

Accurate quantification and confident identification of 12 explosives in soil samples using high-resolution mass spectrometry

Using SWATH acquisition and MRM^{HR} workflow on the SCIEX X500R QTOF system

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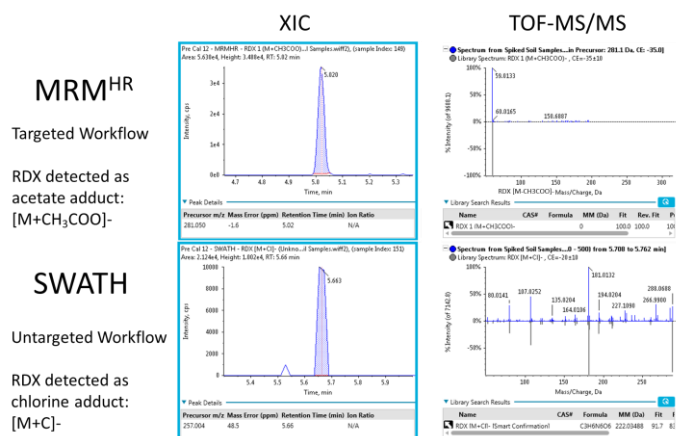
The widespread use of explosive materials in activities such as military training, blasting, mining, explosive production and other explosive-related applications has led to their introduction into the environment. Due to their labile nature, explosive materials and residues can migrate through subsurface soil and cause widespread contamination of soil and groundwater. Their ubiquitous presence in the environment is a growing health and public safety concern worldwide due to their toxic and carcinogenic nature. As a result, the ability to rapidly detect and identify trace amounts of explosives in soil is paramount for forensic science, environmental monitoring projects and legal authorities alike. Being able to accurately quantify explosives and their residues in different soil samples is critical to gain insights into the extent of the contamination and to assess the risk associated with their presence in the environment.

The combination of chromatography with tandem mass spectrometry (LC-MS/MS) provides the required levels of sensitivity and specificity for the detection of explosives. Given their intrinsic richness in electron-withdrawing nitrogen-containing groups and their thermally instable nature, explosives are well-suited for analysis by LC-MS/MS. Here, a high-throughput and sensitive detection method for accurate quantification and confident identification of 12 explosives in soil samples, combining a fast and simple sample preparation procedure with analysis on the SCIEX X500R QTOF system, is described.



Key advantages of LC-MS/MS method for explosives detection in soil samples

- Fast and simple soil sample preparation negates the need for time-consuming sample pre-treatment steps and enables high analyte recoveries (between 65 to 98%)
- Rapid, 7-minute LC run enables high-throughput separation of the 12 explosives
- The SCIEX X500R QTOF system provides the required speed, sensitivity and mass accuracy for high-throughput identification and quantification of explosives, with low (pg/g) LLOQs (lower limits of quantification)
- MRM^{HR} workflow offers optimal workflow flexibility: targeted quantification can be performed through high-resolution extraction of parent ions or through high-resolution extraction of unique fragment ions, which benefits from additional selectivity capabilities
- SWATH acquisition creates a digital record of the sample by acquiring high-quality MS and MS/MS spectra on all detectable compounds in a sample, enabling retrospective analysis and minimizing the risk of missing a critical component during sample analysis
- Overall performance of the system resulted in excellent correlation ($R^2 > 0.99$) with optimal measurement accuracy (with bias $\pm 15\%$) across the calibration range
- Workflow provides a high-throughput and easily implemented method for confident identification and accurate quantification of explosives in soil samples



Methods

Target analytes and solutions: Explosive standards were purchased from Cambridge Isotope Laboratories (Tewksbury, MA) and Accustandard Inc. (New Haven, CT). Two solutions were prepared in acetonitrile/water (50:50) with 2 mM ammonium acetate: a standard mixture containing the 12 explosives and a 1 ng/mL internal standard solution of ^{13}C -2,4,6-trinitrotoluene (^{13}C -TNT). A list of the 12 explosives used in this study is provided in Table 1.

Sample preparation: The sample preparation procedure used to extract the explosives from soil samples is summarized in Figure 2.

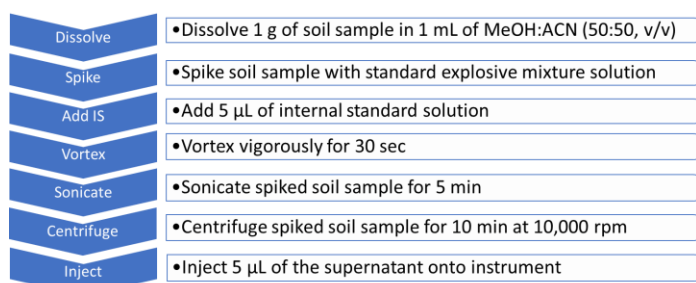


Figure 2. Sample preparation procedure for the 12 explosives in soil samples. A 6-step sample pre-treatment method was used for preparing the soil samples for analysis.

Chromatography: HPLC separation was performed using a Phenomenex Luna Omega C18 column (100 x 2.1 mm, 1.6 μm , 00D-4748-AN) held at 40°C on an ExionLC system. Mobile phase A and mobile phase B consisted of ammonium acetate in water and ammonium acetate in methanol/acetonitrile (50:50), respectively. The flow rate was 0.5 mL/min. The injection volume was 5 μL and the total LC runtime was 7 minutes.

Mass spectrometry: MS and MS/MS data were collected using atmospheric pressure chemical ionization (APCI) in negative mode on the SCIEX X500R QTOF system with SCIEX OS software 2.0. Negative-ion mode APCI provided the best sensitivity for detection of explosives due to their electron-withdrawing nitro group. The MRM^{HR} workflow with the *Apply TOF start/stop* mass feature was used to collect full-scan MS/MS data for identification. One MRM^{HR} transition was monitored for each of the 12 explosives targeted in the panel. SWATH acquisition was also performed using 8 Q1 windows (50 Da each) covering 50-400 m/z, automatically populated in SCIEX OS software 2.0. Both data acquisition methods started with a TOF MS experiment.

Data analysis: Data was processed in SCIEX OS software 2.0. Peak detection and integration was accomplished within the viewing window using the MQ4 algorithm.

Developing a robust targeted workflow for accurate quantification of explosives in soil

Control soil samples were spiked with the 12 explosive compounds of interest at concentrations ranging from 1-1000 pg/g then extracted using the sample preparation procedure highlighted in Figure 2. Prepared samples were then analyzed using MRM^{HR} workflow which enabled quantification of the explosives using the accurate mass of either the parent or the unique fragment ions for each analyte. A data processing method was built using the unique accurate mass fragment for quantification. The acquisition of looped full scan MS/MS spectra also enables confirmation through the use of MS/MS spectral library searching.

Figure 3 shows the extracted ion chromatogram (XIC) of the 12 explosive compounds extracted from a control soil sample. The chromatogram shows separation of the 12 explosives using a 7 minute runtime. Most explosives in the panel were observed as deprotonated molecular form $[\text{M}-\text{H}]^-$, although a number of alternative molecular species such as nitrogen dioxide adducts (i.e., Tetryl at m/z 287.0138 interpreted as $(\text{M}+\text{NO}_2)^-$), ammonium adducts (i.e., AP at m/z 246.0237 interpreted as $(\text{M}+\text{NH}_4)^-$) and acetate adducts (i.e., HMX at m/z 355.060 interpreted as $(\text{M}+\text{CH}_3\text{COO})^-$) were also observed.

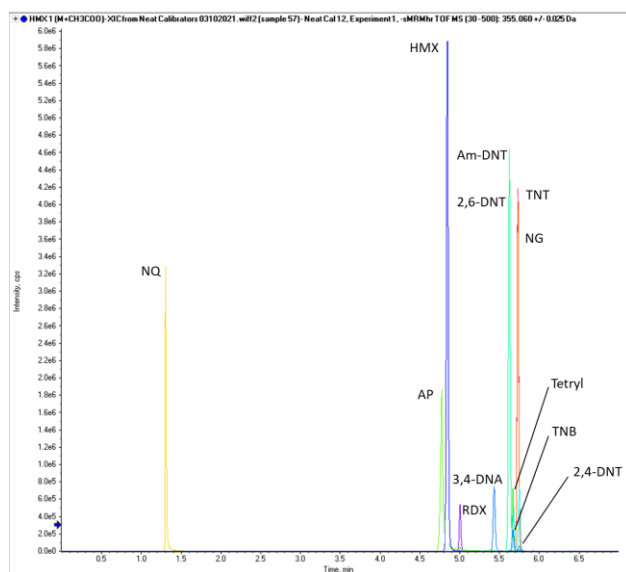


Figure 3. Chromatographic profile of the 12 explosives included in the panel. Extracted ion chromatograms (XICs) resulting from the optimized MRM^{HR} acquisition method showing identification of the 12 explosives using a 7-minute LC runtime.

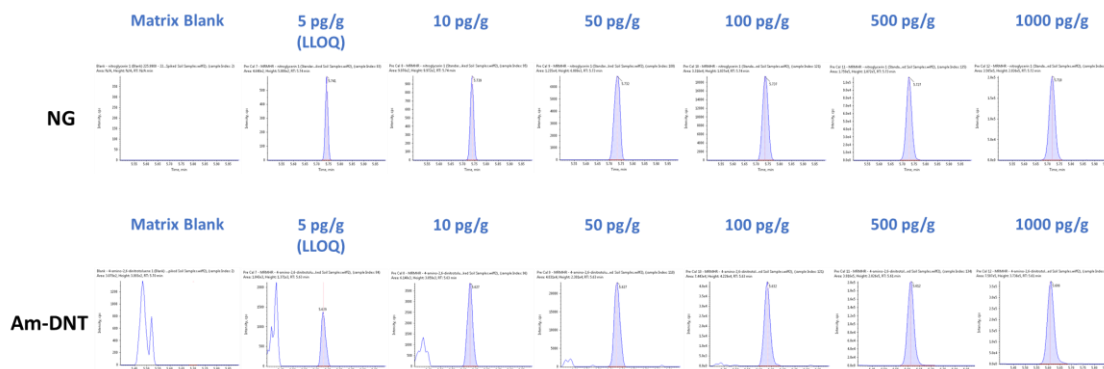


Figure 4. Representative extracted ion chromatograms (XICs) for two selected explosives included in the panel. XICs for NG and Am-DNT from 5 to 1000 pg/g, including the blank injection generated using the MRM^{HR} workflow. The measurements showed excellent precision and accuracy across the calibration range for the 12 explosives targeted in this study.

Analytical performance of the MRM^{HR} workflow for explosive quantification

Accurate quantification of explosives was previously demonstrated by extracting unique fragment ions.¹ This approach benefits from added selectivity when the presence of high background in the TOF-MS spectra interferes with the analyte signal. The efficiency of the sample preparation procedure used in this experiment enabled the use of the unique TOF-MS adduct ions formed during ionization for quantification. Here, three replicate injections of the spiked soil samples at six calibration levels ranging from 1-1000 pg/g were performed to evaluate the linearity and the quantitative performance of the MRM^{HR} workflow. Figure 4 shows the representative extracted ion chromatograms (XICs) for A) nitroglycerin (NG) and B) 4-amino-4,6-dinitrotoluene (Am-DNT) from 5 pg/g (LLOQ) to 1000 pg/g. The signal shown for the 5 pg/g is well above the blank signal and represents the LLOQ for those two compounds. LLOQs were found to be ranging from 5 to 50 pg/g for the 12 explosives targeted in this study (Table 1).

The quantification performance of the MRM^{HR} workflow was investigated by calculating the accuracy and precision of measurements for all the explosives included in the panel across the entire concentration range, including at the LLOQ. Overall, the developed MRM^{HR} method showed excellent precision and accuracy across the reported calibration range, with CV% values below 20% and bias % values in the $\pm 15\%$ interval, respectively. Table 1 includes the precision and accuracy values at two calibration levels (25 and 50 pg/g).

Calibration curves were automatically generated in SCIEX OS software 2.0. Figure 5 shows the resulting regression lines plotted across concentrations ranging from 5 to 1000 pg/g. The calibration curves demonstrate excellent correlation and linearity with correlation coefficients (R^2 values) above 0.99 for all the 12 explosives targeted in this study.

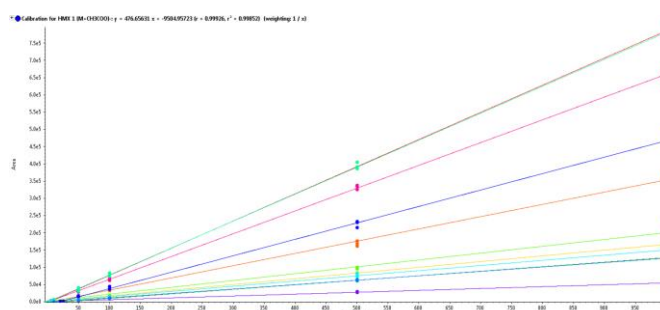


Figure 5. Excellent linearity for the 12 explosives targeted in this study. Regression curves resulting from the calibration series for the 12 explosives compounds across concentrations ranging from 5 to 1000 pg/g. The assay showed excellent linearity with R^2 values greater than 0.99 for all the explosives.

Rapid and efficient sample preparation method yields high explosives recovery

One critical aspect associated with the analysis of explosives in the environment is the presence of matrix interferences that can affect the performance of the assay. As a result, it is critical to use an efficient extraction method that can remove the many matrix components present in soil and optimize the analyte recovery while minimizing sample pre-treatment time. The efficiency of the sample preparation procedure was investigated by calculating the explosives recovery values at two concentration levels (25 and 50 pg/g). The analyte recovery values were calculated for each of the 12 explosives by dividing the average ($N=3$) peak area of the pre-spiked sample by the average peak area of the post-spiked sample at both concentration levels. The explosives recovery values at the two concentration levels ranged between 62 and 98%, demonstrating the efficiency and high-recovery of the sample preparation method.

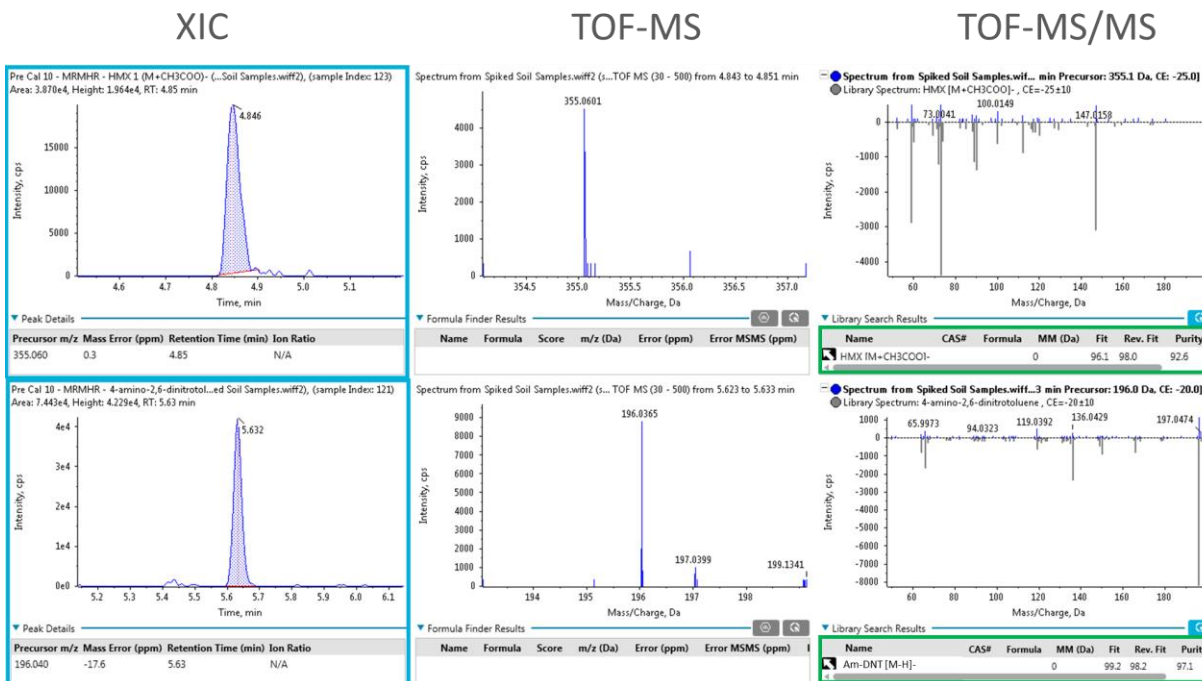


Figure 6. MRM^{HR} workflow enables accurate and confident identification of the targeted explosives extracted from soil samples. XICs, TOF MS and MS/MS spectra with library search match showing confident and detailed identification of HMX (top) and Am-DNT (bottom) spiked in a control soil sample at 50 pg/g. The excellent MS/MS fit values of 96.1% for HMX and 99.2% for Am-DNT provide absolute confidence for the definitive identification of the two explosives.

In addition to yielding high explosives recovery, the sample preparation procedure provided excellent reproducibility across the triplicate injections for the concentration levels. The measurement reproducibility (expressed as %RSD) ranged from 0.61% and 12.78%, proving the robustness and efficiency of the sample preparation procedure. The recovery values and measurement reproducibility values for the 12 explosives targeted in this study are summarized in Table 1.

High-quality MS/MS leads to accurate explosives identification

In addition to providing accurate mass product ions for quantification, the MRM^{HR} workflow also generated high-quality MS/MS spectra that were used for identification of explosives through spectral library matching. Figure 6 shows the TOF MS XIC, TOF MS and TOF MS/MS spectra with library search match showing positive identification of HMX and Am-DNT in the spiked soil samples. The reliable and reproducible fragmentation information generated can be used as an added level of confirmation for the accurate identification of explosives in soil samples. The library fit scores of 96.1% for HMX and 99.2% for Am-DNT indicate high confidence in the detection of those two explosive compounds from the spiked soil sample.

SWATH acquisition was also used to generate high-quality MS/MS spectra. Unlike MRM^{HR}, SWATH acquisition is an untargeted data acquisition method that sequentially acquires MS/MS spectra of all precursor ions across the mass range. This complete digital map of the sample allows retrospective data analysis, minimizing the risk of missing a critical component in the analysis when new information in the future arises. SWATH acquisition is particularly fitting for the study of explosives since they tend to form different types of adducts based on the types of additives used, the interface geometry, the temperature, the gas type and flow rates as well as the voltages used for the corona discharge.

Figure 7 shows two different XICs, TOF MS/MS spectra and resulting calibration curves for TNT, resulting from the ionization and subsequent fragmentation of TNT as two different adducts. Both species eluted around 5.7 minutes. Unlike other explosive compounds, TNT does not produce adducts with any of the reactant ions. Instead, TNT either ionizes by electron attachment or by proton abstraction. This is typical of nitro aromatic explosives like TNT where the NO₂ groups are attached to a benzene ring, and as a result are more stable than the NO₂ and NO₃ groups of other explosive compounds such as RDX and NG. Figure 7A shows the XIC of the precursor ion at m/z 226.011, interpreted as TNT [M-H]⁻ which is labeled in the TOF MS/MS spectrum along with the ions produced at m/z 210.0370 [M-OH]⁻ and m/z 195.999 [M-NO]⁻.

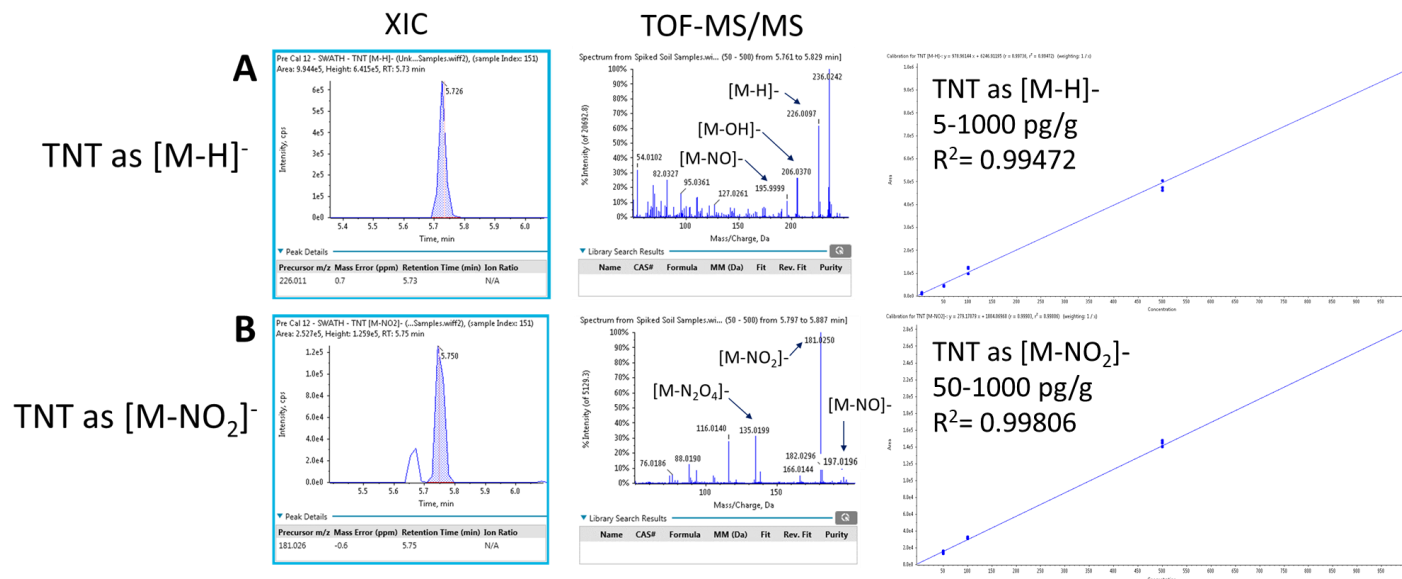


Figure 7. SWATH acquisition enables identification of unique fragment ions produced by the ionization of the explosives and subsequent fragmentation. XICs, MS/MS spectra and resulting calibration curves for A) TNT $[M-H]^-$ at m/z 226.011 and B) TNT $[M-NO_2]^-$ at m/z m/z 181.026 as well as unique fragmentation peaks resulting from TNT's ionization and subsequent fragmentation. SWATH acquisition enabled accurate quantification of these two species across the calibration range with excellent linearity (R^2 values above 0.99).

Figure 7B shows the XIC of the parent ion at m/z 181.026, interpreted as TNT $[M-NO_2]^-$, as well as the ions at m/z 197.020 $[M-NO]^-$ and m/z 135.020 $[M-N_2O_4]^-$. The regression lines show excellent linearity with R^2 values greater than 0.99 for both TNT $[M-H]^-$ and TNT $[M-NO_2]^-$, demonstrating that quantification of both TNT adducts was possible using SWATH acquisition. The results shown in Figure 7 demonstrate SWATH acquisition is a very useful tool for the analysis of explosives or other compounds where adduct formation is likely. Similar observations were observed for the analysis of RDX, another explosive targeted in this study. Figure 1 shows the detection of RDX as an acetate adduct $[M+CH_3COO]^-$ using the MRM^{HR} workflow and as a chlorine adduct $[M+Cl]^-$ using SWATH acquisition. The information extracted from the identification of these unique fragment ions can be further utilized for the development of targeted methods for explosives detection.

Conclusions

A robust workflow for the detection of 12 explosives extracted from soil samples was successfully developed using the SCIEX X500R QTOF system. The combination of a fast and efficient sample preparation procedure and acquisition of high quality MS and MS/MS spectra enabled accurate detection and identification of the explosives. The use of the MRM^{HR} workflow enabled sensitive and accurate quantification of the 12 explosives across six calibration levels ranging from 1 to 1000 pg/g. Reproducibility of injection (measured as %RSD) across two concentration levels was found to be below 15%. Excellent

linearity was also observed across the calibration range, with R^2 values above 0.99 for the 12 explosive compounds targeted in this study. Extraction recovery values were found to be between 62 and 98%, demonstrating the efficiency of the sample pre-treatment used in this workflow. The MRM^{HR} workflow generated high-quality MS/MS spectra which enabled accurate identification of the explosives using spectral library searching.

The untargeted SWATH acquisition approach was used to generate comprehensive TOF MS and MS/MS spectra on all detectable species, such that various adducts of the explosives could also be identified and quantified. This is important to ensure the assay provides an assessment of the total amount of an explosive type in a sample. The identification of different adducts and their unique fragment patterns from the MS/MS spectra can then be leveraged to develop new targeted workflows for future analysis.

Overall, the SCIEX X500R QTOF system with multiple different acquisition workflows was shown to be a flexible, fast, specific and sensitive platform for the analysis of explosives and residues commonly encountered in soil samples.

References

1. High throughput platform for confident identification and quantitation of organic explosives. [SCIEX technical note, RUO-MKT-02-7052-A.](#)

Table 1. Summary table for the analysis of the 12 explosives targeted in this workflow. The table includes compound name and abbreviation, precursor mass of the detected species, calibration range, linear correlation coefficient (R^2 value), as well as the reproducibility of measurement (%RSD) and the analyte recovery value (%) at the two calibration levels (25 and 50 pg/g).

Compound	Abbreviation	Precursor mass (Da) and molecular species	Calibration range (pg/g)	Linear correlation (R^2)	Calibration level (pg/g)	RSD (%)	Recovery (%)
Octahydro-1,3,5,7-tetranitro-1,3,5,7-triazine	HMX	355.06 as $[M+CH_3COO]^-$	25-1000	0.99852	25	2.04	72.62
					50	4.84	75.81
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	281.05 as $[M+CH_3COO]^-$	25-1000	0.99728	25	1.46	64.93
					50	3.73	95.61
2,4,6,N-tetranitro-N-methylaniline	Tetryl	241.02 as $[M+NO_2]^-$	50-1000	0.99675	25	6.85	94.38
					50	10.83	91.48
2,4,6-trinitrotoluene	TNT	226.01 as $[M-H]^-$	5-1000	0.99935	25	6.26	81.66
					50	4.83	78.39
4-Amino-4,6-dinitrotoluene	Am-DNT	196.04 as $[M-H]^-$	5-1000	0.99896	25	5.32	76.91
					50	12.29	75.15
Nitroglycerin	NG	226.03 as $[M-H]^-$	5-1000	0.99702	25	3.41	86.58
					50	10.51	91.74
Nitroguanidine	NQ	103.03 as $[M-H]^-$	25-1000	0.99969	25	1.57	91.87
					50	1.29	97.64
Ammonium Picrate	AP	227.99 as $[M-NH_4]^-$	10-1000	0.99634	25	1.03	86.70
					50	1.75	97.74
1,3,5-Trinitrobenzene	TNB	213.01 as $[M-NO]^-$	50-1000	0.99874	25	2.67	75.74
					50	1.57	96.39
2,6-Dinitrotoluene	2,6-DNT	181.03 as $[M-H]^-$	10-1000	0.99884	25	9.50	76.91
					50	12.78	82.48
2,4-Dinitrotoluene	2,4-DNT	181.03 as $[M-H]^-$	10-1000	0.99859	25	5.81	87.90
					50	3.43	91.92
3,5-Dinitroaniline	3,5-DNA	182.02 as $[M-H]^-$	10-1000	0.99766	25	0.61	82.19
					50	3.31	97.94

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