Supporting Information

Integrating the MasSpec Pen with Sub-Atmospheric Pressure Chemical Ionization for Rapid Chemical Analysis and Forensic Applications

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Supporting Methods

Chemicals

All LCMS grade solvents, including water, methanol, and acetonitrile, were purchased from Fisher Scientific (Waltham, MA). Acetic acid was purchased from Sigma Aldrich (St. Louis, MO). Cocaine and oxycodone standards, as well as their internal standards, cocaine-d3 and oxycodone-d3, were purchased from Cerilliant (Round Rock, TX). Atrazine and azoxystrobin standards were purchased from Supelco Inc. (Bellefonte, PA). Agrochemical internal standards, atrazine-d5 and azoxystrobin-d4, were purchased from HPC Standards Inc. (Atlanta, GA). All explosive compounds, including dinitroglycerin (DNG), dinitrotoluene (DNT), and trinitrotoluene (TNT) were purchased from Accustandard (New Haven, CT).

MasSpec Pen Devices

The MasSpec Pen design has been previously described in detail by Zhang, et al.[1] For all experiments, the MasSpec Pen sampling system consists of a polydimethylsiloxane probe tip, made using a 3D printed mold, with three conduits that connect at a 4mm diameter reservoir. The first conduit is connected to a syringe pump via polytetrafluoroethylene (PTFE) tubing to deliver the solvent to the pen tip reservoir, the second conduit is open to air, and the third conduit is connected to a 0.5 m PTFE transfer tube to introduce the sample into the ionization source. The PTFE transfer tubing is connected to the ionization source by means of a silicone tubing that is opened and closed using a pinch valve controlled by a microcontroller unit. The pen tip and part of the PTFE tubing are housed in a 3D printed pen case. The sampling process is started by pressing a button to activate the microcontroller unit code where first a droplet is delivered to the pen tip followed by sample extraction and the pinch valve opening to transfer the sample droplet to the ionization source by means of the source vacuum pressure.

Standard Preparation for Calibration Curves

Calibrants were prepared in 1:1 methanol:water solution from stock solutions by serial dilution. Eight concentrations were prepared for each analyte at various ranges depending on the analyte (ranges for each

analyte are presented in **Table 1**). Prior to MasSpec Pen analysis, five 5 µL of each solution was deposited on a PTFE coated glass slide. Spotting the solutions onto PTFE prevented the solutions from spreading across the surface and allowed the sample to dry in a small (<2 mm diameter) area that could be fully encompassed by the reservoir within the MasSpec Pen tip. This allowed reasonable assurance that the analyte amount deposited on the slide was within the sampling area for the analysis. Solutions were dried for variable amounts of time depending on the volatility of the analyte and stability of the analyte signal (**Figure S14**), and dry times are included in **Table 1**. Internal standards for each analyte, the identities of which are included within **Table 1**, were added to the extraction solvent system. Four calibration curves were performed for each analyte over 48 hours to evaluate inter- and intra-day variability. Calibrant solutions were stored at -4 ºC when not in use.

For calibration curves constructed directly from the Teflon coated fiberglass and orange peels, 4-5 calibrant solutions and a blank solvent were deposited on the sample surface, allowed to dry for the optimized time (**Table 1**), and analyzed in the same manner as performed for the analyses on the PTFE coated glass slide. The solutions spotted on the Teflon coated fiberglass did not spread, allowing the entire droplet area to be encompassed by the MasSpec Pen tip reservoir. For the orange peel, a hydrophobic pen was used to draw 3 mm circles on the peel to prevent spreading of the calibrant beyond the 4 mm reservoir area.

Percent Recovery studies

Standards were prepared for cocaine, atrazine, and TNT, in the same manner as the previous calibrants, at amounts within the linear range of each analyte's analysis method to be used for droplet collection. Prior to droplet collection, 5µL of each solution was deposited on a PTFE coated glass slide multiple times and allowed to dry according to the analyte's dry time (**Table 1**). Concentrations of 50 ppb, 500 ppb, and 5 ppm were prepared for cocaine which dried to 0.25, 2.5, and 25 ng respectively. Atrazine concentrations of 250 ppb and 1 ppm were prepared which dried to 1.25 and 5 ng respectively. TNT concentrations of 2.5 ppm, 5 ppm, and 10 ppm were prepared and dried to amounts of 12.5, 25, and 50 ng. Droplets were collected by

inserting a vial between the MasSpec Pen sample transport tubing and the source. This vial was connected to the MasSpec Pen sub-APCI system via PTFE tubing in a rubber septum with one PTFE tube connected to the vacuum source and the other connected to the MasSpec Pen. Multiple 5μL deposits were collected for each analyte sample (4 for cocaine and atrazine, 10 for TNT) to collect enough volume for liquid chromatography-mass spectrometry (LC-MS) or LC-UV-Vis analysis. Three separate replicates were collected for each analyte concentration. Control standards were made corresponding to the theoretical 100% recovery of each analyte concentration and each standard was analyzed three times. LC-MS analysis was performed for cocaine and atrazine control standards and their corresponding collected MasSpec Pen sub-APCI droplets using an Agilent Technologies 6546 Accurate-Mass Q-TOF LC-MS (Agilent Technologies, Santa Clara, CA) in the University of Texas at Austin's mass spectrometry facility in the positive ion mode. LC coupled to a UV-Vis detector, using an Agilent Technologies 1200 series LC system (Agilent Technologies, Santa Clara, CA) in the University of Texas at Austin's mass spectrometry facility, was used for the detection of TNT due to poor mass spectrometer signal. Percent recoveries for cocaine and atrazine were calculated by comparing the extracted ion chromatogram of the precursor ion area under the curve (AUC) from the collected droplet and the control standard. Percent recoveries for TNT were calculated using the AUC for the absorbance chromatogram at 240 nm. Percent relative standard deviations (RSD) were calculated for each analytes' concentrations' percent recovery and control standards AUC. Average percent recoveries were calculated using all individual percent recoveries for each analyte (cocaine n=9, atrazine n=6, TNT n=9).

Data collection and processing

MasSpec Pen data were acquired in the ion trap of Thermo Scientific Orbitrap XL and Elite hybrid mass spectrometers (San Jose, CA) using collision induced dissociation tandem MS. Collisional activation energy and adduct used as the precursor ion were optimized for each analyte and are summarized in **Table 1**. For each analysis, the ion signal obtained from the unique and/or most abundant fragment ion for the analyte and corresponding internal standard were integrated using Xcalibur Qual Browser. Calibration

curves were constructed in RStudio using the ggplot2 and plyr R packages. The normalized abundance of the analyte signal was calculated by dividing the integrated signal of the analyte's primary fragment ion by the integrated signal of the internal standard's primary fragment ion. Limits of detection (LOD) were calculated using 3 times the standard error in y (s_y) divided by the slope of the calibration curve.

Supporting Results and Discussion

Traditional MasSpec Pen Design Details and Comparison to sub-APCI – MasSpec Pen Design

The MasSpec Pen was initially designed by directly integrating the pen to the inlet of the mass spectrometer, allowing rapid droplet evaporation and analyte ionization within the mass spectrometer inlet. This ionization method is similar to the process described as "inlet ionization", in which vaporization and ionization is facilitated by the rapid increase in temperature in conjunction with a rapid drop in pressure.[2] This simplistic approach allows untargeted analysis of profiles of lipids and small metabolites from biological tissue samples, relying on mass spectral patterns of the relative abundances of multiple molecular ions simultaneously detected for tissue classification. As molecular patterns are conserved despite potential fluctuations in the total ion abundances, this approach is efficient for tissue analysis and statistical classification, as we have previously reported.[1, 3] On the other hand, quantitative analysis of specific analytes for forensics applications requires low signal variability and LOD. Thus, we explored sub-APCI as an alternative ionization method for targeted chemical analysis.

While the precise ionization mechanism and processes that occur in inlet ionization are largely unknown, data resulting from the analyses appear similar to that obtained with electrospray ionization.[1, 2] Therefore, more polar analytes are generally preferentially ionized with this method. In contrast, APCI has been largely recognized for its ability to ionize analytes with lower polarity. This feature is particularly beneficial for the analysis of agrochemicals, many of which are lipophilic, including atrazine (XLogP3 = 2.6) and azoxystrobin (XLogP3 = 2.5).[3] **Figure S19** illustrates this, showing an analysis of atrazine standard with both the traditional and sub-APCI versions of the MasSpec Pen, with the signal intensity greatly improved when using APCI ionization due to the lipophilic nature of the analyte. Another difference

between these methods is the vaporization, as the sample is vaporized with an external heater for the sub-APCI system rather than within the mass spectrometer inlet. To fully vaporize a sample with the traditional MasSpec Pen, the inlet temperature is typically above 350°C. This high temperature could decrease the stability of heat sensitive molecules and, in fact, we were unable to detect the explosive compounds with the traditional version of the MasSpec Pen, likely due to their instability at high temperatures. Another significant difference between the traditional and sub-APCI versions of the MasSpec Pen is the presence of extraneous ion signals in the spectra when using the sub-APCI version. Prior to analysis with the traditional MasSpec Pen system, there is largely no signal detected as ions are not generated until solvent enters the inlet and is vaporized. In contrast, the corona discharge within the sub-APCI source ionizes molecules within the source both prior to and during analyses, leading to significant background ion signal (**Figure S20**). The source of these signals is unknown, but likely stem from ionization of small amounts of contaminants within the ion source, potentially from the polymers used to vacuum seal the source. The relative intensities of these signals are reduced during analysis and interferences are largely excluded during tandem MS, but their presence complicates the use of this technique for full MS analyses and untargeted applications.

Optimization of the MasSpec Pen sub-APCI System

Many experimental parameters were assessed to evaluate and optimize data quality including solvent composition, solvent volume, source pressure, source temperature, and inlet temperature. Some parameters, such as source pressure and source temperature, were kept constant for all analytes as alterations in them either did not improve signal or prevented proper functioning of the system. The source pressure was held constant across analyte classes (~590 torr at an inlet temperature of 350 °C, for drugs and agrochemicals, and ~620 torr at an inlet temperature of 200 °C, for explosives, due to increased pressure at lower temperature) as decreasing the applied vacuum attached to the source prevented the droplet from rapidly moving through the tubing into the source while increasing the vacuum would decrease the detected analyte signal, presumably from analytes being aspirated into the external vacuum instead of reaching the MS inlet

(**Figure S21**). The source temperature, as controlled by the external heating unit, was maintained at 325 °C across analytes as lower temperatures led to inconsistent desolvation of the sample leading to plasma quenching and higher signal RSD while increased temperatures lead to analyte degradation (**Figure S22**).

Solvent volume, solvent composition, and inlet temperature were the three parameters tested that appeared to be analyte dependent. Inlet temperature primarily impacted analysis of the explosive compounds, as the inlet temperature of 350 °C used for analysis of drugs and agrochemicals caused significant degradation of the explosive compounds. In fact, inlet temperatures above 200 °C prevented detection of the explosive analytes completely (**Figure S23**). Thus, the inlet temperature was maintained at 200 °C for TNT and DNG analyses. For each analyte, multiple solvent compositions were tested to evaluate which solvent allowed effective extraction and ionization. While water was consistently used within the solvent as the high surface tension prevented solvent leakage from the tip prior to and during analysis, multiple organic solvents (acetonitrile, methanol, ethanol, isopropyl alcohol, and acetone) and solvent additives (acetic and formic acid) were tested at various proportions to evaluate the impact of solvent composition in the signal intensity obtained for the analytes. An example optimization procedure for the solvent blend is shown in **Figure S24.** The solvent blends ACN:H_2O w/ 0.1 % acetic acid and MeOH:H_20 w/ 0.1% acetic acid were selected for analysis of agrochemicals and drugs of abuse, respectively, as these solvents yielded the highest signal intensities for the analytes. For explosives, ACN:H_2O (75:25) was selected as the lower inlet temperature used (200 °C, as explained above) required a more volatile solvent composition (**Figure S25**). The solvent volume used (20 µL droplet plus flush volumes of 16µL, 20 µL, 24µL for explosives, agrochemicals, and drugs, respectively) were optimized for each analyte class, depending on the amount of solvent required to prevent sample accumulation in the tubing. Decreased volumes resulted in increased carry-over due to inefficient sample transport and increased volumes resulted in quenching of the corona discharge and thus decreased ion signal (**Figure S26**).

To evaluate carry-over and contamination, three spots of 500 pg of a cocaine standard dried on a PTFE surface were analyzed by the MasSpec Pen sub-APCI system followed by blank analyses for each standard analysis (**Figure S3**). The blank analysis immediately following the cocaine analysis had a non-

negligible signal intensity for the cocaine fragment peak at *m/z* 182.1. On average, the ion signal resulting from blank analyses was 18.2% ± 9.2% (n=3) of the signal from the previous analysis. There was a significant reduction in analyte signal for the second and third blank analyses however, with only 2.7% ± 0.7% and 1.5% ± 0.2% of the original analyte signal being detected. Carry-over can also be reduced by replacing the disposable MasSpec Pen devices, which can be exchanged without stopping data collection, removing the source from the instrument, disrupting the vacuum of the source/instrument, and without significantly delaying analysis (replacements take < 1 minute, as shown in the chronogram of **Figure S3B**). By replacing the MasSpec Pen immediately after analyses of the cocaine standard, an average 8.8% ± 4.4% of the analyte signal was observed in the following blank, which was further reduced to 2.5% ± 0.6% of the original signal upon a second blank. These results show that while some carry-over within the sub-APCI source is observed, especially for more concentrated samples, the carry-over can be significantly reduced and eliminated with a few washes/blanks between analyses.

Additionally, percent recovery studies were performed for each class of analytes to determine the sampling efficiency of the MasSpec Pen sub-APCI system. An average recovery of 40% (n=6, RSD=3.9%) was measured when sampling two different concentrations of atrazine (**Table S1, Figure S5**). Furthermore, an average recovery of 72% (n=9, RSD=4.3%) was measured when sampling three different concentrations of TNT (**Table S1, Figure S6**). Recovery for cocaine can be found in the main text.

Supporting Figures

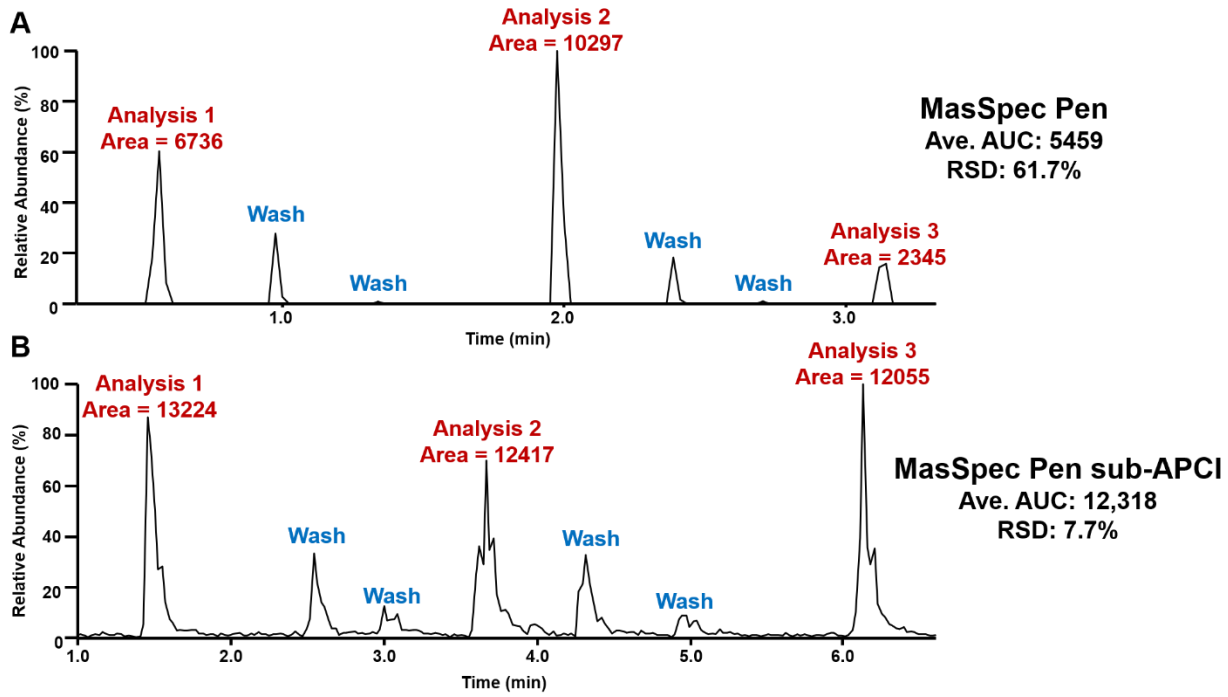

Figure S1. Analysis reliability and signal intensity is improved via integration of the MasSpec Pen with the sub-APCI source. **A)** Chronogram of three analyses of 1.25 ng of cocaine spotted on a PTFE coated glass slide with the traditional MasSpec Pen system. **B)** Chronogram of three analyses of 1.25 ng of cocaine spotted on a PTFE coated glass slide with the MasSpec Pen sub-APCI system. The area of each analysis is the integrated counts for the extracted ion chronogram of *m/z* 182.2, the primary CID fragment of cocaine. The wash analysis results from triggering of the MasSpec Pen system without analysis of a sample.

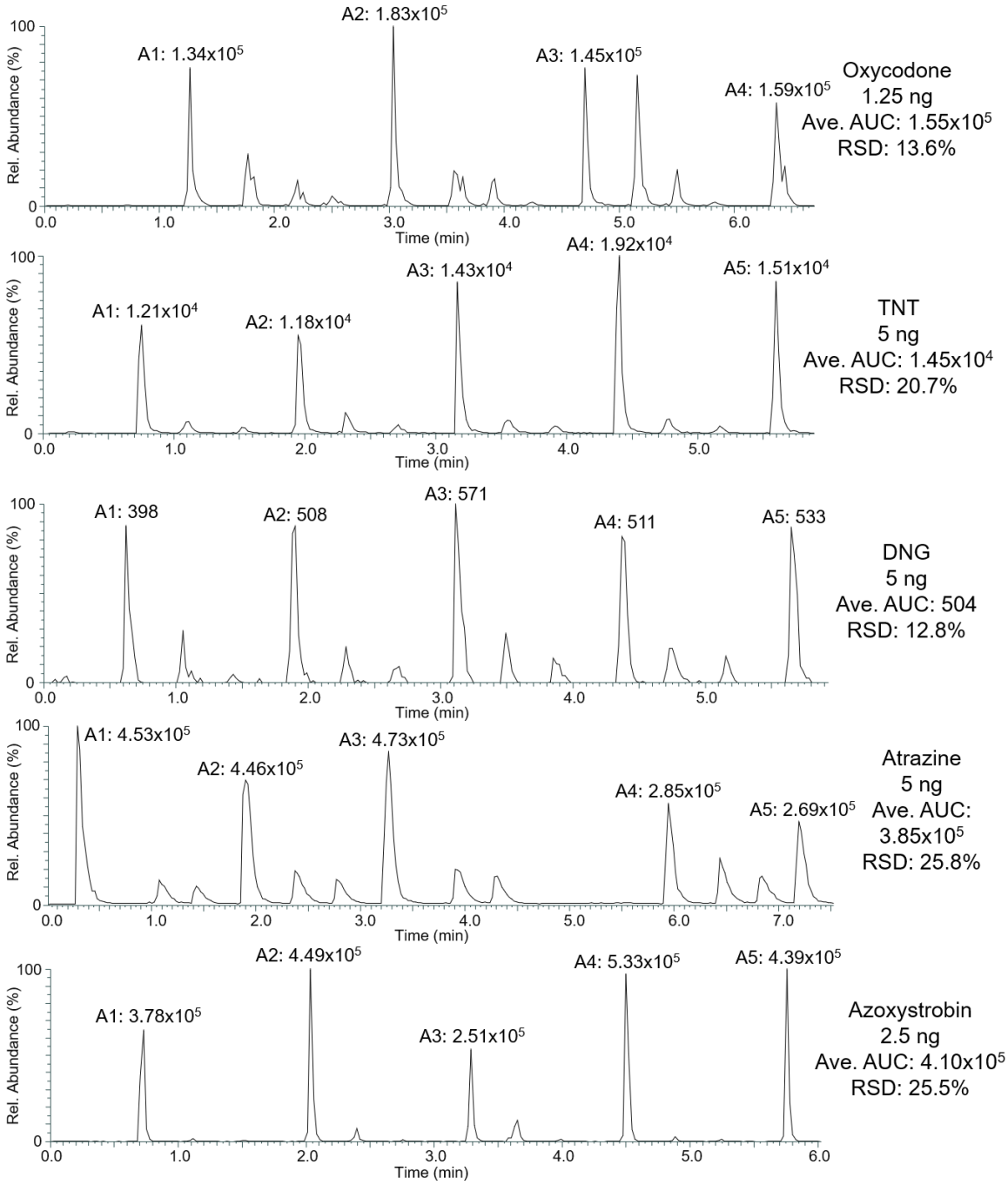

Figure S2: Extracted ion chronograms of the predominant fragment ion for replicate analyses of the analytes discussed within the manuscript. Each peak from a sample analysis (A1 = Analysis 1) is labeled with the peak area. The average integrated peak area from the extracted ion chronograms and the RSD for the measurements are displayed to the right of the spectra.

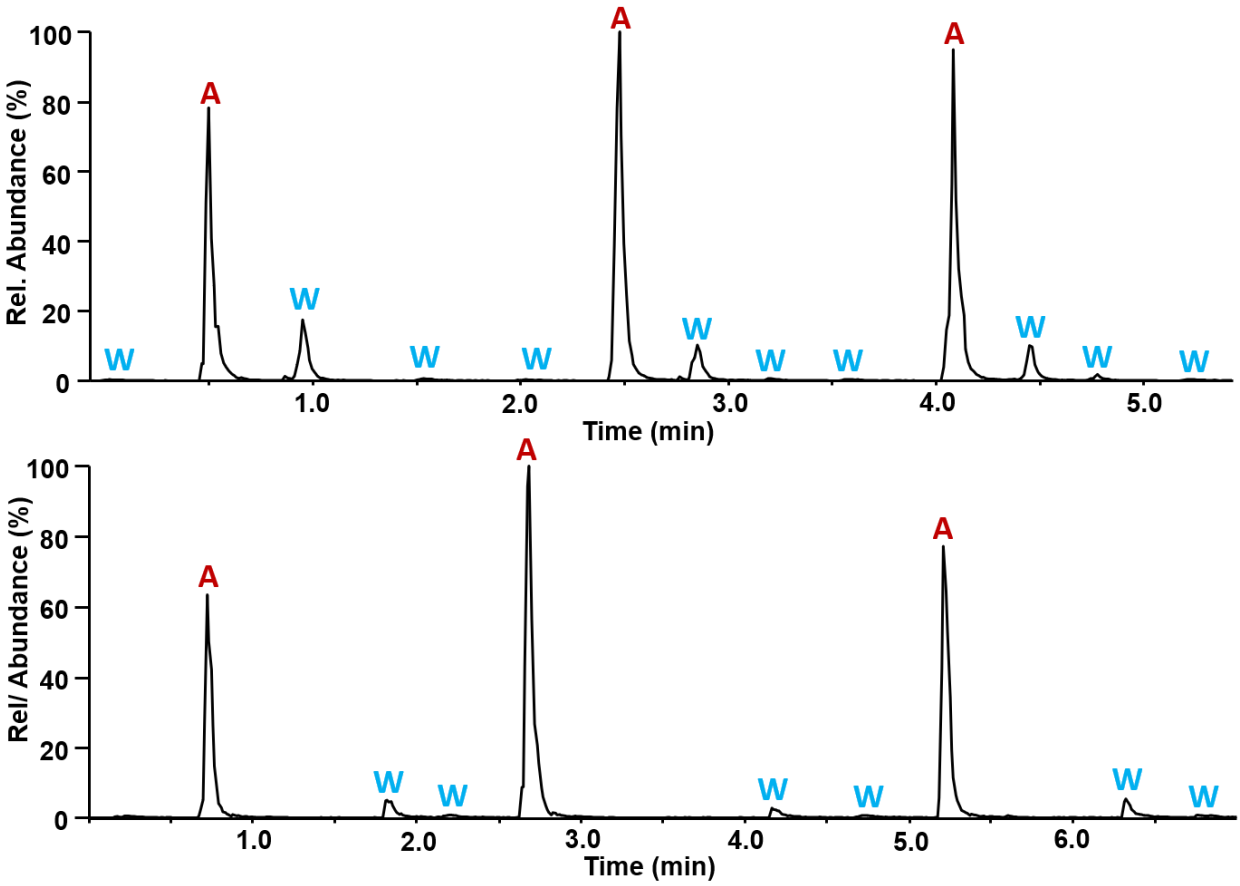

Figure S3. Extracted ion chronogram (*m/z* 182.2) of three analyses of 50 pg of cocaine spotted on a PTFE coated glass slide with the MasSpec Pen sub-APCI system followed by washes without changing (Top) and with changing (Bottom) the tubing after the cocaine analysis. Each analysis was followed by blank analyses in which the system was triggered without analyzing any sample to evaluate the carry-over within the MasSpec Pen tubing and the sub-APCI source.

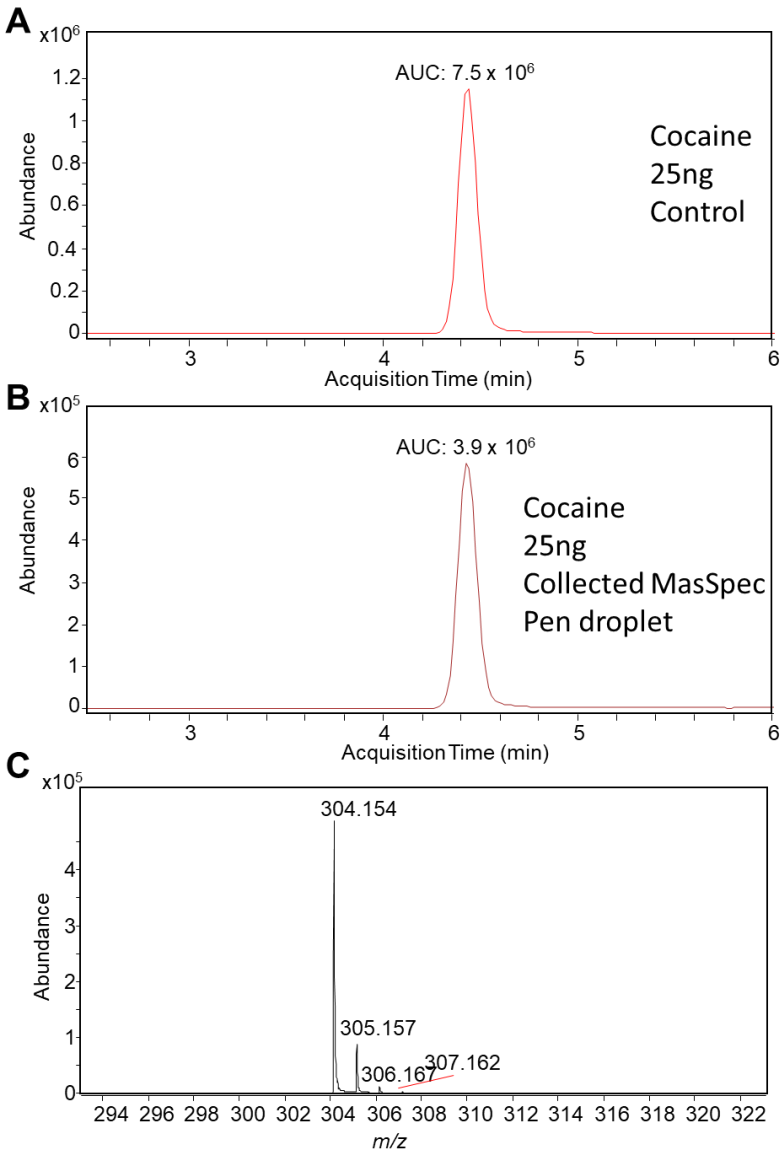

Figure S4. Extracted ion chromatograms at *m/z* 304.154 for **(A)** 25 ng control standard of cocaine and **(B)** a collected MasSpec Pen droplet sampled from 25 ng cocaine. **(C)** Mass spectrum of 25 ng cocaine control standard with observed cocaine isotope pattern.

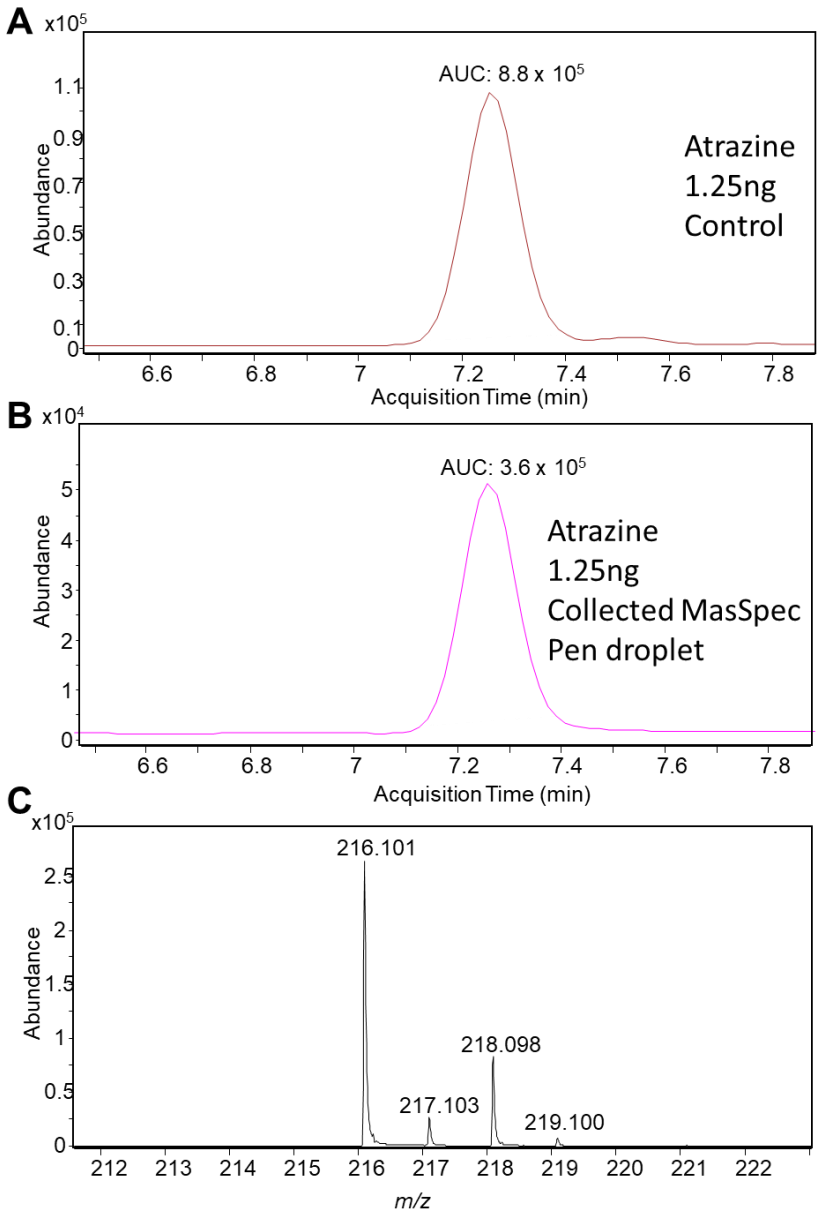

Figure S5. Extracted ion chromatograms at *m/z* 216.101 for **(A)** 1.25 ng control standard of atrazine and **(B)** a collected MasSpec Pen droplet sampled from 1.25 ng atrazine. **(C)** Mass spectrum of 5 ng atrazine control standard with observed atrazine isotope pattern.

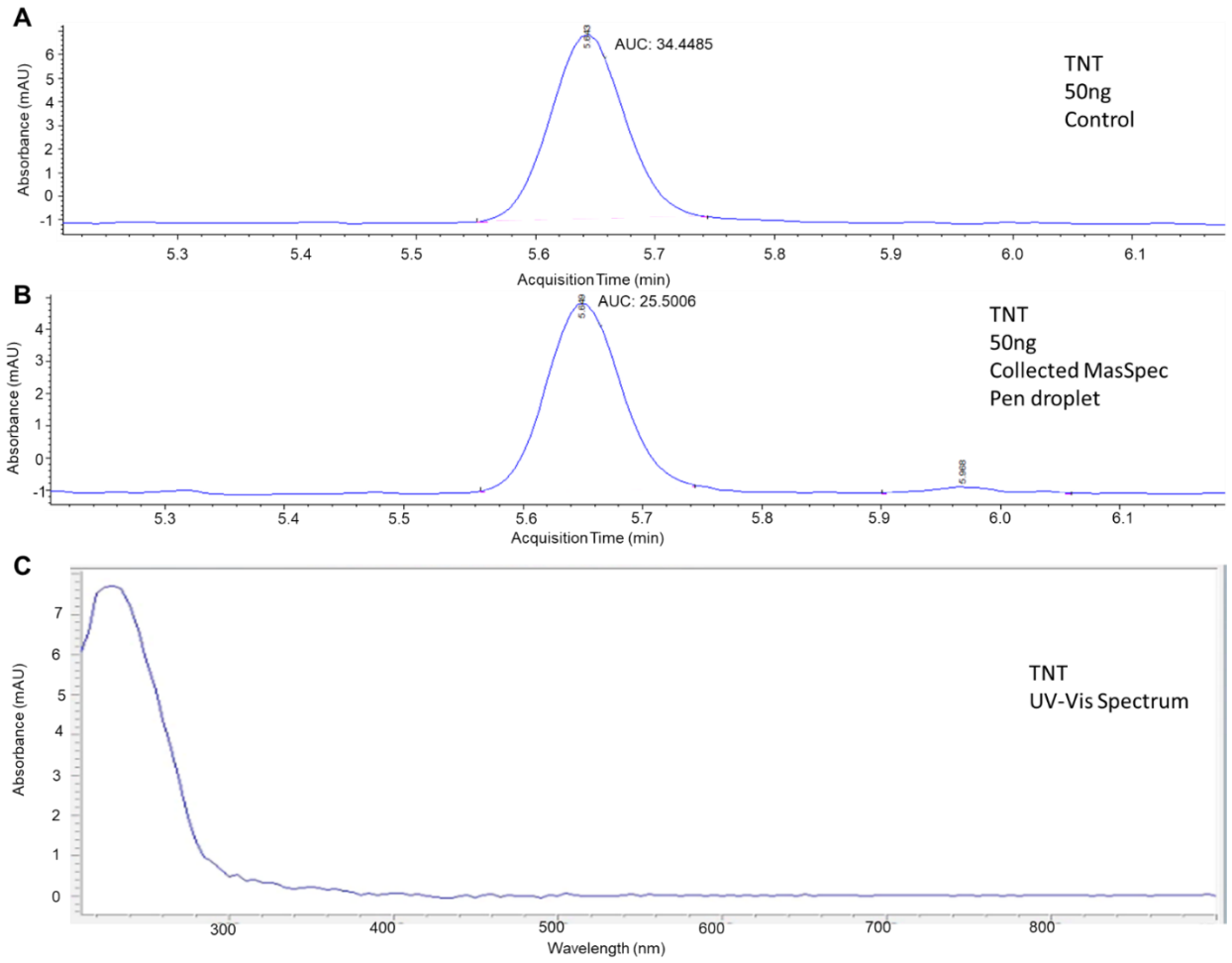

Figure S6. Absorbance (mAU) vs time (min) at 240nm for **(A)** 50 ng control standard of TNT and **(B)** a collected MasSpec Pen droplet sampled from 50 ng TNT. **(C)** UV-Vis spectrum of 50 ng TNT control standard taken from acquisition time 5.6 minutes.

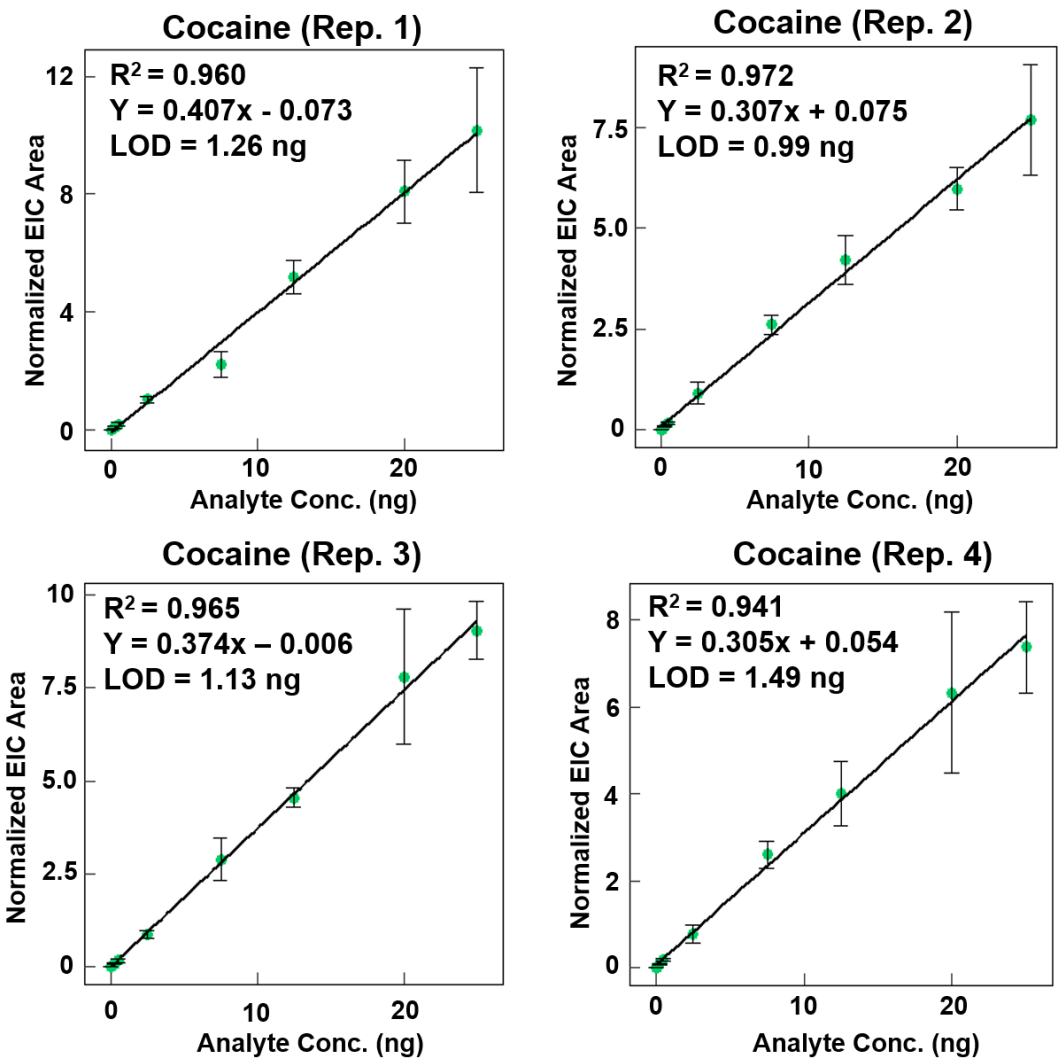

Figure S7. Four replicate calibration curves for cocaine. Replicates were performed over 48 hours, with two calibrations performed each 24 hours using common stock solutions to evaluate both intra- and inter-day variability. The y-axis is the AUC for the analyte quantitative fragment divided by the AUC for the IS quantitative fragment.

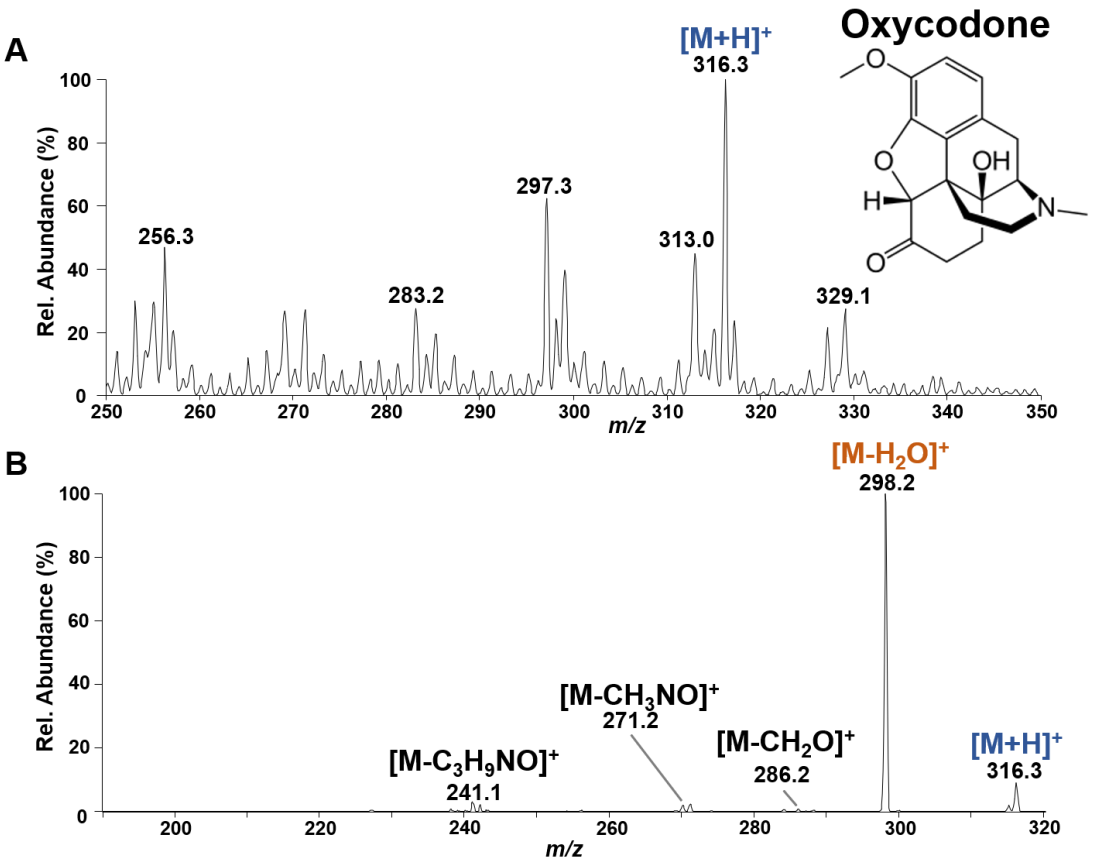

Figure S8. 500 pg of oxycodone analyzed with the MasSpec Pen sub-APCI system. **A)** MS^1 analysis of oxycodone, with the precursor selected for fragmentation, $[oxycodone+H]^+$ highlighted in blue **B)** MS^2 fragmentation of oxycodone, with the fragment used for quantification highlighted in orange.

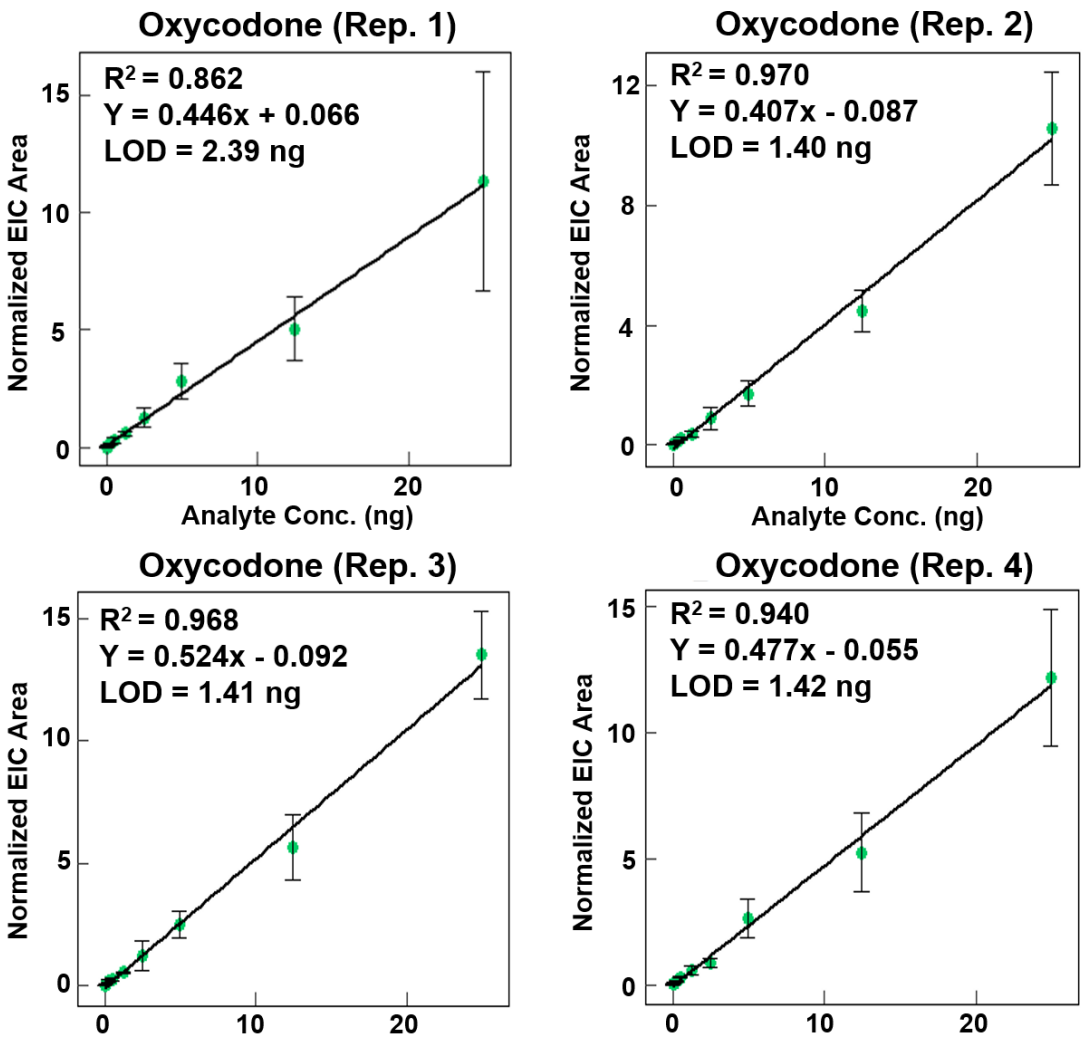

Figure S9. Four replicate calibration curves for oxycodone. Replicates were performed over 48 hours, with two calibrations performed each 24 hours using common stock solutions to evaluate both intra- and inter-day variability. The y-axis is the AUC for the analyte quantitative fragment divided by the AUC for the IS quantitative fragment.

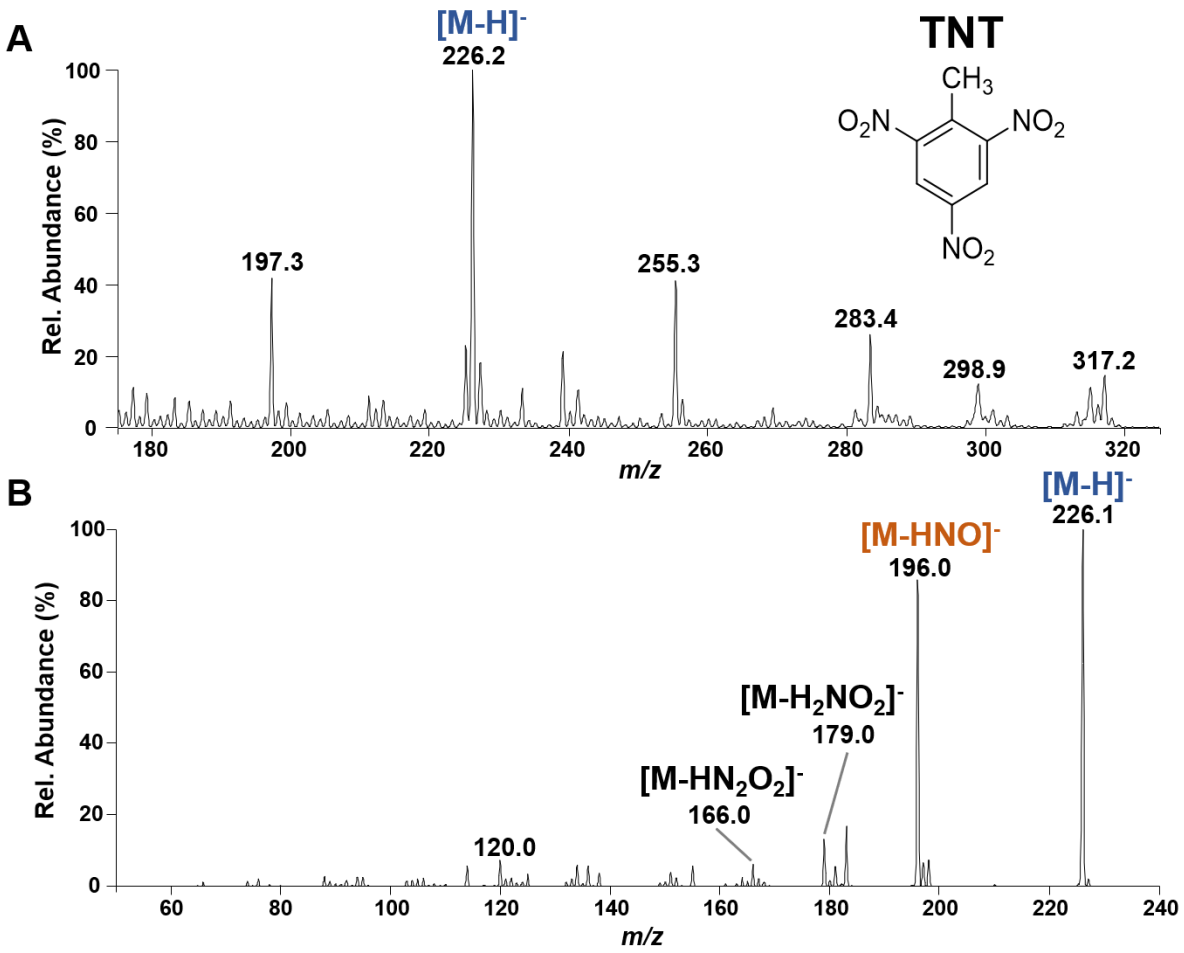

Figure S10. 5ng of TNT analyzed with the MasSpec Pen sub-APCI system **A)** MS^1 analysis TNT, with the precursor selected for fragmentation, $[TNT-H]^-$ highlighted in blue **B)** MS^2 fragmentation of TNT, with the fragment used for quantification highlighted in orange.

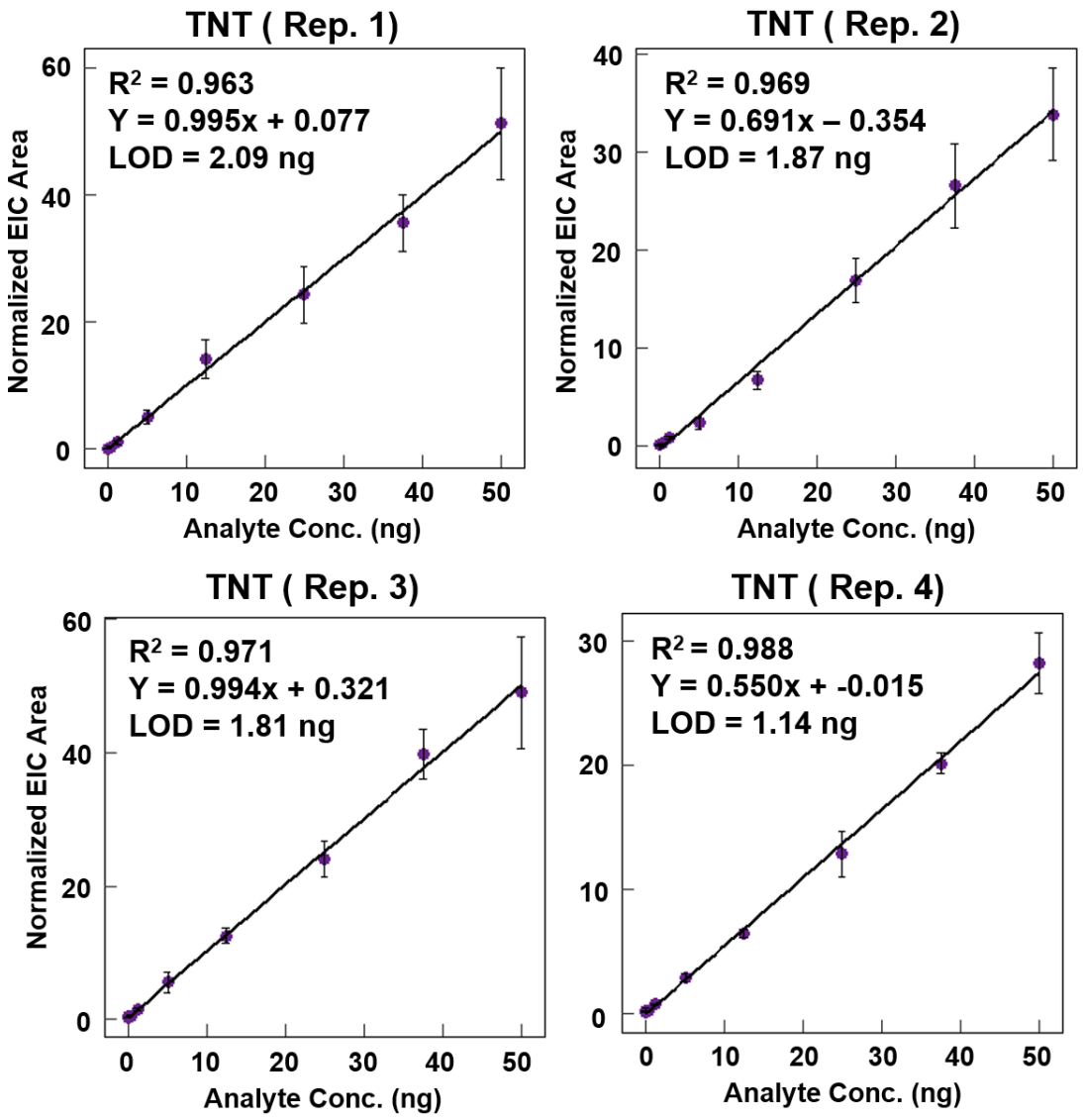

Figure S11. Four replicate calibration curves for TNT. Replicates were performed over 48 hours, with two calibrations performed each 24 hours using common stock solutions to evaluate both intra- and inter-day variability. The y-axis is the AUC for the analyte quantitative fragment divided by the AUC for the IS quantitative fragment.

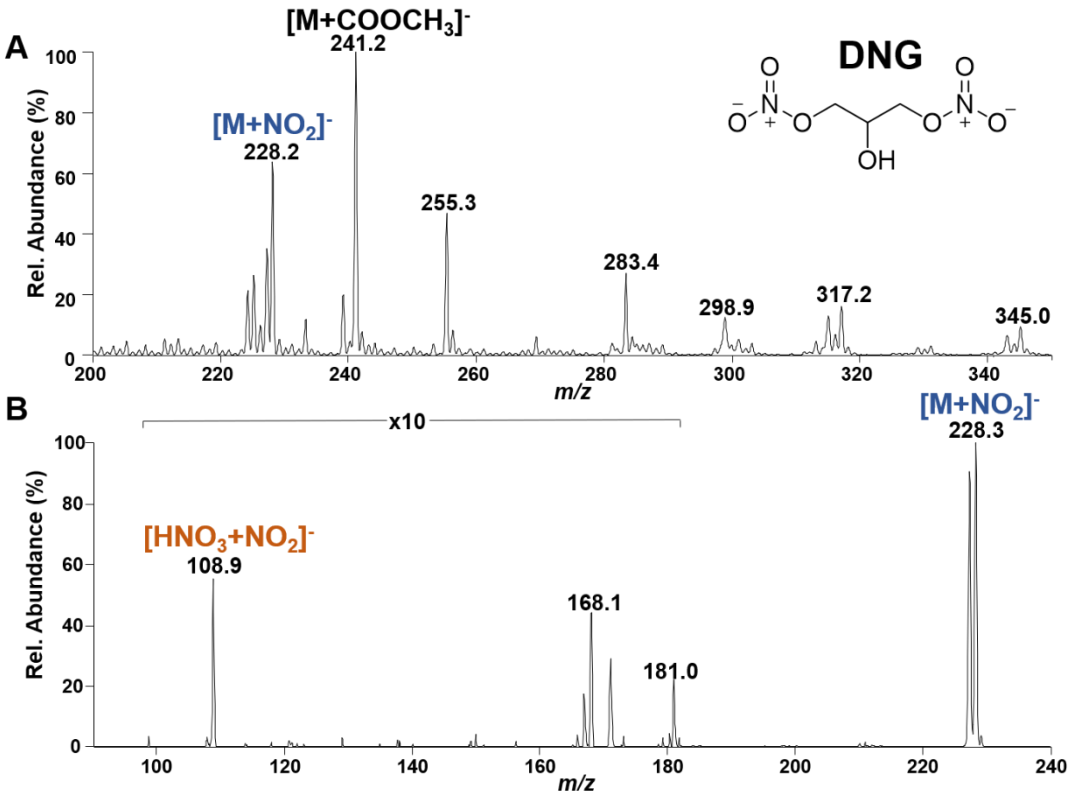

Figure S12. 5ng of DNG analyzed with the MasSpec Pen sub-APCI system **A)** MS1 analysis DNG, with the precursor selected for fragmentation, $[DNG+NO_2]^-$ highlighted in blue **B)** MS2 fragmentation of DNG, with the fragment used for quantification highlighted in orange.

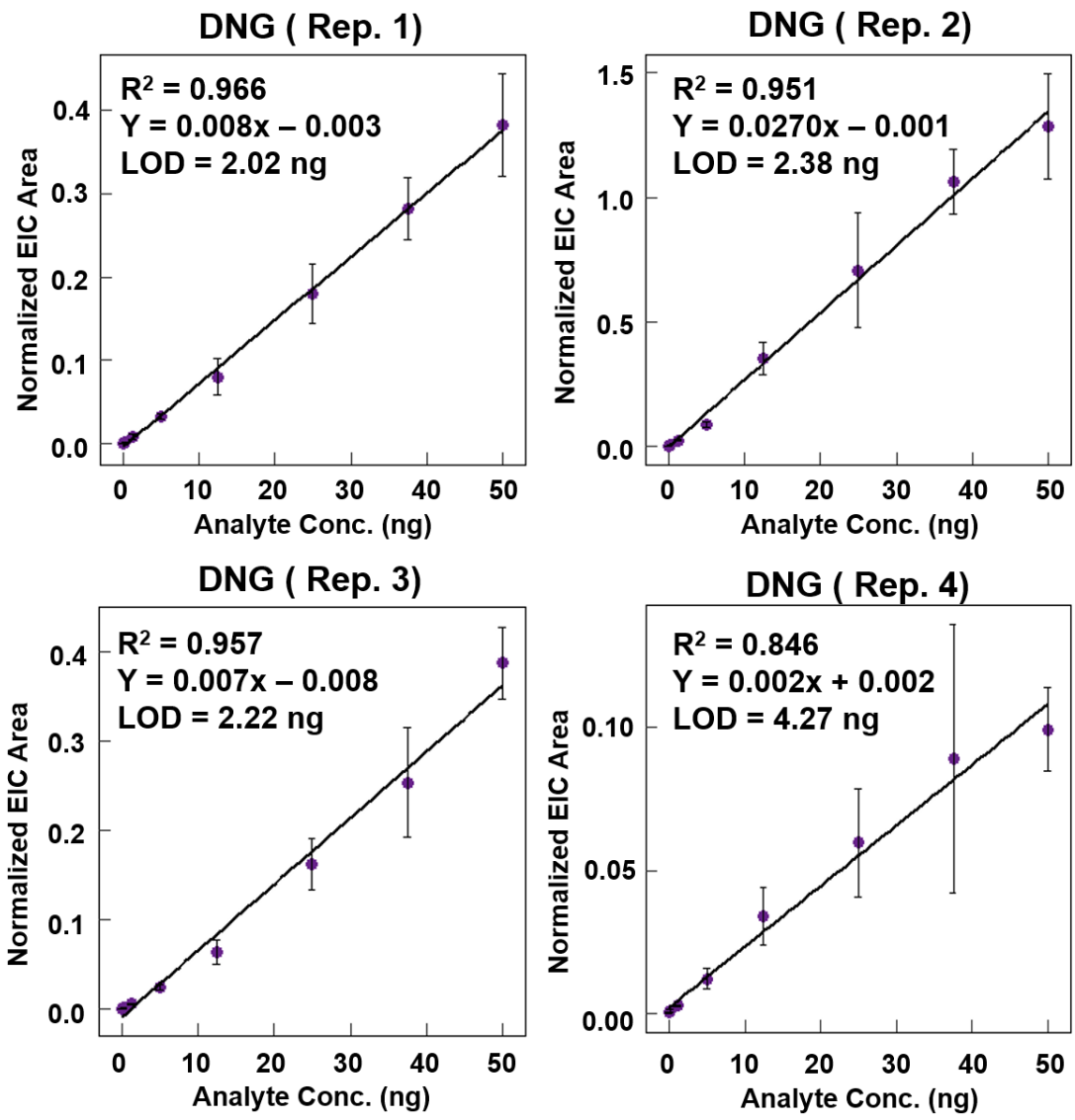

Figure S13. Four replicate calibration curves for DNG. Replicates were performed over 48 hours, with two calibrations performed each 24 hours using common stock solutions to evaluate both intra- and inter-day variability. The y-axis is the AUC for the analyte quantitative fragment divided by the AUC for the IS quantitative fragment.

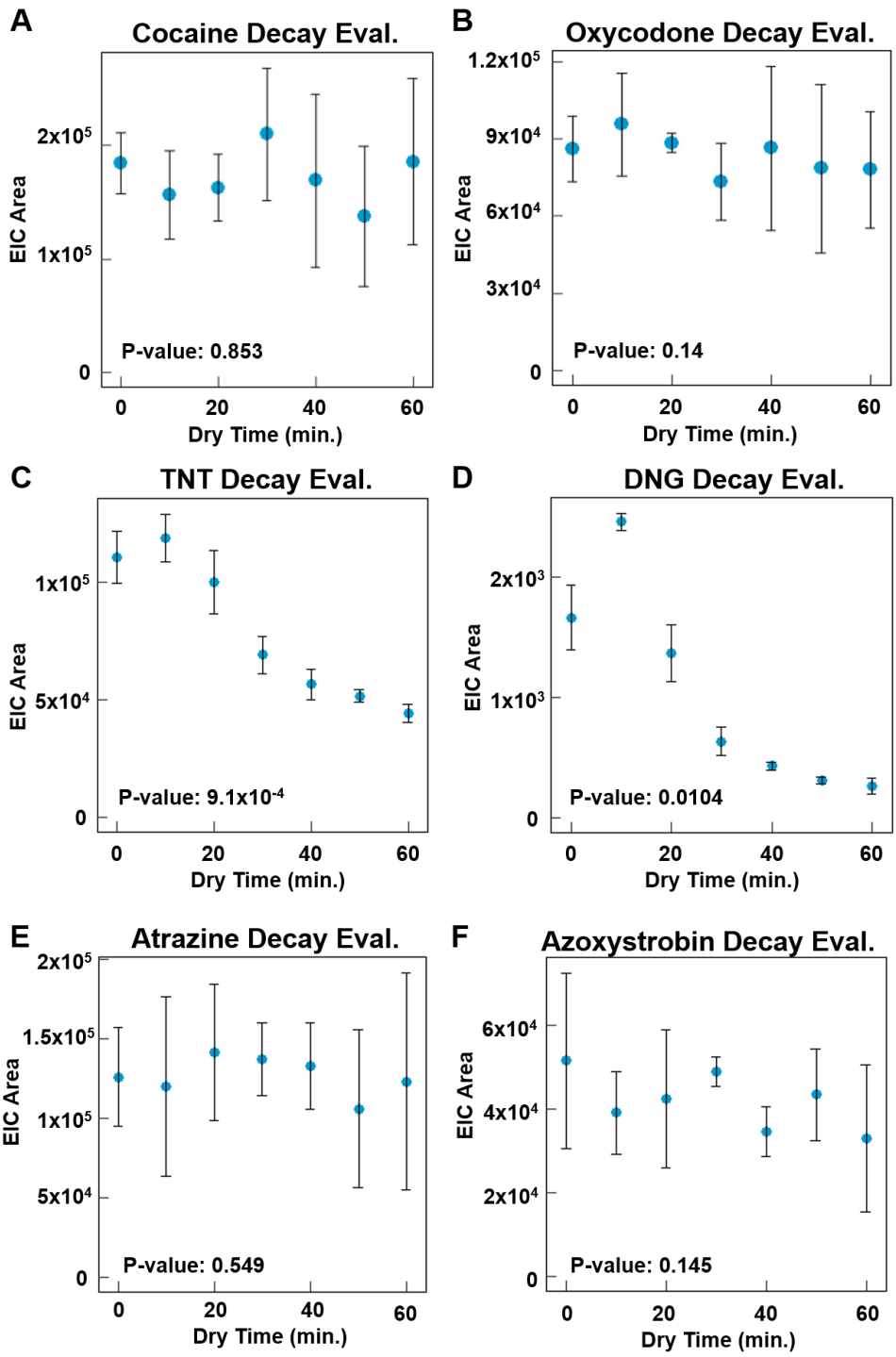

Figure S14. Signal decay over 1 hour time period for all analytes analyzed to evaluate if the analyte can reliably be analyzed while drying to determine at what time within the drying process the samples should be analyzed. ANOVA was used to evaluate the statistical difference in signal between time points.

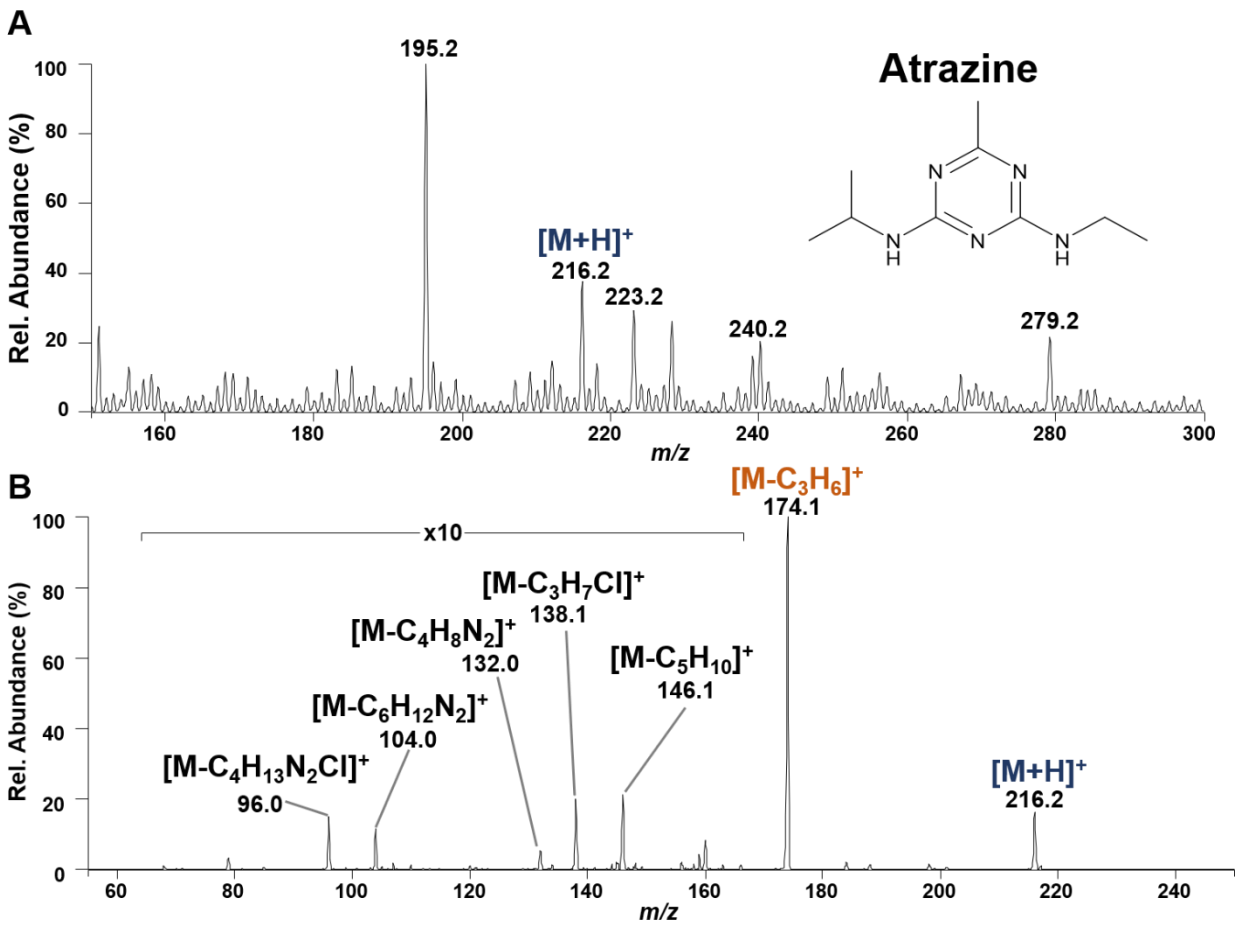

Figure S15. 500 pg of atrazine analyzed with the MasSpec Pen sub-APCI system **A)** MS1 analysis atrazine, with the precursor selected for fragmentation, $[atrazine+H]^+$ highlighted in blue **B)** MS2 fragmentation of atrazine, with the fragment used for quantification highlighted in orange.

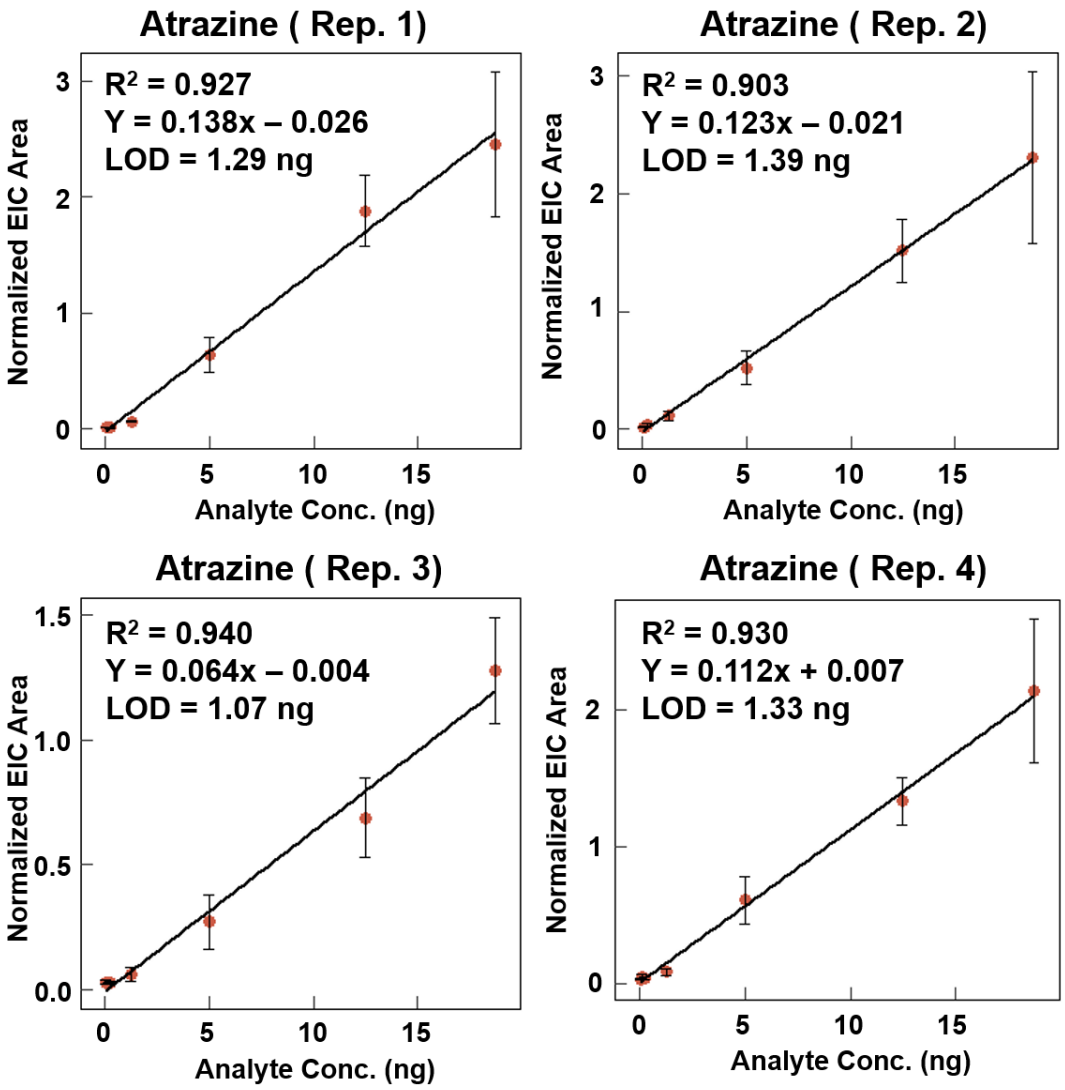

Figure S16. Four replicate calibration curves for atrazine. Replicates were performed over 48 hours, with two calibrations performed each 24 hours using common stock solutions to evaluate both intra- and inter-day variability. The y-axis is the AUC for the analyte quantitative fragment divided by the AUC for the IS quantitative fragment.

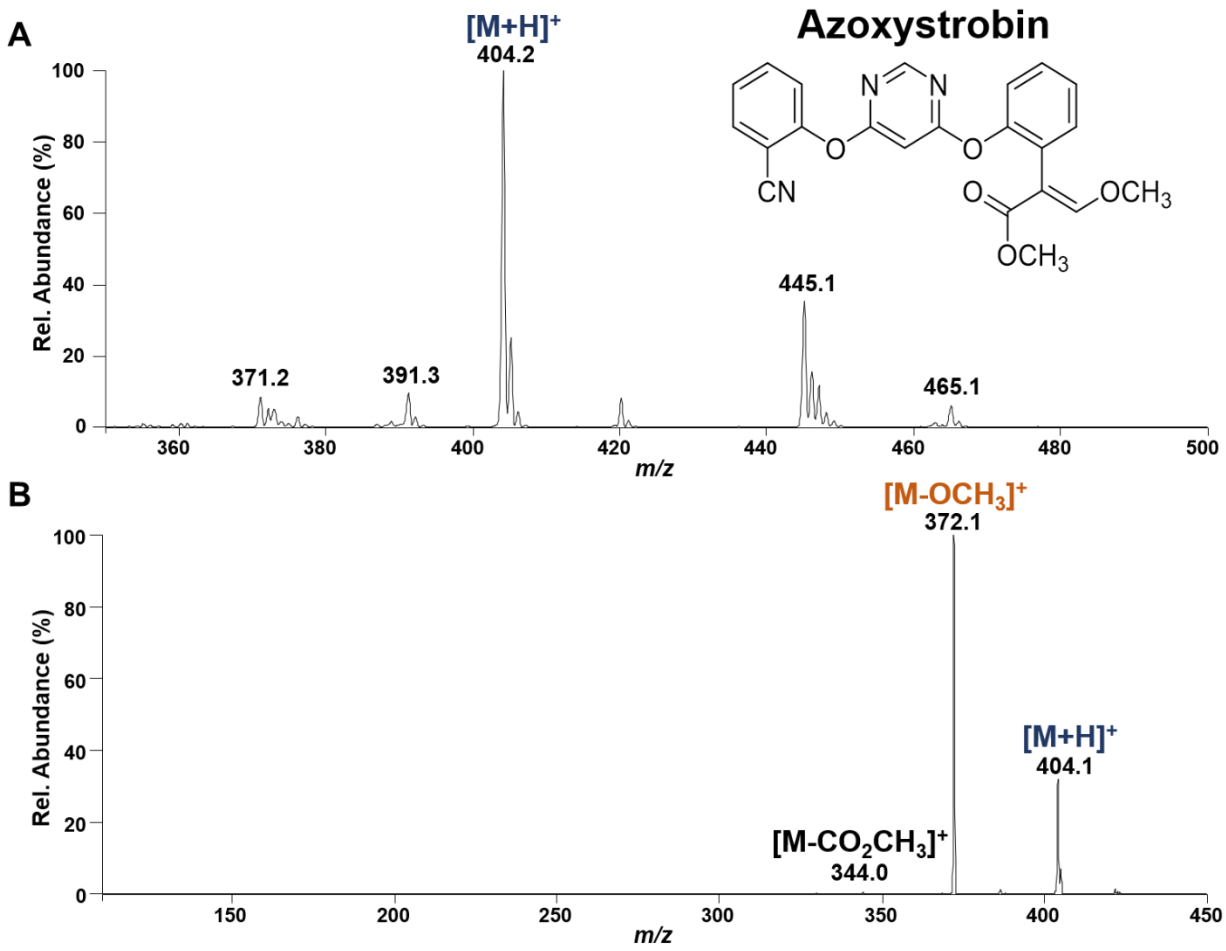

Figure S17. 500 pg of azoxystrobin analyzed with the MasSpec Pen sub-APCI system **A)** MS1 analysis azoxystrobin, with the precursor selected for fragmentation, [azoxystrobin+H]$^+$ highlighted in blue **B)** MS2 fragmentation of azoxystrobin, with the fragment used for quantification highlighted in orange.

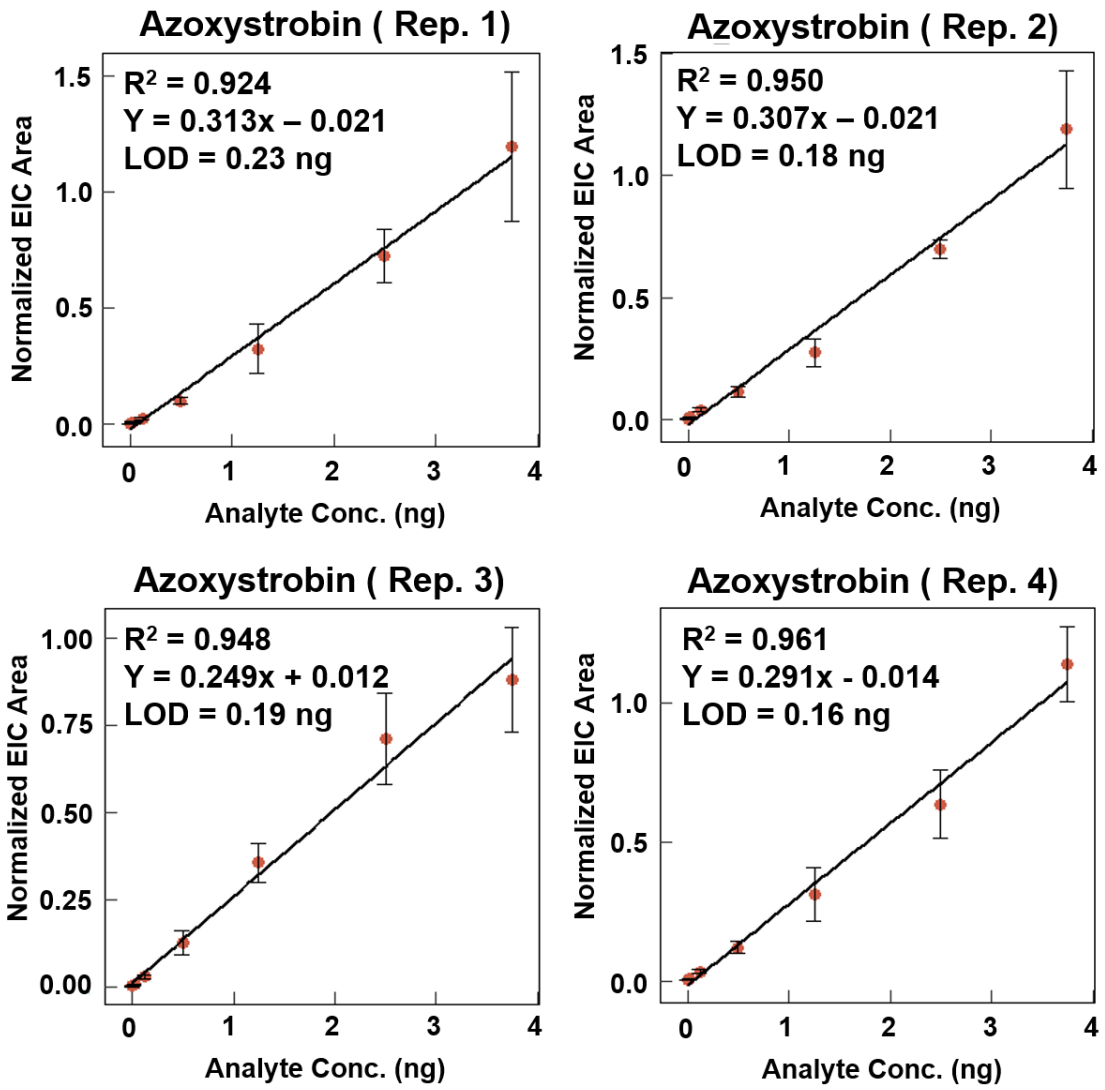

Figure S18. Four replicate calibration curves for azoxystrobin. Replicates were performed over 48 hours, with two calibrations performed each 24 hours using common stock solutions to evaluate both intra- and inter-day variability. The y-axis is the AUC for the analyte quantitative fragment divided by the AUC for the IS quantitative fragment.

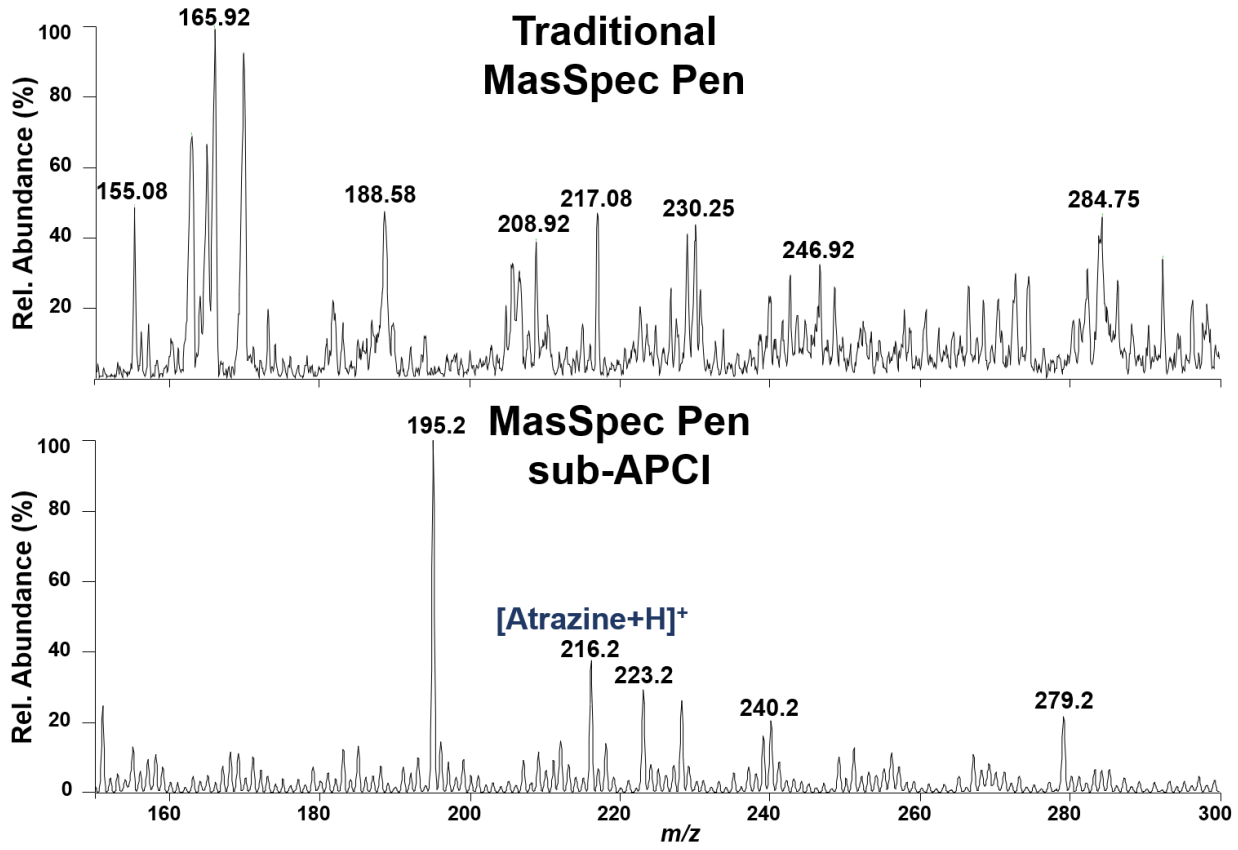

Figure S19. Analysis of 0.5 ng of atrazine, a lipophilic agrochemical, with both the traditional MasSpec Pen and the MasSpec Pen sub-APCI systems, illustrating that sub-APCI allows for enhanced sensitivity due to the ability of APCI to ionize less polar analytes.

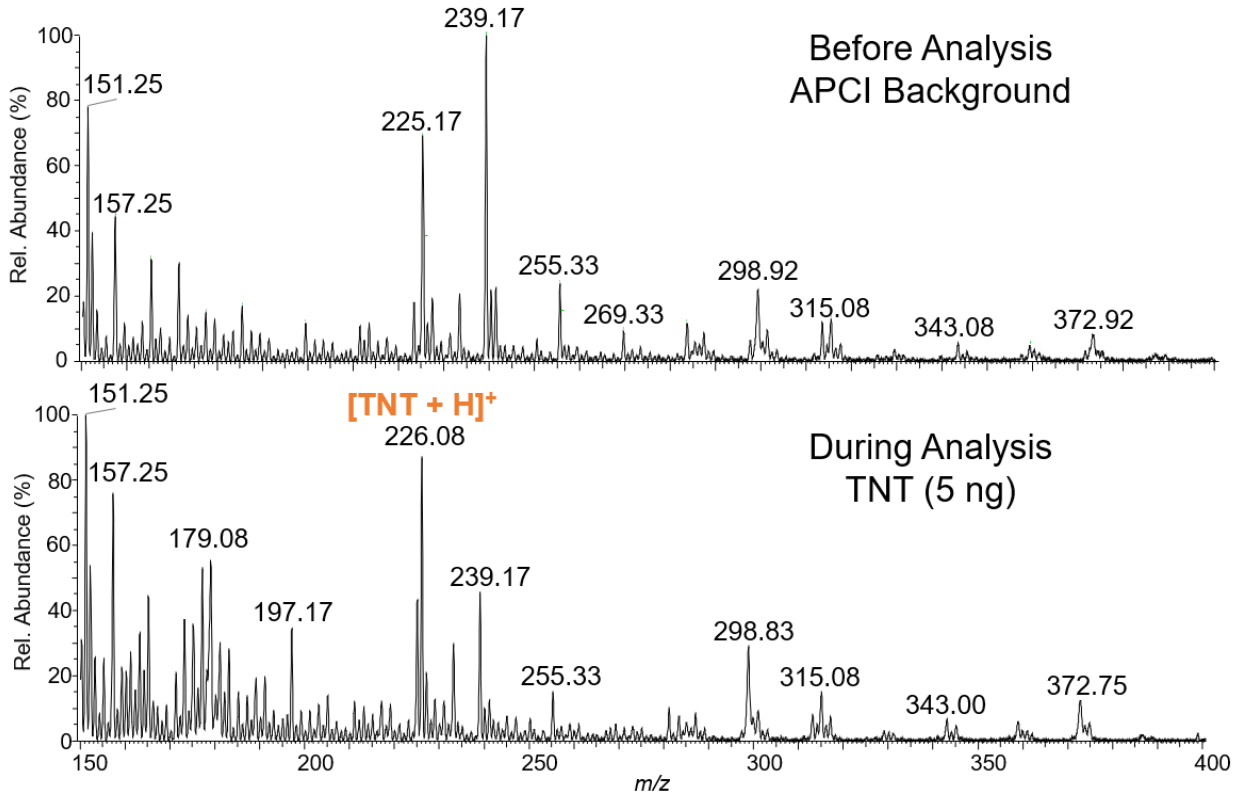

Figure S20. Spectra before (Top) and during (Bottom) a MasSpec Pen sub-APCI analysis of TNT, illustrating the background signal produced by the corona discharge throughout the analysis process.

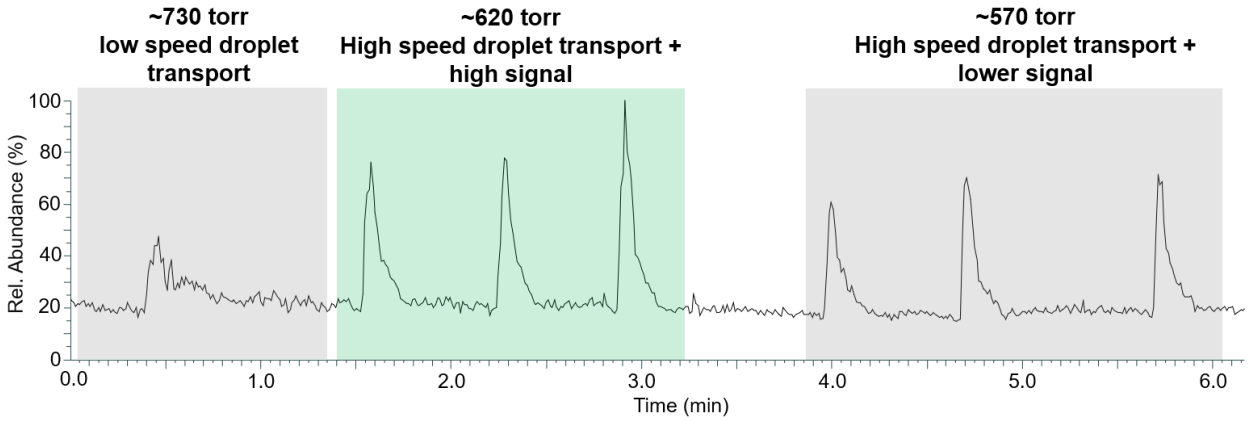

Figure S21. Ion chronogram of the isolated precursor ion of TNT (m/z 226.2) at decreasing source pressures (i.e. high supplied external vacuum), with optimal pressure highlighted in green. At higher pressures, droplet transport through the PTFE tube to the source is slow and signal is irreproducible. At lower pressures, signal intensity is decreased as the external vacuum begins to aspirate the analytes. Note that pressures readings are dependent of the inlet temperature (200° C for explosives) and will vary for higher inlet temperatures (350° C for drugs and agrochemicals).

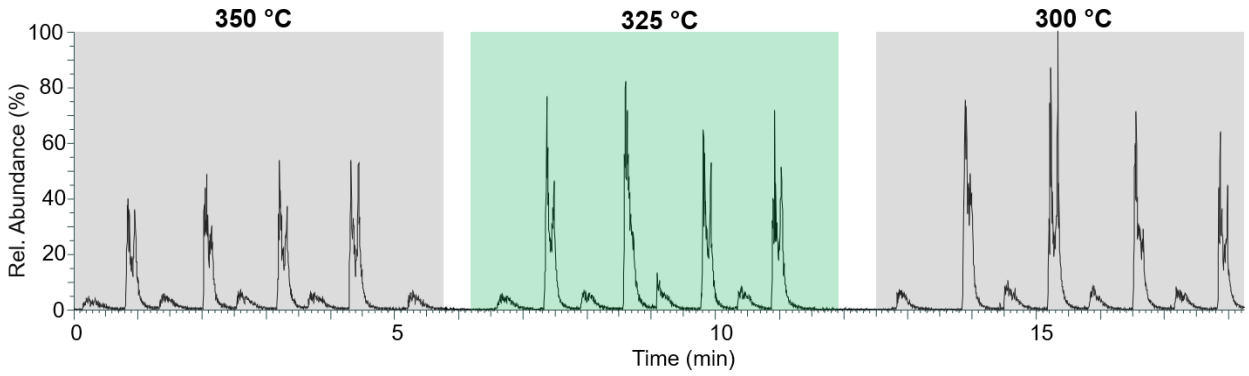

Figure S22. Ion chronogram of the precursor mass of an explosive analyte during analyses at various sub-APCI source heater temperatures, with the optimal temperature of 325 °C, highlighted in green. At higher temperatures (350 °C), the signal intensity of the ion was noticeably lower, likely due to degradation of the explosive at high temperatures. At lower temperatures (300 °C), the signal intensity was high but has a higher RSD value (RSD = 20.6%) for the AUC than what was obtained for 325 °C (RSD = 10.6%), potentially due to lower levels of solvent vaporization that impact ionization efficiency.

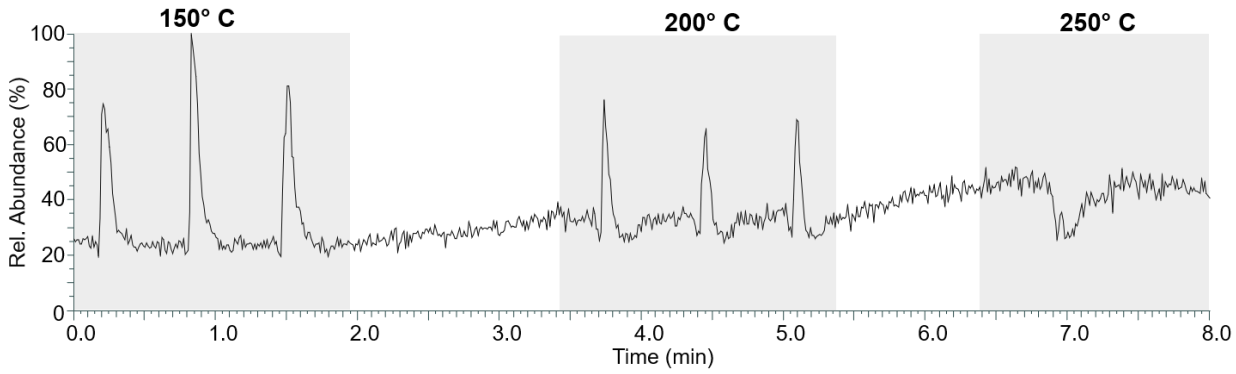

Figure S23. Ion chronogram of the isolated precursor ion of TNT (m/z 226.2) at increasing inlet temperatures, illustrating a significant decrease in detected ion signal at high inlet temperatures.

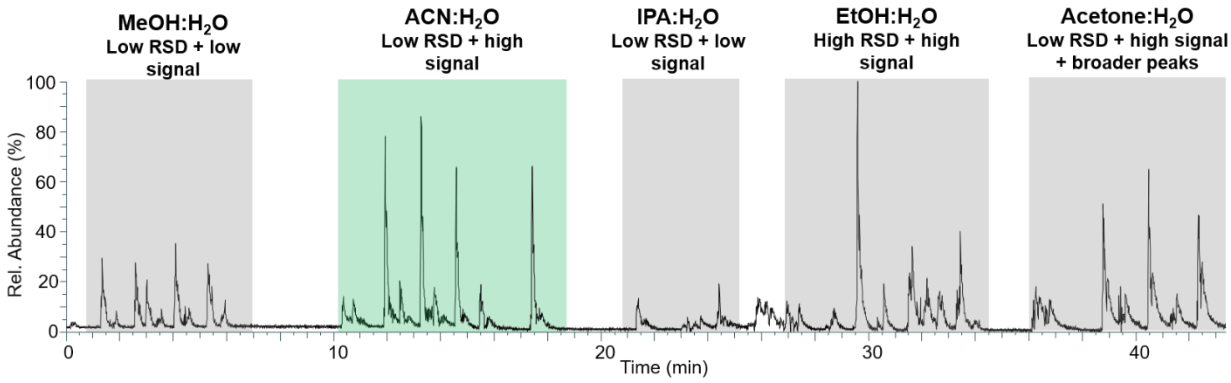

Figure S24. Ion chronogram of the isolated fragment ion of atrazine (m/z 174.0) during analyses with various solvent system, with optimal solvent blend highlighted in green. Solvents were evaluated based on signal intensity and reproducibility. Both ACN:H_2O and Acetone:H_2O yielded similar results for those two parameters, but ACN:H_2O yielded more narrow signal peaks, leading to decreased analysis time, and was thus selected as the solvent blend for this application.

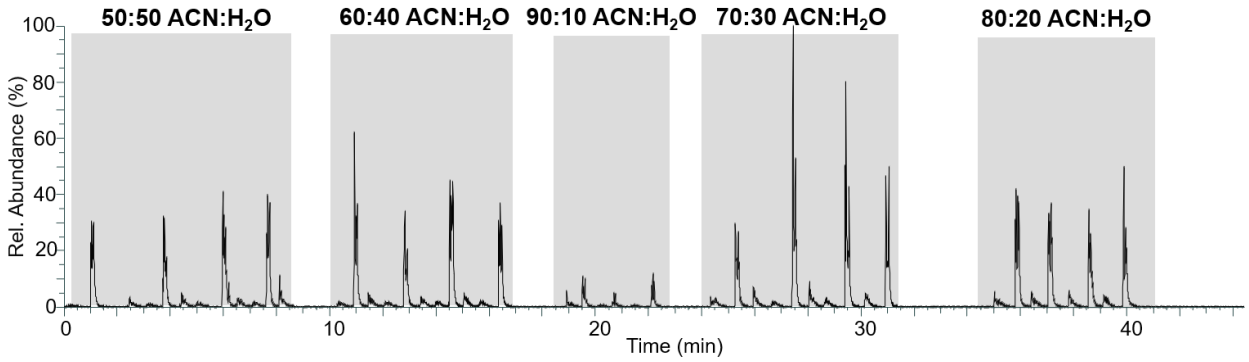

Figure S25. Ion chronogram of the precursor mass of an explosive analyte during analyses at different solvent compositions ranging from 50:50 ACN:H_2O to 90:10 ACN:H_2O. The 50:50 ACN:H_2O solvent system yielded lower average signal intensity (AUC = 7.31×10^5, n=4) than the solvent systems with higher proportion of ACN, for example the 70:30 ACN:H_2O solvent system (AUC = 9.33×10^5, n=4). This is likely due to the increased volatility of the solvent and improved vaporization in the sub-APCI source. However, the 90:10 ACN:H_2O produced very poor results, potentially due to low solubility of the analyte in ACN, decreased surface tension of the solvent prevented effective transport of the analyte through the tubing, or degradation of the analyte by overheating in the source after vaporization. Therefore, a solvent system composition between these extremes of 75:25 ACN:H_2O was selected for the explosive compounds.

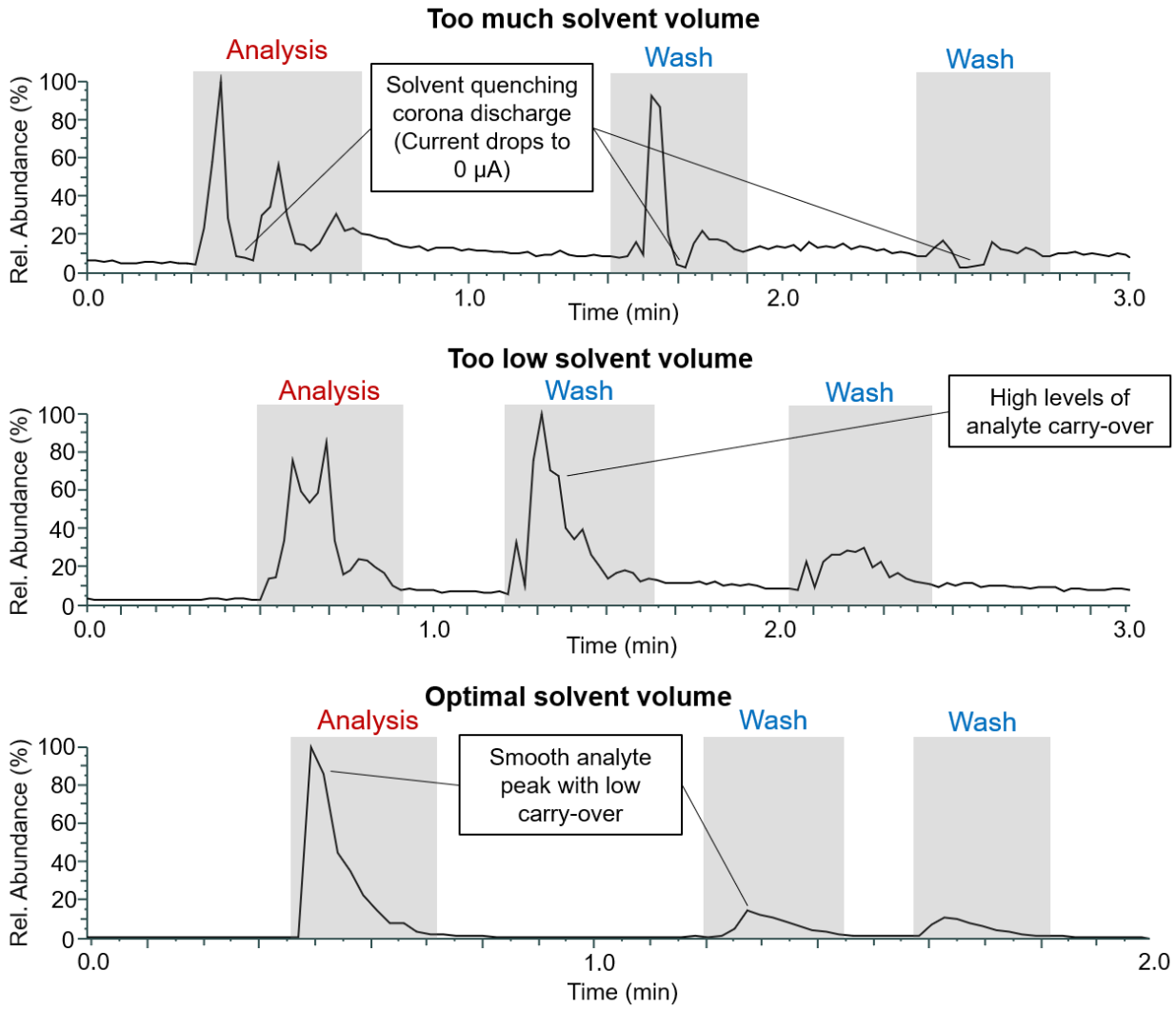

Figure S26. Ion chronogram of the fragment ion of an atrazine *(m/z* 174.0) during analyses with varying solvent flush volumes. The top chronogram illustrates impacts of using too much solvent within the flush, resulting in quenching of the corona discharge, visualized sharp decreases in peak intensity and drops in current measured by the instrument. The middle chronogram shows the impact of too low solvent flush volumes, resulting in inefficient analyte transfer during the initial analysis and subsequent detection in the wash analyses. The bottom chronogram illustrates optimal solvent flush volume, yielding smooth, high signal intensity analysis peaks without extensive carry-over in wash analyses.

Supporting Tables

Table S1. Percent recovery table for each molecule selected from the drugs, pesticides, and explosives. Average percent recovery, percent relative standard deviation (%RSD) of recovery, and %RSD of control standard is listed for each tested amount of cocaine, atrazine, and TNT.

Cocaine			
Amount (ng)	**Average of Recovery**	**%RSD of Recovery**	**%RSD of Control Standard**
0.25	**51%**	**6.3%**	**1.7%**
2.5	**51%**	**2.0%**	**4.3%**
25	**52%**	**4.9%**	**2.5%**
Atrazine			
Amount (ng)	**Average Recovery**	**%RSD of Recovery**	**%RSD of Control Standard**
1.25	**41%**	**3.8%**	**2.6%**
5	**39%**	**1.5%**	**3.5%**
TNT			
Amount (ng)	**Average Recovery**	**%RSD of Recovery**	**%RSD of Control Standard**
12.5	**71%**	**4.9%**	**4.2%**
25	**73%**	**4.3%**	**4.9%**
50	**72%**	**5.4%**	**5.4%**

Supporting References

(1) Zhang, J.L.; Rector, J.; Lin, J.Q.; Young, J.H.; Sans, M.; Katta, N.; Giese, N.; Yu, W.D.; Nagi, C.; Suliburk, J., et al., Nondestructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system. *Science Translational Medicine* **2017,** *9*, (406).
(2) Pagnotti, V.S.; Inutan, E.D.; Marshall, D.D.; McEwen, C.N.; Trimpin, S., Inlet Ionization: A New Highly Sensitive Approach for Liquid Chromatography/Mass Spectrometry of Small and Large Molecules. *Analytical Chemistry* **2011,** *83*, (20), 7591-7594.
(3) Sans, M.; Zhang, J.L.; Lin, J.Q.; Feider, C.L.; Giese, N.; Breen, M.T.; Sebastian, K.; Liu, J.S.; Sood, A.K.; Eberlin, L.S., Performance of the MasSpec Pen for Rapid Diagnosis of Ovarian Cancer. *Clinical Chemistry* **2019,** *65*, (5), 674-683.
(4) Cheng, T.; Zhao, Y.; Li, X.; Lin, F.; Xu, Y.; Zhang, X.; Li, Y.; Wang, R.; Lai, L., Computation of Octanol−Water Partition Coefficients by Guiding an Additive Model with Knowledge. *Journal of Chemical Information and Modeling* **2007,** *47*, (6), 2140-2148.