



<b>ATF-LS-E23.2</b> <b>Liquid Chromatography-Mass Spectrometry (LC-MS)</b> <b>Method: Analysis of Explosives on an Orthogonal</b> <b>Acceleration q-TOF Mass Spectrometer</b>	Published Online: <b>March 2018</b>
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## I. SCOPE

This method covers the use of ultra-high performance liquid chromatography (UHPLC) - mass spectrometry (MS) and tandem mass spectrometry (MSMS) in explosive casework on an orthogonal acceleration (oa), quadrupole-time-of-flight (q-TOF) mass spectrometer.

Specific conditions are provided for the analysis of five high explosives, including: cyclotetramethylene tetranitramine (HMX), cyclotrimethylene trinitramine (RDX), pentaerythritol tetranitrate (PETN), 2, 4, 6-trinitrotoluene (TNT) and nitroglycerin (NG). It can be applied to solutions of these explosives or to extracts suspected to contain them. It can also be applied with or without the use of an ancillary photodiode array (PDA) detector. The protocol does not limit the adjustment of parameters.

## II. REFERENCES

“Accurate Masses and Structure Confirmation”, M.L. Gross, J. Am. Soc. Mass Spectrom., (Feb 1994) 5, p.57

“Orthogonal Acceleration Time-Of-Flight Mass Spectrometry”, M. Guilhaus, D. Selby, and V. Mlynski, Mass Spectrometry Reviews, 2000, 19, pp 65 – 107.

“Guidance for Industry/ Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues/ FINAL GUIDANCE”, U.S. Department of Health and Human Services Food and Drug Administration Center for Veterinary Medicine May 1, 2003

<http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052658.pdf>

“Applications of a Travelling Wave-Based Radio-Frequency-Only Stacked Ring Ion Guide”, K. Giles, S. D. Pringle, K. R. Worthington, D. Little, J. L. Wildgoose and R. H. Bateman, Rapid Commun. Mass Spectrom. (2004) pp. 2401-2414

“Ultra Performance Liquid Chromatography (UHPLC): An Introduction”, M. Schwartz, Separation Science Redefined May 2005, pp 8-14.

“Comparison of Two High Explosive LC/MSMS Methods”, P.A. Dreifuss and R. A. Strobel, Proceedings of the 9th International Symposium on the Analysis and Detection of Explosives (2007)

## III. VALIDATION

The following were evaluated over several years at the FSL-W: enhanced duty cycle, targeted compound analyses using multiple time-based, functions, temperature parameters, cone voltage parameters, RF parameters, ESI spray parameters; alternative collision gases and their gas flow rates. Validation using standards, blind samples and casework was completed. Results were compared to those obtained by EGIS, a triple quadrupole method and GC-MS. All data used in the validation study is preserved in two binders, entitled, Method Development Waters q-TOF Mass Spectrometer and q-TOF Premier Alternative Collision Gases. Casework extracts analyzed by LC-MS and GC-MS produced consistent results, with LC-MS providing much greater resilience to the deleterious effects of real life matrices than GC-MS.

#### IV. APPARATUS/REAGENTS

- Waters oa q-TOF Premier mass spectrometer with ESI and APCI probes and MassLynx 4.1
- Waters Acquity Binary Solvent Manager, Waters Acquity Sample Manager
- Waters eλ (eLambda) Detector Diode Array Detector (optional)

#### Standard Solutions, Reagents and Supplies:

<u>Item</u>	<u>Suggested Supplier(s)</u>
• Explosive stock solutions, such as HMX, RDX Accustandard (New Haven, CT) NG, TNT, PETN.	Cerilliant (Round Rock, TX)
• Zero grade or equivalent N2 gas Roberts # RLN380	Roberts Oxygen, Rockville, MD
• Screw top vials with pre-slit septa	Waters, #186000327DV
• HPLC solvents	Riedel-deHaen Chromasolv
• 99.999% ammonium acetate	Sigma Aldrich
• Sodium formate mass scale calibrant	Waters #700002647-6

#### Instrumental Parameters:

The parameters given below are a good starting point for an explosives mixture of HMX, RDX, NG, TNT and PETN. The parameters for this analysis can be varied and the parameters used will be kept in the case jacket. Changes in sensitivity, spectral relative abundances and chromatographic separation are acceptable if the requirements in Quality Assurance/Quality Control section below are achieved.

Chromatography conditions:

Column: Waters Aquity UPLC BEH Phenyl 1.7 $\mu$ , 2.1 x 100 mm	Solvent A: 85:15 Water:Methanol with 10 mM ammonium acetate; Solvent B: 25:75 Water:Methanol with 10 mM ammonium acetate
Column Temperature: 55°C	Linear gradient: 37.5% B ( 0 min) to 93.8% B (4 min) 100% B (5.15 min), 50% B (5.25 min), 50% B (8 min)
Sample Manager Temperature: 8°C	Flow rate = 400 $\mu$ L/min
10 $\mu$ L loop – injection volume varies, typically 1 $\mu$ L, set to Partial loop with needle overfill.	Weak solvent wash: 20% methanol Strong solvent wash: 80% methanol Seal Wash: 50% methanol

Mass Spectrometry Parameters:

Negative ion Electrospray	Desolvation Temperature: 215C												
V Optics, resolution $\geq$ 11,000	API gas: N <sub>2</sub>												
Collision gas: N <sub>2</sub> @ 0.60 mL/min or He @1 mL/min or Ar @0.32 mL/min	Data collection (for all functions): m/z 30-500 in 0.3 seconds, interscan delay of 0.05 seconds, analysis time of 5 minutes												
LM and HM resolution: 4.7 and 15 (provides ca. $\pm$ 3 Dalton set mass window)	Cone flow rate: 25 L/hr												
Source Temperature: 100C	Capillary voltage: 0.5 kV												
RF settings are set to manual using the profile: <table border="1"><thead><tr><th>Mass</th><th>Dwell Time</th><th>Ramp Time</th></tr></thead><tbody><tr><td>40</td><td>30</td><td>10</td></tr><tr><td>100</td><td>30</td><td>30</td></tr><tr><td>200</td><td>-</td><td>-</td></tr></tbody></table>	Mass	Dwell Time	Ramp Time	40	30	10	100	30	30	200	-	-	Desolvation Temperature: 275C Desolvation flow rate: 600 L/hr
Mass	Dwell Time	Ramp Time											
40	30	10											
100	30	30											
200	-	-											

The method is typically divided into six method functions (one for each explosive analyzed and one to serve as an optional general MS scan window).

The following table describes some suggested parameters for each function when nitrogen is used as collision gas.

Function	Description	Scan Type	Set Mass	Cone Voltage	Collision Energy	EDC* Mass
1	General	MS	-	0.5	0	180
2	HMX	MSMS	355	20	7.7	300
3	RDX	MSMS	281	13	5.5	221
4	NG	MS	-	5.5	0	180
5	TNT	MSMS	226	35	16	185
6	PETN	MSMS	375	7	1	350

\*EDC = enhanced duty cycle

For the use of helium as a collision gas, the following conditions have been used:

Function	Description	Scan Type	Set Mass	Cone Voltage	Collision Energy	EDC Mass
1	General	MS	-	15	0	-
2	HMX	MSMS	355	20	6.5	300
3	RDX	MSMS	281	11	5	221
4	NG	MSMS	286	7	5	180
5	TNT	MSMS	226	35	15	226
6	PETN	MSMS	375	14	1	315

Nitrogen provides good fragmentation for most explosives. Nitrogen does not provide ions higher than m/z 180 for NG and MSMS scans on NG are best performed using helium. While best for NG, helium often does not exhibit sufficient fragmentation to identify other explosives.

## V. PROCEDURES

### Tuning and Calibration

A Waters engineer will tune and calibrate the instrument during the annual periodic maintenance. If exact mass measurements are not required this tuning and calibration is sufficient for at least one year as the spectrometer is extremely stable. A trained primary operator can also perform these tasks. Tuning and calibration in the 1000 mass range is done with sodium formate or another calibration material for which the software contains reference tables. A recalibration of the instrument is recommended (not required) midway through the year. Periodic adjustments of tuning may be desirable when low levels of an explosive are anticipated. Documentation of all tuning, calibrations and adjustments should be made in the instrument maintenance logbook and the parameter sheet should be updated.

### Acceptable conditions

An analysis of a mixed standard of explosives will be done before and after the analysis of all casework (in some case, a series of individual standards may be substituted). The method can be used for the analysis of samples after good quality spectra and chromatography of the targeted species can be produced.

### Casework Analyses

Methanol/water mixtures provide the best results. Dilution of methanol samples and standards with water to approximately 50% methanol will provide the best chromatography. For best retention time reproducibility, it is recommended that the solvent used match that of the standards.

For every preparation or extraction of a case exhibit a “Materials Control” sample representative of its production will be run. Solvent blanks must precede standards, samples and material controls. A solvent blank can be 100% methanol or an approximately 50/50 methanol/water solution.

For negative ion electrospray with nitrogen as the collision gas the following ions are monitored:

Explosive	Ions
HMX	355 [M+CH <sub>3</sub> COO] <sup>-</sup> 221 [M-H-CH <sub>2</sub> NNO <sub>2</sub> ] <sup>-</sup> 174 147 [M-H-2CH <sub>2</sub> NNO <sub>2</sub> ] <sup>-</sup>
RDX	281 [M+CH <sub>3</sub> COO] <sup>-</sup> 221 [M-H] <sup>-</sup> 106 [NNO <sub>2</sub> + NO <sub>2</sub> ] <sup>-</sup> 93 [2NO <sub>2</sub> + H] <sup>-</sup> 46 [NO <sub>2</sub> ] <sup>-</sup>
NG	180 [M-HNO <sub>2</sub> ] <sup>-</sup> 93 [2NO <sub>2</sub> + H] <sup>-</sup> 86 62 [NO <sub>3</sub> ] <sup>-</sup>
TNT	227 [M] <sup>-</sup> 226 [M-H] <sup>-</sup> 196 [(M-H)-NO] <sup>-</sup>
PETN	375 [M+CH <sub>3</sub> COO] <sup>-</sup> ; 315 [M-H] <sup>-</sup> 62 [NO <sub>3</sub> ] <sup>-</sup>

### Instrument and Periodic Maintenance

All instrument maintenance necessary for proper instrument performance such as column replacement or cone cleaning will be documented in an instrument maintenance log.

### Quality requirements for identification

The identification requirements listed below follow guidance provided for MS identifications found in “Guidance for Industry/ Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues/ FINAL GUIDANCE”, listed in the references. In LC-MSMS product ion spectra, a structurally significant ion is any ion including the precursor ion with a relative abundance exceeding ca 3 %, except for isotopes. For LC-MS (without MSMS) spectra, a structurally significant ion is any ion with a relative abundance exceeding approximately 10%, except for isotopes

1. A reconstructed ion chromatogram (extracted ion profile) of a structurally significant ion or the sum of a series of structurally significant ions should exceed a signal-to-noise (s/n) threshold of 3:1.
2. In order to report a positive result, the retention time of a targeted peak(s) shall agree with that of the standard within 5%.
3. All structurally-specific ions should be present, except those whose relative abundances (RA) are close to the lower limit of detection (for example product ions below m/z 196 in the LC-MSMS spectra of TNT). These ions do not need to be detected as long as there are at least three structurally significant ions in both the unknown and standard. Minor deviations from this requirement are acceptable if justification is given in the case jacket.
4. The spectra from unknowns should visually match the spectra from the standard. There should be a general correspondence between the RA for the unknown and standard (for example plus/minus 10% of each ion RA).
5. Solvent blanks immediately preceding an unknown analysis and material controls must be devoid of these responses from the component of interest.