are updated as necessary and kept on the instrumental parameter sheets.

## II. REFERENCES

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“Atmospheric Pressure Ionization LC/MS Methods for the Analysis of Black Powder Substitutes”, P.A. Dreifuss and J. V. Goodpaster, Proceedings of the 8<sup>th</sup> International Symposium on the Analysis and Detection of Explosives (2004) pp.168 -180.

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ISO Standard 22892 (2006) – Soil Quality - Guidelines for the identification of target compounds by gas chromatography and mass spectrometry.

“Identification and confirmation of chemical residues in food by chromatography-mass spectrometry and other techniques”, Lehotay, S.J., Mastovsak, K., Amirav, A., Fialkov, A.B., Alon, T., Martos, P.A., de Kok, A., Fernandez-Alba, A., Trends in Analytical Chemistry, (2008), Vol. 27, No. 11, 1070-1090.

### III. APPARATUS/REAGENTS

LC-MS/MS systems come in a variety of configurations. The mass spectrometer and its ionization probe, the chromatography system, column, and eluents and required standards are given in specific methods or parameter sheets.

### IV. SAFETY PRECAUTIONS

- Pump exhaust should be vented to a fume hood or other vent whenever facilities permit.
- Used pump oil may contain chemicals that could pose potential health hazards and should be disposed of properly.
- All gas cylinders should be properly secured and stored. Pressure regulators should be inspected whenever the cylinders are replaced.
- Avoid reaching into an autosampler compartment while it is running.
- Operators should exercise care when cleaning heated components.
- Maintenance or inspection of electrical circuits is to be performed by or under the supervision of operators familiar with the specific hazards.
- Lifting of heavy equipment (pumps, etc.) should be performed by at least two operators.
- The hazards of chemicals and solvents must be understood before working with them.
- The waste solvent containers should be emptied when full and solvents should be disposed of properly.

### V. PROCEDURES

Aqueous sample extracts should be diluted with running solvents/buffers when possible. Filtration of samples with disposable syringe filters is recommended, particularly for UHPLC where pore sizes of 0.22  $\mu\text{m}$  or smaller are preferred. Instrumental conditions and procedures are given in the individual methods.

### VI. QUALITY ASSURANCE AND CONTROLS

To ensure that the chromatography and mass spectra data will be of acceptable quality, checks on instrumental performance should be completed prior to running a sample. These include, tuning the instrument, mass calibration, analysis of the background spectrum, analysis of a blank sample and a reference standard. The frequency of tuning and calibration is instrument dependent and will be given in the method procedures and/or work instructions. A reference standard will be analyzed before and after the analysis of casework exhibits and a copy of one of the standards should be kept near the instrument. Newly acquired data should be compared with previously acquired data. The reference

standard data should be examined to determine if the sensitivity/response, peak shape and retention times are satisfactory as outlined in the method and/or work instructions.

#### *Parameter Sheet and Allowance for Variation*

A copy of the parameter sheet detailing the instrument and analytical settings used in case work must be included in the case jacket. This will include at a minimum the date and instrument used, the ionization type and polarity, reagents and eluents, column, injection volume and chromatographic and mass spectrometer settings/parameters. Minor adjustments to instrument settings are common in LC-MS/MS.

#### *Identification*

Criteria for retention times, percent relative abundances of ions (RA), and acceptable signal to noise levels (S/N) are determined during method development. Because variation in LC-MS/MS is common, contemporaneous comparison of sample retention times and mass spectra (including RA and S/N) to a reference is required for identification. The absence or presence of significant ions (as determined by their relative abundance) in a sample which is inconsistent with the reference standard will prevent an identification unless it can be explained comprehensively. For example, ions from filters and solvents may be explained in the case jacket and not prevent an identification. If possible, the molecular ion or an adduct (pseudo-molecular ion) should be selected as a diagnostic ion, where the term diagnostic ion indicates an ion that provides information about a compound's structure.

Identification points include retention time, diagnostic ions with the correct RA and S/N, information from other detectors such as UV data, and other known sample information. The number of points and/or ions required for identification is specific to a method, but should not be less than 4.

Sample information that can be considered an identification point includes physical and chemical properties. For example, in the case of a yellow, viscous liquid which yields only two diagnostic ions and a retention time matching that of nitroglycerin, the examiner may identify nitroglycerin based on 4 identification points; the retention time and two characteristic ions in the MS matching in relative abundance to a reference compound, and the physical characteristic of the material. If the physical properties of the sample are not known, for instance in post blast samples, additional identification point(s) are required for samples having only two structurally significant ions and a retention time matching a reference. Additional identification points may be attained through alternate ionization and/or chromatographic methods or analysis on another instrument. When high resolution mass spectrometry is used ( $\leq$  a few ppm, such as a time of flight instrument) two identification points may be assigned for each diagnostic ion in the correct relative abundance to a standard. Regardless of the number of diagnostic ions or identification points obtained, comparison of both the retention time and the ions to a reference standard is required.

#### VII. GLOSSARY

Atmospheric Pressure Chemical Ionization (APCI) - an API ionization technique used in LC-MS, generally for less polar compounds. Analytes are ionized by plasma (ionized gas) using argon or helium gas.

Atmospheric Pressure Ionization (API) - the term used to describe LC/MS or LC-MS/MS ionization methods such as Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI).

Calibration - electronic adjustment of a mass spectrometer's analyzer to measure the correct m/z values.

Duty Cycle – the fraction or percentage of ions of a particular m/z produced in the source which is effectively analyzed. This is generally the proportion of time the analyzer spends analyzing this value of m/z. The duty cycle is dependent on the mass analyzer and its mode of operation.

Electrospray Ionization (ESI) - an API ionization technique used in LC-MS, generally for more polar analytes. Analytes are ionized by desolvation in solution.

Enhanced Duty Cycle (EDC) - synchronization of ion arrival with the voltage pulse into the time-of-flight mass analyzer, which is possible on an oa q-TOF mass spectrometer. This can produce up to ten times the signal enhancement over a region surrounding a selected mass.

Liquid Chromatography-Mass Spectrometry (LC-MS) - interfacing of a liquid chromatograph with a mass spectrometer. The term LC-MS/MS is used when the mass spectrometer is a tandem instrument

Mass Spectrum - a plot of ion concentration versus mass-to-charge ratio for a single mass spectrometer scan or series of scans, reflecting scan summation or background subtraction.

Quadrupole - a common mass analyzer used in mass spectrometry to analyze the mass-to-charge ratio (m/z) of ions produced in the ion source. The quadrupole consists of four metal rods which carry radio frequency and electric fields which are varied in such a way to sequentially and rapidly transmit ions of known mass. Ions can be detected at that point or pass onto another mass analyzer.

Retention Time (RT) - the time which it takes a component to travel through a GC or LC column.

Reverse Phase (RP) Liquid Chromatography- form of liquid chromatography where the stationary phase is non-polar. Historically, RP was done on a silica or alumina phase

Selected Reaction Monitoring (SRM) - a targeted mass spectrometry technique performed on triple quadrupole instruments in which collision-induced dissociation is used as a means to increase selectivity. In SRM, two mass analyzers are used as static mass filters, to monitor a particular fragment ion of a selected precursor ion. The specific pair of m/z values associated to the precursor and fragment ions selected is referred to as a "transition" and can be written as parent m/z > fragment m/z (e.g. for TNT: 227>152, 197, 210 ). No mass spectra are recorded in a SRM analysis. Instead, the detector acts as counting device for the ions matching the selected transition thereby returning an intensity value over time.

Tandem mass spectrometry (MS/MS) - Mass analysis performed on a mass spectrometer resulting from the interfacing of similar or different (hybrid) types of mass analyzers to enhance specificity of an

analysis. The most common form of MS/MS is product ion analysis which produces a spectrum resulting in product ions produced from an ion window selected by the first analyzer.

Triple Stage Quadrupole (TSQ) - A tandem mass spectrometer in which both mass analyzer and the refocusing element in the collision cell are all quadrupole.

Tune - adjustment of mass spectrometer lens voltages and other parameters to maximize and normalize ion production, transmission and detection.

Ultra-High Performance Liquid Chromatography (UHPLC or UPLC) – form of liquid chromatography using columns packed with smaller particles and/or using higher flow rates for increased speed, superior resolution and sensitivity.