

# Qualitative Comparison of Selected Explosives in LC/MS Using APCI & APPI

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## Overview

Using a new and novel atmospheric pressure chemical ionization and photoionization combination source in negative ion mode (Figure 1), two compounds commonly found in explosives have been analyzed. The data presented here shows a dramatic increase in ion signal for both the parent ions and parent fragment ions. The increase (2x - 7x) results in a substantial gain in sensitivity, and significantly lower detection limits for this class of compounds by using APCI/APPI.



## Introduction

Electrospray ionization (ESI) and atmospheric chemical ionization (APCI) are routinely used to interface LC to MS. Neither is optimized for all compounds, and both rely on affinity methods. Affinity methods cause the analyte to either protonate or form adducts which may lead to signal suppression by compounds with higher charge affinities, such as in the presence of LC eluents. The ionization mechanism of atmospheric pressure photoionization (APPI) is quite different from that of APCI and ESI.

APPI imparts an energy that is greater than the ionization potentials of most typical compounds, while being lower than the ionization potential of air and most typical solvents. This reduces the noise factor, resulting in greater dynamic range. APPI also shows a clear linear relationship between signal and concentration with minimal charge competition effects. The principal mechanism involves molecule M undergoing photo absorption and electron ejection to form a molecular ion M<sup>+</sup>. In the presence of water vapor or other protonated solvents, the molecular ion M<sup>+</sup> attracts an H to form the MH<sup>+</sup> ion. The higher the proton affinity of the molecular ion, the more likely the MH<sup>+</sup> ion will form.

Photoionization ionizes a wide range of compounds and yields minimal fragmentation, making APPI a complementary ionization technique to APCI and ESI.

FIGURE 1:  
(+) mode APPI Ionization Mechanism

### Direct APPI



Analyte molecule M is ionized to M<sup>+</sup>



In presence of protic solvents M<sup>+</sup> may extract a H to form MH<sup>+</sup>

## Methods

Explosive samples, 2,4,6-trinitrotoluene (TNT) and 2-amino 4,6-dinitrotoluene (Figures 2 & 3) were analyzed on a Finnigan LCQ™ Deca XP Plus mass spectrometer coupled to a Surveyor® LC system. LC/MS measurements were made using the APCI/APPI combo source. LC/MS conditions were optimized for each compound in both APCI and APCI/APPI combo mode. Table 1 shows the optimized parameters for the APCI/APPI combo source.

FIGURE 2:  
2,4,6-trinitrotoluene (227 amu) structure & 2,4,6-trinitrotoluene fragment

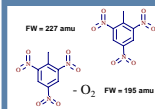


FIGURE 3:  
2-amino 4,6-dinitrotoluene (197 amu) & possible 2-amino 4,6-dinitrotoluene nitro cluster (242 amu) structure

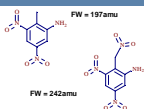


TABLE 1: Source parameters for TNT & 2-Amino 4,6-DNT in APCI/APPI mode

APCI Source Parameters		APCI Source Parameters	
Neapson Temp (°C)	400	Neapson Temp (°C)	350
Sheath Gas Flow (ml)	30	Sheath Gas Flow (ml)	25
Auxiliary Gas Flow Rate	0	Auxiliary Gas Flow Rate	0
Discharge Current	5.00	Discharge Current	4.00
Capillary Temp (°C)	200	Capillary Temp (°C)	200
Capillary Voltage (V)	-50	Capillary Voltage (V)	-57
Tube Lens Offset (V)	-18	Tube Lens Offset (V)	-60

Separation is achieved by using a Thermo Hypersil-Keystone Fluophase® PFP column (5µm 100 x 2.1mm).

Gradient program:

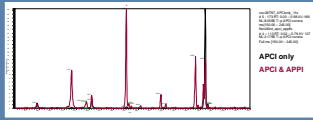
Time:	% Water	% Methanol
0.00	100	0
10.00	0	100
12.00	0	100
12.50	100	0
14.00	100	0

## Results

Upon infusion of each compound, an increase in the number of fragments of a parent ion or an increase in the actual parent ion concentration of interest can be readily seen with the APCI/APPI combination source. In the case of 2,4,6-trinitrotoluene, the addition of the APPI source creates a large signal boost for a parent fragment ion at m/z 195 (Figure 4). The large increase in signal of the m/z 195 fragment ion which arises from a concerted photoionization reaction allows for an overall signal increase (by summation of the parent ion and the fragment ion), leading to an overall increase in sensitivity of the 2,4,6-trinitrotoluene analysis.

FIGURE 4:

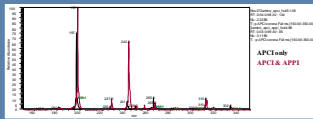
Sample infusion of 2,4,6-trinitrotoluene (5µl/min, sample concentration: 10µg/ml)



In the case of a 2-amino 4,6-dinitrotoluene infusion as shown in Figure 5, an increase in total signal can be achieved from the summation of the parent ion (m/z 197) and the parent plus m/z 45 ion (m/z 242) adduct signals. The m/z 242 ion is possibly explained by the softer ionization of the APPI allowing the detection of the 2-amino 4,6-dinitrotoluene parent attached to a nitro group (Figure 3).

FIGURE 5:

Sample infusion of 2-amino 4,6-DNT (5µl/min, sample concentration: 10µg/ml)



Comparison of a 5 ng 2,4,6-trinitrotoluene standard on column shows a substantial increase in the peak area of the m/z 227 ion in combined APCI/APPI mode (Figure 6). The additional signal (m/z 195 ion fragment) observed in the infusion experiment is not seen in the chromatography run. This is possibly due to suppression and change in surface tension caused by the presence of water as a mobile phase versus MeOH used in the infusion. Even with the disappearance of the m/z 195 ion, the parent ion still shows an increase in peak area which translates to an increase in sensitivity of the compound of interest. By comparing the APCI-only mode peak area data to the APCI/APPI combo mode peak area data, a factor of 2.2 x gain in sensitivity is observed (Table 2).

The LC/MS data for the 2-amino 4,6-dinitrotoluene sample does not show signal suppression in the nitro-adduct ion in the presence of water (Figure 7), thus both ions can be summed to give an additional boost in signal leading to an even greater increase in sensitivity. A factor of 7.4 x is shown in Table 2.

FIGURE 6:

2,4,6-trinitrotoluene ion extraction from a fullscan MS of 5 ng on column (APCI/APPI and APCI only mode).

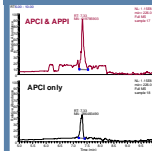


FIGURE 7:

2-amino 4,6-dinitrotoluene ion extraction from a fullscan MS of 5 ng on column (APCI/APPI and APCI only mode).

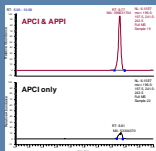


TABLE 2:

Peak area comparison between the APCI and the APCI/APPI source (Full Scan MS)

Compound	Source Type	RT	Peak Area	Enhancement
2,4,6-trinitrotoluene	APCI/APPI	7.33	619,785,903	2.2 x
	APCI	7.33	286,483,490	
2-amino 4,6-dinitrotoluene	APCI/APPI	8.81	396,631,704	7.4 x
	APCI	8.77	53,304,370	

## Conclusion

The data presented clearly shows the advantage of an APCI/APPI source over an APCI source. The presence of additional fragments and parent cluster ions allows for the summation of various ions to increase the signal and thus the sensitivity of a compound of interest. The exact contribution of the fully organic chromatographic mobile phase (as mimicked by the infusion), versus one with an aqueous component deserves further study to help explain the presence of the large signal fragment ion (m/z 195 ion in TNT).

## References

- (1) Evaluation of a Combination Atmospheric-Pressure Photo- and Chemical Ionization Source, Julie A. Horner and Rohan Thakur, Thermo Finnigan, 50<sup>th</sup> ASMS Conference 2002.
- (2) Photoionization Mass Spectrometry, Jack A. Syage et al., American Laboratory, December 2000.
- (3) Photoionization Mass Spectrometry: A Powerful Tool for Drug Discovery, Jack A. Syage and Matthew D. Evans, Pharmacometrics, August 2001.

## Acknowledgements

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