



<b>ATF-LS-E23.1</b> <b>Analysis of Explosives by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) Utilizing a Triple Quadrupole Mass Spectrometer</b>	Published Online: <b>March 2018</b>
Authority: Technical Leader	
Unofficial Copy; May Not Be Most Current Version	Page: 1 of 4

## I. SCOPE

This method is applicable for the detection of commonly encountered high explosives by use of Liquid Chromatography - Mass Spectrometry (LC-MS), Tandem Mass Spectrometry (MS/MS), and Selective Reaction Monitoring (SRM) on a triple quadrupole mass spectrometer. An Electrospray Ionization (ESI) or Atmospheric Pressure Chemical Ionization (APCI) ion interface is employed in the negative or positive ion mode.

For the analysis of high explosives, including: cyclotetramethylene tetranitramine (HMX), cyclotrimethylene trinitramine (RDX), pentaerythritol tetranitrate (PETN), 2, 4, 6-trinitrotoluene (TNT), 2, 4-dinitrotoluene (2, 4-DNT) and nitroglycerine (NG), the operating conditions are listed in the parameter sheet. The same conditions can be applied to solutions of these explosives or to extracts, including post-blast evidence. Other high explosives, such as triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD) could be analyzed by applying similar conditions.

## II. REFERENCES

“Highly Sensitive Screening Method for Nitroaromatic, Nitramine and Nitrate Ester Explosives by High Performance Liquid Chromatography-Atmospheric Pressure Ionization-Mass Spectrometry (HPLC-API-MS) in Forensic Applications”, Xiaoma XU, Anick M. Van de Craats and Peter C.A.M. de Bruyn, J. Forensic Sci. Nov.2004, Vol 49, No.6, pp 1171-1180.

“Rapid Forensic Selected Reaction Monitoring Liquid Chromatography/Mass Spectrometry Determination of Ionophore Antibiotics Found at Toxic Levels in Animal Feeds”, J. G. Ebel, T Wachs and J. D. Henion, J. of AOAC. Internat. (2004) 87 pp 25-30.

“Investigation of an Enhanced Resolution Triple Quadrupole Mass Spectrometer for High-Throughput Liquid Chromatography/Tandem Mass Spectrometry Assays”, L. Yang, W. M. Winnek, A. E. Schoen, H. Scheingruber, L. Mylchreest and P. J. Rudiewicz, Rap. Comm. Mass Spectrom. (2002) 16 pp 2060- 2066.

“Development of an LC/MS method for the trace analysis of triacetone triperoxide (TATP)”, Leo Widmer, Stuart Watson, Konrad Schlatter and Andrew Crowson, Analyst, 2002, 127, pp 1627–1632.

## III. VALIDATION

LC-MS, like other mass spectrometry methods, is a well-known and scientifically accepted method for the confirmation of organic components. The method described in this protocol uses MS/MS to make (M-H)<sup>-</sup>, (M)<sup>-</sup> or fragment adducts ions of explosives. The Selected Reaction Monitoring (SRM) technique which is employed in this method is frequently cited for its very high specificity and sensitivity. A minimum of three mass transitions are monitored chromatographically and all of the ions are inspected for correct retention time and for their relative abundances including that of the precursor ion.

Extensive in-house testing of the method with known explosives, blind samples and authentic casework extracts have confirmed its validity. Extracts of casework evidence were screened by EGIS and confirmed by LC-MS/MS, producing consistent results.

#### IV. APPARATUS/REAGENTS

##### Liquid Chromatograph:

A liquid chromatograph (LC) system, such as Thermo Scientific "Accela 1250", an autosampler module and column oven. PEEK® tubing is used for column connections and for transfer line to the MS.

Thermo Scientific "Hypersil Gold Phenyl" 50mm long, 2.1mm ID and 1.9um particle size column or equivalent analytical LC column is used. The column can be run at ambient conditions or may be temperature controlled.

Mobile Phase: Suitable acetate or formate in water or methanol.

Recommended injection solvent is similar to the initial mobile phase composition.

##### Mass Spectrometer:

A triple quadrupole mass spectrometer system with ESI or APCI probes which can be operated in negative or positive ion mode, such as Thermo Scientific "Finnigan TSQ Quantum Ultra"

The data system software for controlling and operating the LC, MS and autosampler which is also capable of plotting chromatograms and spectra for multiple ion transitions.

##### Standard Solutions, Reagents and Supplies:

Standard solutions of explosives are obtained from Accustandard (New Haven, CT), Cerilliant (Round Rock, TX) or equivalent.

High purity nitrogen gas for the mass spectrometer API sheath and auxiliary gases is supplied by a nitrogen generator (PEAK Scientific or equivalent) or a cylinder.

Ultra High Purity (UHP) Argon for the collision gas is supplied by connecting the gas line to a UHP argon cylinder (Nexair or equivalent).

LC/MS grade water (such as that purified by a Millipore Milli-Q® Gradient A10 water purification system or Fisher Scientific Optima LC/MS® water).

LC/MS grade or higher methanol (such as Fisher Scientific Optima LC/MS® methanol).

99.999% ammonium acetate (Catalogue #: 372331-100G from Sigma Aldrich) or equivalent.

1,3,6-Polytyrosine calibration standard available from Thermo Electron (catalogue #: 00301-22925) or another source.

##### Making of LC Mobile Phases and Standard:

4mM ammonium acetate methanol - add 308mg of ammonium acetate in 1000mL of LC/MS grade methanol.

4mM ammonium acetate water - add 308mg of ammonium acetate in 1000mL of LC/MS grade water.

Adjusted concentration and/or substitutions (such as with ammonium formate) may also be used.

A standard mix of known concentration of HMX, RDX, NG, 2,4-DNT, TNT and PETN in the range from 200 to 480 pg/μl (ppb) should be prepared for use with the method. Appropriate substitution could be made when another analyte is identified.

## V. PROCEDURES

### Tuning

The TSQ Quantum Ultra mass spectrometer tuning is extremely stable when operating in ESI or APCI mode. This is accomplished either manually or automatically using 1, 3, 6 - Polytyrosine calibration standard or other similar calibrant. Periodic adjustments of tuning can also be made as determined by the primary operator. Tuning must be done after periodic maintenance, major instrument repair or at least once a year by a primary operator or the manufacturer's engineers. Documentation of the all tuning and periodic adjustments should be made in an instrument logbook.

### Acceptable conditions

A mixed explosives standard (or series of known individual standards) will be analyzed before and after the analysis of all casework exhibits. In order to monitor the performance of instrument, the standard will be evaluated and a copy of data should be kept by the instrument. Newly acquired spectra should be compared with that of a previously acquired data. The data should also be included within the case jacket. The LC-MS/MS system can be used for the analysis of samples only after good quality data of the targeted species can be produced.

### Sample Analysis

For conditioning purpose, at least ½ hour before use of the instrument, it should be turn "ON" (scanning) from standby mode and mobile phases should be flowing through the column to prime the solvent lines. For every sample prepared for mass spectral analysis related to casework, an experimental control sample will be analyzed in a similar fashion to determine potential contamination sources resulting from solvents, labware, concentration steps, or other sources. An instrument blank (50:50 water/methanol) must be run before every sample to confirm that the instrument is free from any contamination. A blank is acceptable if the mass chromatogram for the chosen ions is such that there are no peaks within the appropriate retention time (RT) window with a signal-to-noise ratio greater than 3. If any peak of interest is detected in the blank analysis then the sample immediately following it must be reanalyzed (with blank injection as appropriate). The experimental control can also be used as the instrument blank.

Operating conditions and settings of the instrument are provided in the instrument parameter sheets. A copy of method or parameter sheet detailing parameters that were used in casework must be included in case jacket. Every sample analyzed as part of casework should also be documented in an instrument logbook.

For the detection of explosives, the following masses, as determined by published work and during the initial research work are used:

APCI (negative) parameters

<u>Explosive</u>	<u>Mol. Wt.</u>	<u>Precursor Ion (m/z)</u>	<u>Transition Ions (m/z)</u>
HMX	296.16	355 (M+CH <sub>3</sub> COO) <sup>-</sup>	46, 147, 174
RDX	222.12	281(M+CH <sub>3</sub> COO) <sup>-</sup>	46, 93, 106
NG	227.09	286 (M+CH <sub>3</sub> COO) <sup>-</sup>	46, 62, 180
2,4-DNT	182.14	181 (M-H) <sup>-</sup>	105, 116, 135
TNT	227 (M) <sup>-</sup>		152, 197, 210
PETN	316.14	375 (M+CH <sub>3</sub> COO) <sup>-</sup>	46, 62, 315

ESI (negative) parameters

<u>Explosive</u>	<u>Mol. Wt.</u>	<u>Precursor Ion (m/z)</u>	<u>Transition Ions (m/z)</u>
HMX	296.16	355 (M+CH <sub>3</sub> COO) <sup>-</sup>	73, 147, 174
RDX	222.12	281(M+CH <sub>3</sub> COO) <sup>-</sup>	46, 82, 93
NG	227.09	286 (M+CH <sub>3</sub> COO) <sup>-</sup>	46, 62, 86
2,4-DNT	182.14	181 (M-H) <sup>-</sup>	46, 116, 135
TNT	227.13	226 (M) <sup>-</sup>	46, 104, 196
PETN	316.14	375 (M+CH <sub>3</sub> COO) <sup>-</sup>	46, 62, 109

The retention time (RT) of peaks observed in the sample chromatogram is compared to the chromatogram of the targeted explosive standard. If the RT and spectral data acquired correspond to that of a standard explosive then explosive detected in the sample is reported.

To report a positive identification of an explosive the sample should meet the criteria as specified in ATF-LS-E23.

Control sample and solvent blank immediately preceding the sample is clean from analyte of interest. LC-MS/MS analyses meeting the above criteria can serve as standalone positive identification for the presence of an explosive.

Instrument and Periodic Maintenance

All instrument maintenance which is necessary for proper instrument performance, such as column replacement, pump oil replacement, ion source or analyzer cleaning will be documented in an instrument maintenance log. The log will also document the maintenance and symptoms from any electrical component failures which might occur.