

THE NEW ROLE FOR LC/MS AND LC/MS/MS IN EXPLOSIVES INVESTIGATIONS

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INTRODUCTION

Liquid chromatography/mass spectrometry (LC/MS) is an instrumental technique that has been applied to the analysis of explosives for many years. Our laboratory has used LC/MS for explosives testing for approximately 10 years. Older LC/MS instruments provided detection limits comparable to the conventional HPLC/UV method (EPA Method 8330) and were more selective. However, because of the comparatively higher cost, the primary role for LC/MS was confirmation of samples in which interfering chemicals made interpretation of HPLC/UV results difficult. This is changing. Advances in LC/MS instrumentation afford limits of detection 10-20 times or more lower than before. This combined with lower risk-based PRGs, new regulatory concerns, and encroachment of communities into the immediate vicinity of military sites, has created a new role for LC/MS as the primary method for definitive analysis in some explosives investigations. Recent examples will be presented in which a common military site contaminant and biogenic substances created analytical difficulties effectively solved by LC/MS and LC/MS/MS.

Most explosive compounds or explosive related compounds have unique physical and chemical characteristics that make them unsuitable for analysis by the conventional EPA gas chromatography (GC) methods. They are relatively polar, but have low solubility in water. They have a low vapor pressure, but tend to be heat labile, i.e., they tend to breakdown at temperatures typically used in GC injectors. Liquid chromatography (LC) methods are well suited to the analysis of polar, non-volatile, and heat sensitive compounds. In general, LC can be used for analysis of a much wider range of compounds than is possible by GC methods. As a result, the standard method for analysis of explosives has been EPA Method 8330, which uses dual column high pressure liquid chromatography (HPLC) with an ultraviolet (UV) detector. The method detection limits (MDLs) for water samples analyzed by Method 8330 are approximately 0.1 to 0.2 ug/L for most compounds.

Another HPLC instrumental technique that has been used for many years is LC/MS with a thermospray interface, EPA Method 8321. Using the thermospray interface, the older mass spectrometers, produced MDLs roughly equivalent to those obtained by Method 8330, 0.1 to 0.3 ug/L. The advantage to the LC/MS technique is that the mass spectrometer is a much more selective detector. It can resolve low concentrations of the explosive compounds in the presence of co-contaminants and interferences that defeat analysis by UV detector, whether fixed wavelength or the more modern diode array (DAD). Using tandem mass spectrometers (LC/MS/MS), it is possible to detect compounds while establishing information about the chemical structure of the compounds being tested, which is not possible with a UV detector. However, LC/MS equipment is more expensive than HPLC/UV, the equipment requires considerably more maintenance than the standard HPLC/UV, and the level of training and experience required of the operator is much higher. As a result, the cost of an analysis for explosives by LC/MS is higher than by HPLC/UV, and so LC/MS was most often relegated to the role of confirming HPLC/UV results for difficult samples. HPLC/UV was invariably the method of choice for definitive analysis explosives where interferences were not a significant problem.

New LC/MS Capability

The situation is changing in part because of the development of commercial LC/MS equipment using electrospray and/or atmospheric pressure chemical ionization (APCI) interfaces. These interfaces apply less energy to the spray coming out of the HPLC column, i.e., they use a softer ionization technique, which can result in less fragmentation of the compounds being analyzed. Unlike the older thermospray interface, the newer interfaces produce the molecular ion or adducts of the molecular ion for the explosives at greater than 90% abundance. Modern LC/MS/MS equipment is also more efficient in transporting the ions created at the interface into and through the mass spectrometer, which makes analysis at the second quadrupole more efficient. The result is that the sensitivity of LC/MS and LC/MS/MS for the analysis of explosive compounds has recently improved by a factor of ten to twenty times. LC/MS and LC/MS/MS instruments are now capable of detecting explosive compounds at levels more than an order of magnitude below levels that can be detected by the standard HPLC/UV method. This is apparent in the example in Figure 1, which shows HPLC/UV and LC/MS chromatograms for a 10 ug/L calibration standard containing HMX and RDX. The same C18 column was used on both instruments. The LC/MS signal for both HMX and RDX are well above background noise levels, whereas the HPLC/UV signal is marginally above background levels.

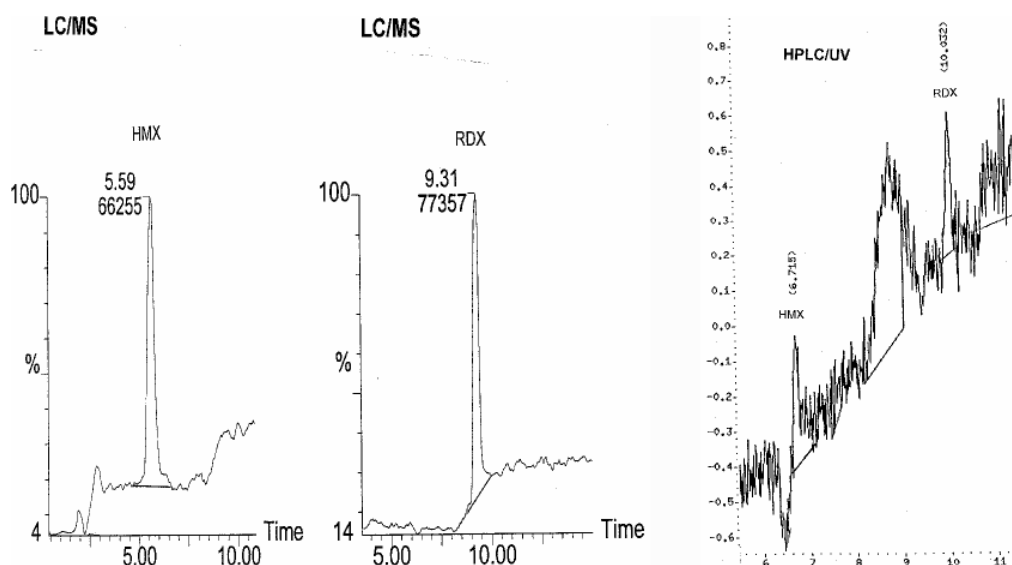


Fig. 1 LC/MS vs. HPLC/UV (10 ug/L HMX / RDX Standard)

New Risk Levels

The role of LC/MS in explosives investigations is also being driven by new risk levels. Through the 1990s new toxicology data was made available to the EPA resulting in lower risk levels assigned to tap water and groundwater sources that might be drinking water sources. As an example, the EPA Region IX Preliminary Remediation Goal (PRG) for 2,4-dinitrotoluene was changed from 73 ug/L to 0.099 ug/L, a 740 times decrease from earlier years. Toxicological studies are incomplete for many compounds, particularly the amino and nitroso breakdown products of the most commonly used explosives, TNT and RDX. At some military sites, remedial action levels are being set at these new lower risk levels for the conventional explosive compounds and levels are being considered for others. As shown in

Figure 2, there are perhaps seven explosives compounds with levels of interest for remedial investigation studies that are below the quantitative limits of HPLC/UV and the older LC/MS technology. In the case of at least three of the compounds (2,4-dinitrotoluene, 2,6-dinitrotoluene, and 1,3-dinitrobenzene), current risk levels are below limits of detection for the HPLC/UV and older LC/MS technology.

Risk-based water cleanup levels
vs
Sensitivity for LC/MS & HPLC/UV
(in order by concentrations of concern)

Risk/Cleanup Criteria				--Methods of Analysis--	
EPA Region IX Tap Water PRG 11/22/00 update (ug/L)	USEPA IRIS 1x10 ⁶ Risk (ug/L)	USEPA DW Sources 1x10 ⁶ Risk (ug/L)	1999 Cleanup Levels (ug/L)	LC/MS SPE / 8321A MDL (ug/L)	HPLC/UV SPE / 8330 MDL (ug/L)
1800	---	---	602	0.02	0.1
1100	---	---	361	0.02	0.06
---	---	---	---	0.02	0.8
---	---	---	168	0.01	0.1
---	---	---	120	0.02	0.2
---	---	---	120	0.02	0.1
61	---	---	118	0.02	0.08
61	---	---	118	0.02	0.2
61	---	---	118	0.02	0.2
3.4	0.35	3.5	3.5	0.02	0.1
2.2	1	---	2.01	0.05	0.08
0.61	0.3	---	0.55	0.03	0.2
---	---	---	---	0.01	0.2
---	---	---	---	0.01	0.2
---	---	---	---	0.03	0.2
0.099	0.05	0.11	0.0885	0.02	0.1
0.099	0.05	---	0.0885	0.01	0.2
0.099	---	---	1.2	0.02	0.1

Fig. 2

Notes: MDLs > 1/3 of cleanup levels are highlighted (3xMDL = ACS Limit of Quantitation)

DW = Drinking Water

GW = Ground Water

IRIS = Integrated Risk Information System

MDL = Method Detection Limit Study performed at STL Denver per 40CFR136B

MNX, DNX, & TNX preliminary levels of interest equivalent to RDX per EPA Region X PRG = Preliminary Remediation Goal

SPE = Solid Phase Extraction, EPA Method 3535

1999 Cleanup Levels = Action levels for one active Army site DNB = Dinitrobenzene

DNT = Dinitrotoluene

DNX = 1,3-Dinitroso-5-nitro-1,3,5-triazacyclohexane HMX = Octahydro-1,3,5,7-tetranitro-1,3,5-triazine

MNX = 1-Nitroso-3,5-dinitro-1,3,5-triazacyclohexane PETN = Pentaerythritol tetranitrate RDX = Hexahydro-1,3,5-trinitro-1,3,5-triazine TNB = 1,3,5-Trinitrobenzene

TNX = 1,3,5-Trinitroso-3,5-dinitro-1,3,5-triazacyclohexane

Short Case Study

For many years, the STL Denver laboratory has been involved in remedial investigation studies at an U.S. Army munitions site built in World War II. The need for environmental studies at and around the site was recognized in the 1980s and earlier. Regulatory actions to investigate the full extent of contamination were delayed 15 years due, in part, to concerns with unexploded ordinance (UXOs). During those years, to an extent even today, focus was on UXO detection and soil characterization related to UXOs.

As soon as EPA Method 8330 was promulgated in 1989, it became the method of choice for definitive analysis of explosives at this site. RDX and TNT were the primary compounds of concern. Colorimetric and immunoassay methods were put into use as soon as they became available (1990-1995), and they continue to be used as field screening methods.

In the mid 1990s concern about groundwater down gradient from the site began to grow in the nearby community. The community had grown over the years, and land immediately adjacent to the fence line of the site is in use. Studies found that particles of TNT remained on the surface for decades. RDX proved to be persistent and mobile once dissolved in rainwater percolating into the water table.

Action levels were lowered in the late 1990s to the levels shown in Figure 2. 8330 MDLs were then near or above levels of concern. In late 2000 state regulators began to demand quantitative results at or below action levels. As a result, in 2001 LC/MS and LC/MS/MS became the definitive method for groundwater characterization, and our laboratory was certified by the US Army Corps of Engineers for analysis of explosives using LC/MS and LC/MS/MS.

Two Example Applications

Early this year, we tested a groundwater sample from the site containing 10 mg/L of JP4 jet fuel. Figure 3 shows an HPLC/UV chromatogram for the sample alongside a chromatogram for a laboratory control sample (LCS) run immediately before the sample on the same instrument. Although peaks were detected in the first 13 minutes of the sample chromatogram and the baseline was elevated, this appeared to be due to material from the JP4 because no explosive compounds were detected. Figure 4 shows an LC/MS chromatogram for the same sample, with a peak for RDX easily detected at 0.05 ug/L.

Groundwater with 10 mg/L Jet Fuel (JP4) by HPLC/ UV

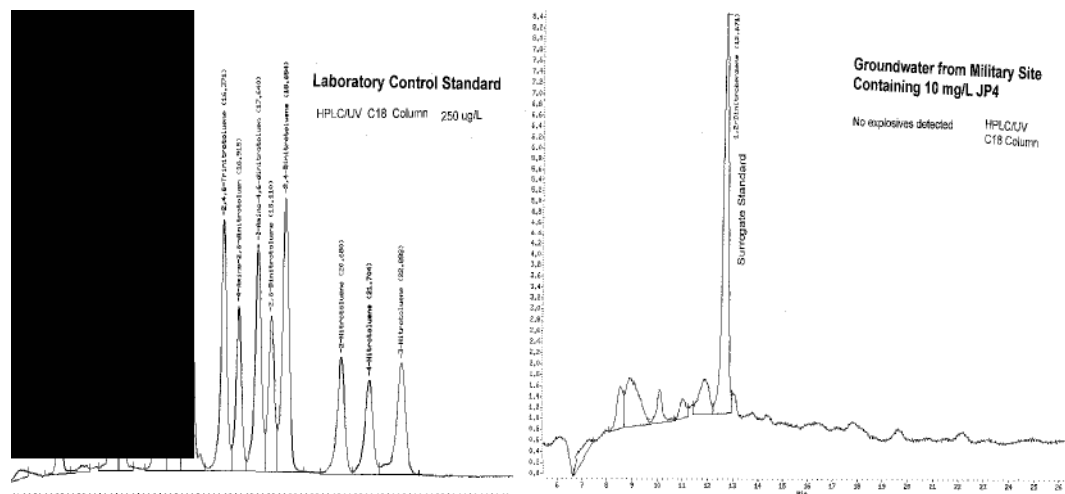


Fig. 3

GW with 10mg/L Jet Fuel (JP4) Analyzed by LC/MS

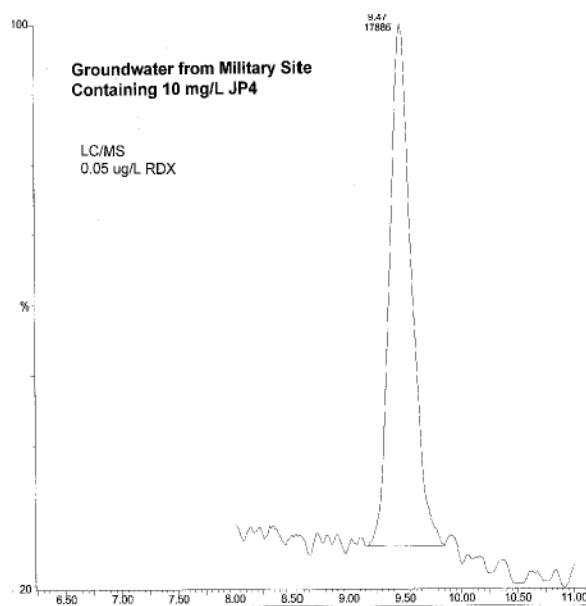


Fig. 4

Also this year, we were asked to test an acetonitrile extract from plant tissue. The extract had been prepared from a cleaned and freeze dried sample prepared by the U.S. Army Waterways laboratory. We were told that an earlier analysis using older LC/MS instrumentation had detected low concentrations of RDX and 2,4,6-trinitrotoluene (TNT) in the extract. We were asked to confirm those results.

Using an HPLC/UV we first obtained the chromatogram shown in Figure 5. A number of poorly resolved peaks were detected, but no explosives were identified. Using a Micromass Quattro Ultima tandem mass spectrometer operated with an APCI interface in the negative ion mode we obtained the single ion chromatograms shown in Figure 6. The 281 amu RDX parent ion was detected, but not the required 46.15 amu daughter ion; the RDX detection by the older LC/MS was not confirmed. However, the TNT result was confirmed. Both the parent ion and the required 109 amu daughter ion were detected. Subsequently the laboratory performed a spike addition experiment by adding a known amount of RDX and TNT to the extract and reanalyzing. The extract spiked with RDX produced a peak at 46.15 amu and a split peak pattern at 59.1 amu, indicating that the compound in the unspiked sample was similar to, but not RDX. The extract spiked with TNT produced a single peak, with a recovery of 115%, which further confirmed the presence of TNT. Surprisingly, the analysis indicated that the original dried plant tissue contained approximately 7 mg/kg of TNT.

Plant Tissue from Phytoremediation Analyzed by HPLC/UV

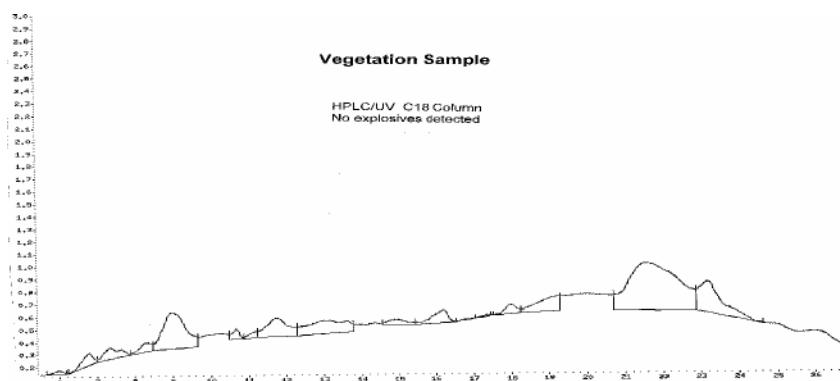


Fig. 5

Analysis of Plant Tissue by LC/MS/MS

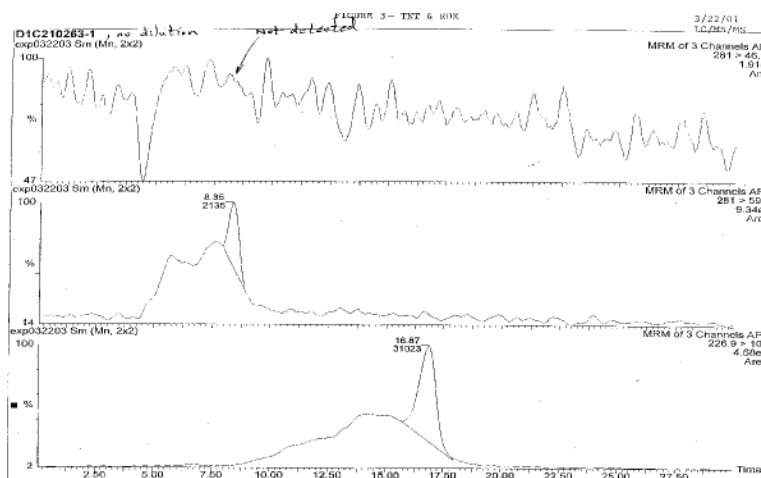


Fig. 6

Conclusion

Our laboratory continues to explore interesting and new applications of this powerful new analytical tool. Analysis of the amino and nitroso degradants of the explosives as markers for contaminant plumes is one application we are pursuing. Clearly there is a need for this more sensitive explosives compound method in groundwater studies. This need is driven by the awareness of the longevity of some explosives compounds, the growing awareness of the mobility of some explosives, new regulations, e.g., the Munitions Rule, community involvement in remedial investigations, and the new risk levels.

Obviously the older field methods and the conventional HPLC/UV method will continue to play a key role. However, our own experience has shown that LC/MS and LC/MS/MS analysis using the newer instruments are the only established techniques for difficult real-world samples requiring both a high degree of sensitivity and accuracy.