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Qualitative Analysis of Post-blast Residue using the Double Hypphenated UHPLC-(HESI)-MS/MS Technique

التحليل النوعي لبقايا ما بعد الانفجار باستخدام تقنية الكروماتوغرافيا السائلة فائقة الأداء مزدوجة

الاقتران والمقترنة بمطياف الكتلة الترادفي مع التأين بالرداذ الإلكتروني

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Abstract

In this paper, the identification and forensic analysis of post-blast residues recovered from controlled blast sites has been presented. The targeted sample was extracted from post-blast soil by using the Accelerated Solvent Extraction technique. The average recovery varies from 86-93% at 250, 500, 750 and 1000 ng/g concentration level. The target compound was primarily identified to be PETN (Penta Erythritol Tetra Nitrate) by a color test and TLC (Thin Layer Chromatography). The confirmatory test for the target analyte was done by using LC-MS/MS (Liquid Chromatography - Tandem Mass Spectrometry). Heated Electron Spray Ionization (HESI) interface with negative mode was employed and SRM (Selected Reaction Monitoring) on Triple Quadruple Mass Analyzer was used for confirmation of PETN in the sample. The Limit of Detection (LOD) obtained from the analysis of post-blast residue by using this method was 7.9 ng/g. This manuscript demonstrates the viability of LC-MS/MS for a fast, accurate and quality-assured analysis of post-blast residue.

Keywords: Forensic Sciences, Accelerated Solvent Extraction, Explosives, Post-blast residue, LC-MS/MS.

المستخلص

تستعرض هذه الورقة طريقة كشف وتحليل جنائي لبقايا ما بعد الانفجار المستعادة من مواقع الانفجار الخاضعة للرقابة. فلقد تم استخلاص العينات المستهدفة من تربة ما بعد الانفجار باستخدام تقنية الاستخلاص المعجل بالمذيبات، حيث تراوح متوسط استرداد الاستخلاص بين 86-93% عند مستوى تركيز 250, 500, 750 و1000 ng/g. وحدد المركب المستهدف بشكل أساسي ليكون ليكون PETN (Penta Erythritol Tetra Nitrate) عن طريق الاختبار اللوني وكروماتوغرافيا الطبقة الرقيقة TLC. وأجري الاختبار التأكيدي للتحليل المستهدف باستخدام تقنية الكروماتوغرافيا السائلة المقترنة بمطياف الكتلة الترادفي LC-MS / MS، واستخدم التأين بالرداذ الإلكتروني المسخن (HESI) بالوضع السليبي مع آلية الكشف بالرصد الأيوني المتعدد SRM لتأكيد وجود PETN في العينة. ولقد كان حد الكشف النوعي (LOD) الذي تم الحصول عليه من تحليل بقايا ما بعد الانفجار باستخدام هذه الطريقة 7,9 ng/g، وتوضح هذه الورقة جدوى العمل بتقنية LC-MS / MS لإجراء تحليل سريع ودقيق ومضمون الجودة لبقايا ما بعد الانفجار.

الكلمات المفتاحية: علوم الأدلة الجنائية، المتفجرات، الاستخلاص المعجل بالمذيبات، بقايا ما بعد الانفجار، الكروماتوغرافيا السائلة، مطياف الكتلة الترادفي.



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1. Introduction

The investigation of bomb incidents, either after the device has exploded or it has been rendered safe, is a requirement for the Criminal Justice System and national security. Forensic laboratory provides the investigator with valuable investigative leads, ultimately connecting the suspect to the crime. The laboratory can only examine what is recovered from the blast scene and submitted in the form of samples or evidence. Hence the collection of evidence plays an important role in the investigation and forensic analysis of post-blast residue. Evidence is used to aid in the solving of a case, to develop leads, provide or eliminate suspects, establish a connection to the suspect, establish matches such as tool marks, residue etc., substantiate a theory of the case and establish the device designer's "signature" [1,2]. The post-blast residue analysis gives information on the origin of the explosives used in the preparation of a bomb and gives law enforcement a specific direction for investigation.

Explosives are chemical compounds/ mixtures that contain a huge amount of potential energy. An explosion is accompanied with a release of heat energy, light, loud sound, blast pressure and occurs when these substances are exposed to mechanical or thermal shock [3-5]. An explosion in a controlled environment can be used for constructive applications such as mining, building demolition, pyrotechnics, carving (e.g. Mount Rushmore), controlling avalanches etc. Micro-explosions are even used medicinally to break-up kidney stones. The destructive effects of unrestrained explosions make explosives a very good choice for terrorists when carrying out illegal activities. For this destructive activity high secondary explosives are mostly used, such as RDX, TNT, PETN, PEK because of their high velocity of detonation (2000-8000 meter/second).

The forensic analysis of post-blast residue helps investigators and law enforcement agencies in narrowing down an investigation and unwrapping

terrorist activity. Forensic scientists face several challenges during the analysis of post-blast residue because of the limited availability of the targeted compound in the sample, i.e in picogram (10-12 gram) or femtogram (10-12 gram) level and the sample received for analysis is a soil sample from the blast site, which is a very complex matrix [6]. To overcome these challenges, reliable extraction techniques and sensitive instrumental procedures need to be used for the analysis of post-blast residue. Table-1 indicates the analytical techniques used for the analysis of explosives.

The LC-MS/MS is a double hyphenated analytical technique best suited for thermal labile non-volatile mixture compounds. The Liquid Chromatograph separates the components, and the Mass Spectrometer analyses the mass. There are certain explosives which possess almost similar molecular mass, such as; RDX & TATP (Molar mass 222.12 g/mol & 222.24 g/mol respectively), TNT & Nitro glycerine (227.13 g/mol & 227.08 g/mol). The MS/MS (Mass Spectrometer with tandem Mass) is capable of overcoming the difficulty in differentiating between two molecules that have similar molar mass by fragmenting the molecular ions and analysing the product/daughter ions. This analytical technique has proved to be very promising for its high resolving power, high mass stability, high mass accuracy, fast polarity switching and minimum scan to scan variance [11, 14]. There is no trade-off between resolution & sensitivity, both can be achieved in LC-MS/MS. The MS/MS technique is capable of detecting compounds in ppt level [15].

In this article, the important practical systematic procedure for the identification of post-blast residues of explosives are presented.

2. Materials and Methods

2.1. Standards and Chemicals

The reference standards of RDX (1,3,5-Trinitro-1,3,5-triazinane), TNT (Trinitrotoluene), DNT



Table 1- Analytical Techniques used for the analysis of Explosives [7-13].

Analytical Technique	Acronym	Targeted Explosive	LOD	Advantages	Drawbacks
Thin Layer Chromatography	TLC	Organic	$\mu\text{g/g}$	Easy-to-use, Cost-effective	Low resolution and sensitivity, susceptible to contaminants
Ion Chromatography	IC	Inorganic	ng/g	Reliable	Only inorganic ions can be identified
Ion Mobility Spectrometry	IMS	Organic and inorganic	ng/g	High Sensitivity	The sample needs to be ionized
High Performance Liquid Chromatography with UV detector	HPLC-UV	Organic	ng/g	Moderate Sensitivity	Moderate Selectivity
Gas chromatography with electron capture detector	GC-ECD	Nitro-group Explosives	pg/g	Fast, High Selectivity, High Sensitivity	Requirement of volatile analytes, insensitivity to hydroxy carbons
Fourier Transform Infrared Spectroscopy	FTIR	Organic and inorganic	pg/g	Universal	Complex Spectra
Mass Spectrometry	MS	Organic and inorganic	pg/g to ng/g	High Sensitivity, High Selectivity	Difficulty in interpreting the spectra
Gas chromatography-mass spectrometry	GC-MS	Organic	pg/g to ng/g	High Sensitivity, High Selectivity	Requirement of volatile analytes
Direct-Analysis-in-Real-Time and Mass Spectrometry	DART-MS	Organic	pg/g	High Sensitivity	Difficulty in interpreting the spectra
Liquid Chromatography - Tandem Mass Spectrometry	LC-MS/MS	Organic	pg/g to fg/g	High Sensitivity	Complex Spectra

(2,4-Dinitrotoluene), PETN (PentaErythritol TetraNitrate), Nitro Cellulose, Nitro Glycerin, Cordite, Tetryl and three soil samples were received from the explosives cell, Delhi (pen name). Table-2 indicates the chemical and physical structures of these standards [9].

The chemicals alpha-naphthylamine, sulphanilic acid, acetic acid, sulphuric acid, diphenylamine, potassium hydroxide, ammonium nitrate and the solvents ethanol, methanol, acetonitrile and water were used for this analytical study. The chemicals were of ACS (American Chemical Society) grade with percentage purity $\geq 95\%$. The solvents used were of HPLC (High Performance Liquid Chromatography) grade. The Millipore membrane filter with a 47 mm diameter and 0.22 μm pore size was used for filtration of the sample.

2.2. Sample Description

The presence of different metallurgic inorganic materials, organic substances, liquids and gases affect the physical and chemical properties of soil, making it a complex matrix. The physical and chemical properties of soil interfere with post-blast residue analysis [12, 13]. Hence some of these properties from the post-blast soil sample (S1, S2, S3) were determined. Table-3 indicates the physical and chemical properties of post-blast soil samples.

2.3. Extraction of post-blast residue

ASE (Accelerated Solvent Extraction) method was used to extract the post-blast explosive residue from the soil sample. ASE is a solvent extraction system that uses a combination of tem-



Table 2- Structure of different explosive standards.

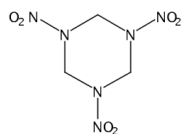
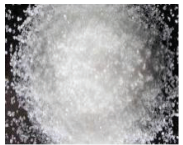
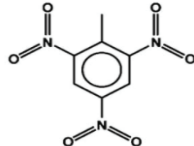

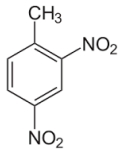

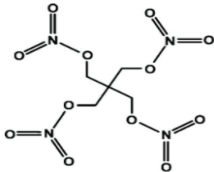

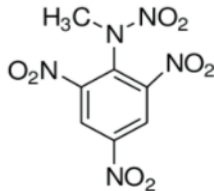

Standard	Chemical Name	Chemical Structure	Physical Appearance
RDX	1,3,5-Trinitroperhydro-1,3,5-triazine		
TNT	2,4,6-Trinitrotoluene		
DNT	2,4-Dinitrotoluene		
PETN	Pentaerythritol tetranitrate		
TETRYL	2,4,6-trinitrophenyl-n methylnitramine		

Table 3- Physical and chemical properties of post-blast soil samples.

Physical Property	Soil Sample (S1)	Soil Sample (S2)	Soil Sample (S3)
Type of soil	Sandy	Sandy	Sandy
Color	Black	Black	Black
PH	7.6	8.1	7.3
Soil moisture (%)	17	22	25
Bulk Density (g/cm ³)	1.4	1.55	1.6
Particle Density (g/cm ³)	2.7	2.55	2.7
Porosity	0.42	0.36	0.43



perature (RT or 40-200 °C), pressure (100 bar - fixed) and standard liquid solvents to extract solid and semi-solid samples (1-100 g). This extraction technique was preferred for this analytical technique as it requires a significantly lower amount of organic solvent, providing low cost per extraction and is able to extract the maximum target analyte. It takes less time and less manpower, providing a platform for automation. It is equivalent or superior to classical extraction methods like Soxhlet and sonication etc. In this study, Thermo Scientific Dionex ASE 350 Accelerated Solvent Extractor has been used for the extraction of explosives from the post-blast soil sample. Table-4 indicates ASE conditions for the extraction of unburned explosives from post-blast soil samples.

2.4. Preliminary analysis of post-blast residue by colour test

The analysis of post-blast residue by colour test offers a preliminary idea about the explosive material used in a bomb incident. In the present work,

Table 4- ASE conditions for the extraction of unburned explosives from post-blast soil samples.

ASE Condition	Value
System Pressure	1500 psi
Oven Temperature	1000C
Sample Size	5 g.
Oven Heat-up Time	5 min
Static Time	5 min
Flush Volume	60%
Solvent	Acetone
Purge	150 psi N ₂ for 200 S
Static Cycle	1
Total Extraction Time	14 min
Solvent usage	20 l per sample

four reagents are used for the colour test of the post-blast soil samples (S1, S2 & S3). Table-5 indicates the method of preparation of reagents [16].

2.5. Tentative identification of post-blast residue by Thin Layer Chromatography (TLC)

TLC is the simplest separation technique well known for its rapidity, feasibility of visualizing all the components in UV light, cost effectiveness and less sample size [17]. In the present work, the post-blast soil samples (S1, S2 & S3) were tentatively identified by comparing with standards of RDX and PETN (as from the colour test the samples were suspected to be RDX or PETN). A precoated TLC plate with Silica Gel-G as stationary phase activated at 1100C in the pre-heated oven was used in this work. The spotted plate was developed using

Table 5- Reagent used in the colour test and its method of preparation.

Reagent	Method of preparation
Alcoholic Potassium Hydroxide (Alc. KOH)	10% of potassium hydroxide is prepared in 100 ml of ethanol.
Griess Reagent	Reagent is prepared using two Reagents A and B separately. Reagent A- 1% sulphanilic acid is prepared by dissolving 1gm of sulphanillic acid in 100 ml of 33% acetic acid. Reagent B- Dissolve 1gm of alpha-naphthylamine in 100ml of boiling distilled water, cool and decant the colourless supernatant liquid and mix with 110ml of glacial acetic acid.
Diphenyl amine (DPA) Reagent	1% solution of DPA is prepared using 1gm DPA in 10 ml of concentrated sulphuric acid.
Thymol test	A few crystals of Thymol dissolved in concentrated sulphuric acid.



the mobile phase Trichloroethane and Acetonitrile at 9:1. The plate was observed under UV light using both short and long wavelength after development. The developed plate was sprayed using 10% ethanolic solution of Diphenyl amine reagent followed by exposure of the plate to UV light for about 20 mins.

2.6. Confirmatory analysis of post-blast residue by LC-MS/MS

LC-MS/MS is a reliable technique for the trace analysis of post-blast residue [18-20]. The confirmatory test for the analysis of post-blast residue was performed by the LC-MS/MS system (M/s Thermofisher Scientific, Germany). In this system the TSQ Quantum™ Access MAX Triple Quadrupole Mass Spectrometer is interfaced with the Thermo Scientific Accela Ultra High-Performance Liquid Chromatography (UHPLC) system. The UHPLC system comprises of a quaternary pump with operating pressure up to 1250 bar, an autosampler with 200 sample positions and a C18 reverse phase column (Hypersil Gold, Dimension-50 x 2.1 mm, particle size-1.9 μm). The UHPLC system and the Triple Quadrupole Mass Spectrometer are

interfaced using Heated Electrospray Ionization system (HESI). The ions are detected by Electron Multiplier Tubes (EMT) in the mass spectrometer.

The solvents used in the preparation of the mobile phase are HPLC grade Methanol and water (Thermofisher Scientific). The HPLC grade Acetonitril (Thermofisher Scientific) is used for washing the autosampler. All the solvents, water and the extracted sample were further purified using a Millipore membrane filter (0.22 μm pore size) before applying them to the LC-MS/MS. The column temperature was set at 450C. The mobile phase consisted of 2.0 mM aqueous ammonium nitrate (Merck) and methanol. Table-6 indicates the gradient pump program developed for a better chromatographic separation. Table-7 indicates the optimized MS parameters or tune method data set for confirmation of PETN in the post-blast residue.

The highly pure N₂ gas used as the sheath gas (1.6 L/min, 3.8 x 10⁻⁵ torr) and auxiliary gas (1.2 L/min) are generated in the laboratory using a PEAK SCIENTIFIC NM30LA-MS Nitrogen Generator.

3. Results and Discussion

The observations of the colour test performed on the extracted sample are mentioned in Table-8.

Table 6- UHPLC Solvent Gradient pump programme.

S. No.	Time (min)	2.0 mM aqueous ammonium nitrate (%)	Methanol (%)	Mobile phase flow rate ($\mu\text{l}/\text{min}$)
1	0.0	95	5	300
2	4.0	50	50	300
3	7.5	5	95	300
4	8.5	5	95	300
5	8.7	95	5	300
6	10.0	95	5	300



Table 7- Tune Method Parameters for MS.

Tune Method Data	
Ion Source Type	HESI
Polarity	Negative
Spray Voltage	4 kv
Vaporizer Temperature	100 °C
Sheath Gas Pressure	40 AU
Ion Sweep Gas Pressure	0.0 AU
Auxiliary Gas Pressure	12 AU
Capillary Temperature	200 °C
Capillary offset	-35 V

Positive results were found when the post-blast soil samples (S1, S2 & S3) were tested with Griess Reagent, Diphenyl amine (DPA) Reagent and Thymol, dissolved in concentrated sulphuric acid.

The greenish grey spots were developed for extracted soil samples and standard PETN, while purplish grey with that of standard RDX. Figure-1(a) shows the developed TLC plate under UV light and Figure-1(b) shows the developed TLC plate after the application of the DPA reagent. The post-blast residue was tentatively identified to be PETN by the Thin Layer Chromatographic technique. The retention factor (Rf) was found to be 0.96.

The UHPLC column effluent was diverted to the HESI source at a flow rate of 300 μ l/min. In order to acquire the mass spectrum of the PETN standard and the extraction of the soil sample suspected to be PETN, Q1 scanning mode was performed. The retention times of the standard and sample were found to be 5.4 minutes each. The m/z with the highest relative abundance was found at 377 in each case. It was suspected to be the m/z of the PETN +Nitrate group. To confirm this an MS/MS analysis

was done for 377 precursor ions. By SRM (Selected Reaction Monitoring) scan mode, the retention time for NO₂ at m/z 62 was found to be 5.4 minutes. Hence the m/z 377 (considered 378, as negative ESI mode is used) was confirmed to be [PETN (316 g/mol) +Nitrate (62 g/mol)]. Table-9 indicates the MS/MS conditions for the identification of the selected analyte in LC tandem MS. Figure-2(a) shows the mass chromatogram of PETN standard. The retention time of the PETN standard was found to be 5.4 minutes. The Selected Reaction Monitoring (SRM) scan of PETN standard for mass 377.9 is shown in Figure-2(b). Figure-3(a), 4(a), 5(a) presents the mass chromatogram of extracted soil samples S1, S2 & S3, respectively. It is clear from the chromatogram that these three extracted soil samples S1, S2 & S3 have the same retention time, i.e., 5.4 minutes which is similar to the retention time of the PETN standard. Figure-3(b), 4(b) & 5(b) shows the mass spectra of the peak RT 5.4 of extracted soil samples S1, S2 & S3, respectively. For further confirmation, the SRM scan of product ion, i.e., NO₃⁻ ion was done. The chromatogram and SRM scan of NO₃⁻ ion are shown in Figure- 6(a) & 6(b) respectively. The retention time of the product ion, i.e., NO₃⁻ ion was also found to be 5.4 minutes.

The chromatogram of the standard PETN solution was compared with the chromatogram of the blank acetone, and the chromatogram of the post-blast soil extract was compared with the

chromatogram of the blank soil extract for each analyte, to check the specificity. There was no interaction observed between the matrix and solvent at retention time 5.4 minutes. The recovery from this technique was found by the comparison of four pre-extracted & post-extracted samples, analyzed at four different concentrations, i.e., 250, 500, 750 and 1000 ng/g. The average recovery ranged from



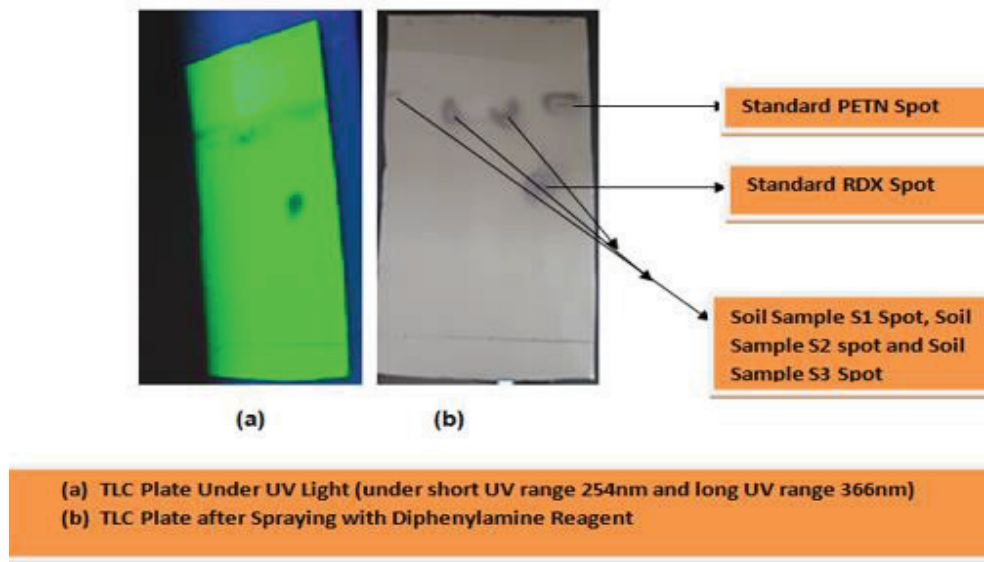


Figure 1(a, b)- Thin Layer Chromatography of post-blast soil samples.

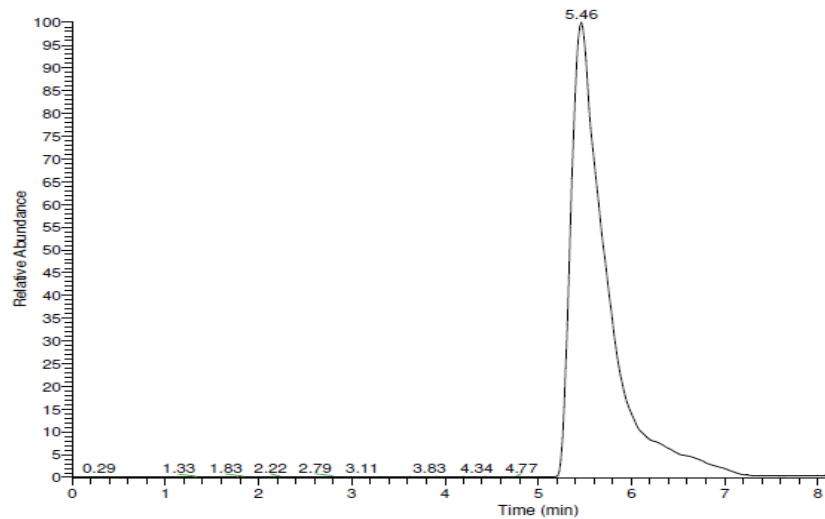


Figure 2(a)- Mass Chromatogram of PETN standard.

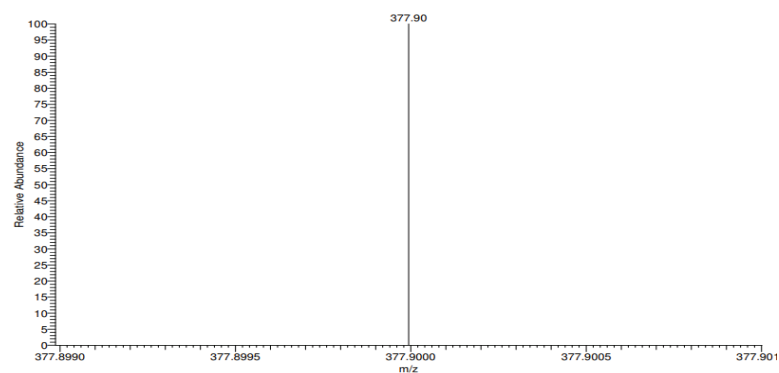


Figure 2(b)- Selected Reaction Monitoring (SRM) scan of PETN Standard for m/z 377.



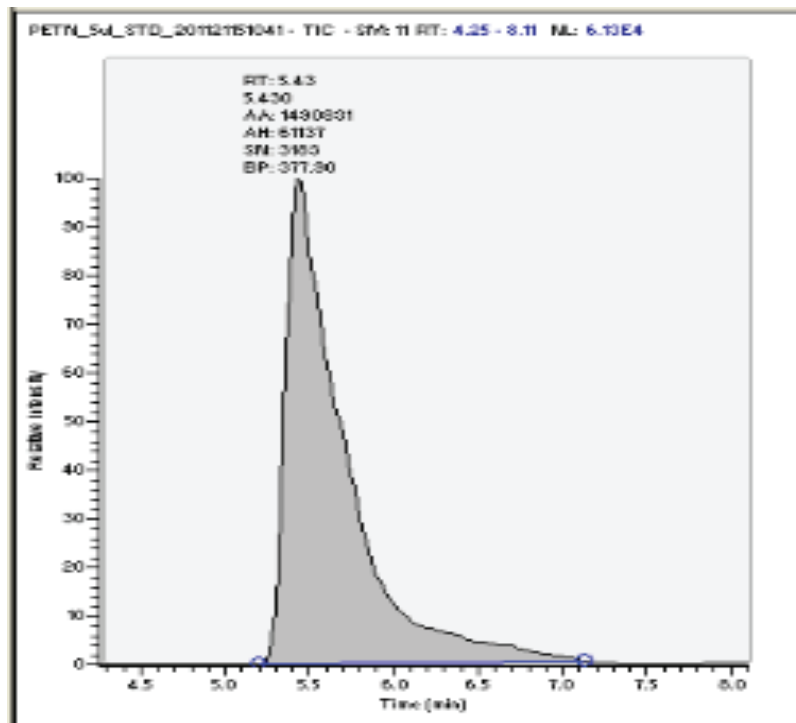


Figure 3(a)- Mass Chromatogram of extracted soil sample S1.

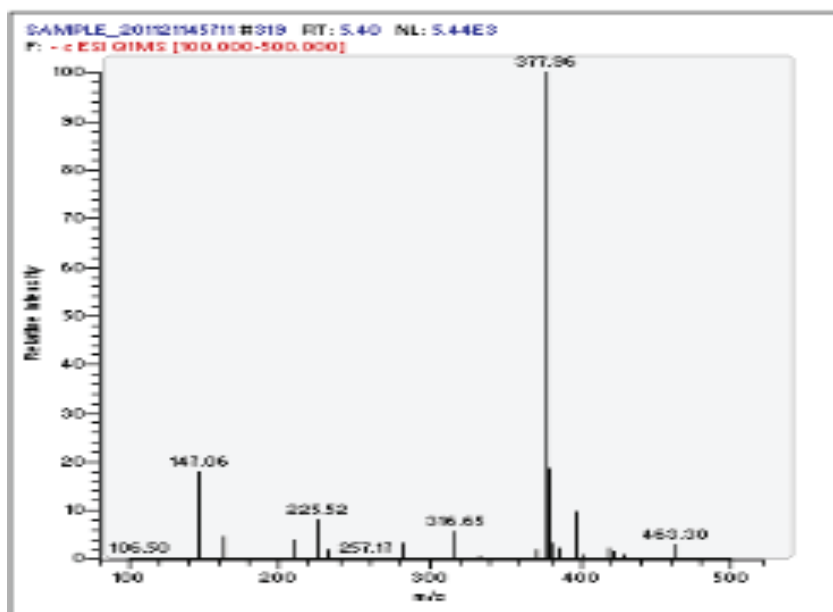


Figure 3(b)- Mass Spectra of extracted soil sample S1.



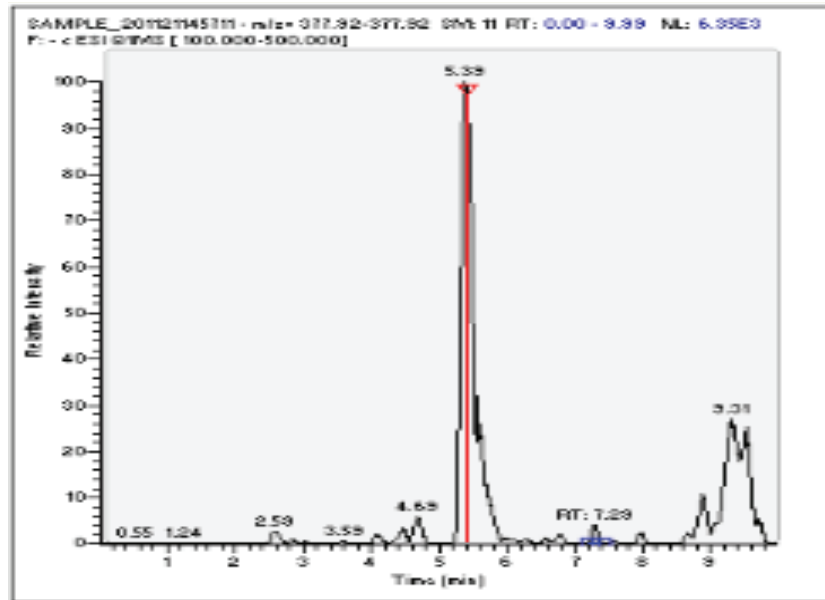


Figure 4(a)- Mass Chromatogram of extracted soil sample S2.

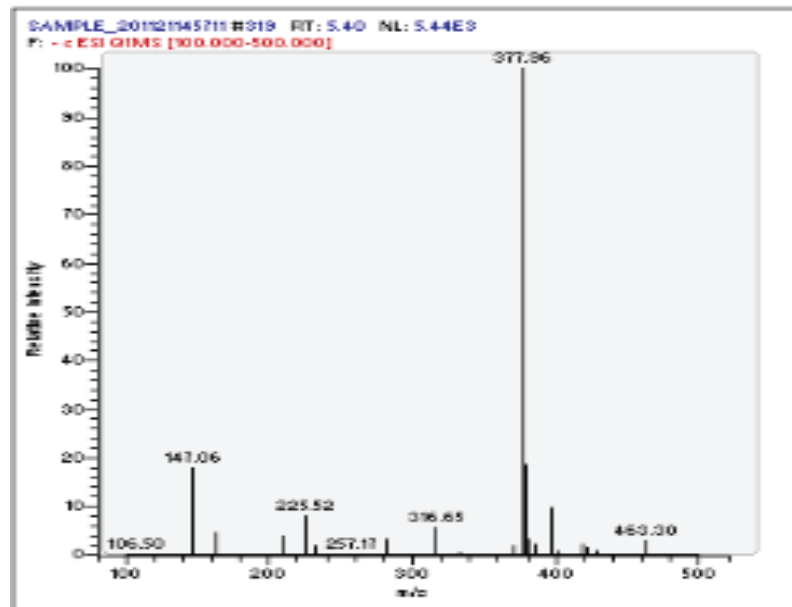


Figure 4(b)- Mass Spectra of extracted soil sample S2.



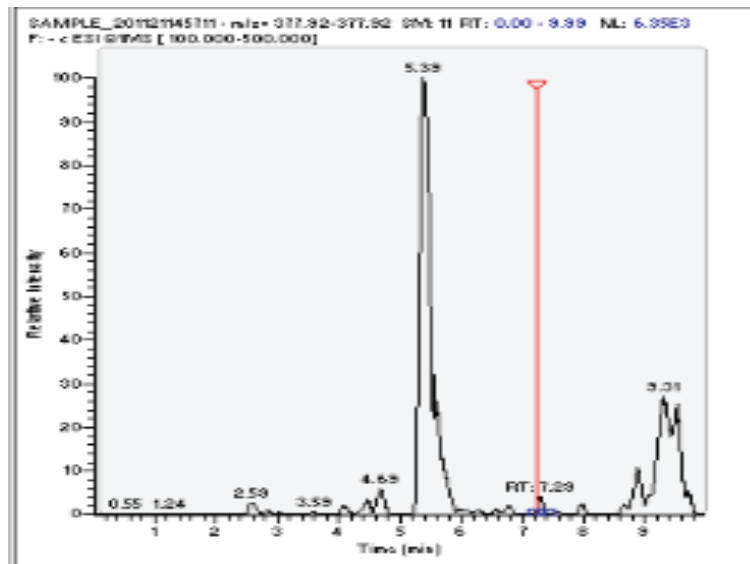


Figure 5(a)- Mass Chromatogram of extracted soil sample S3.

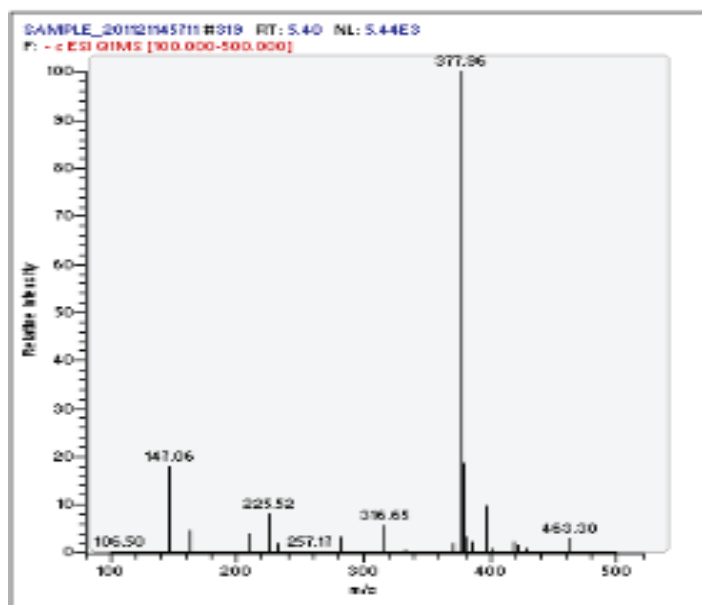


Figure 5(b)- Mass Spectrum of the peak RT 5.40 of extracted soil sample S3.



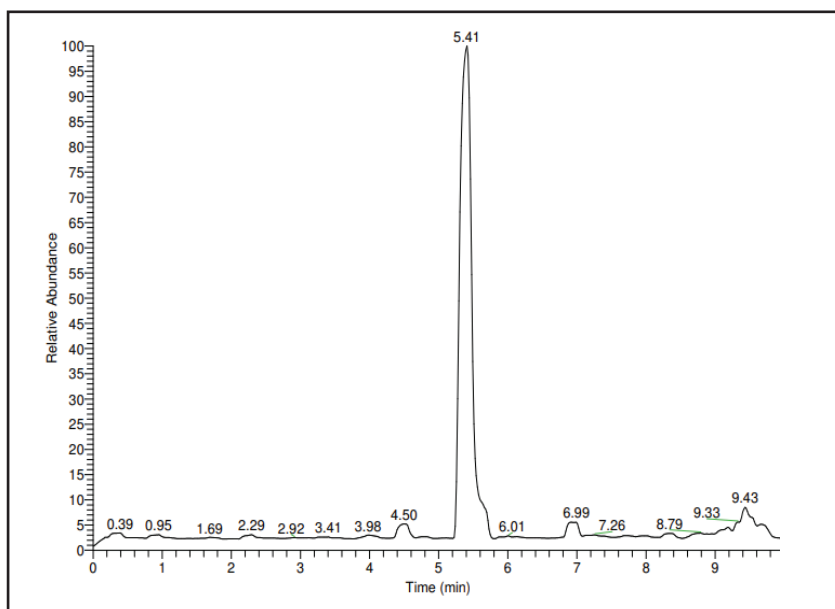


Figure 6(a)- Chromatogram for Nitrate Ion.

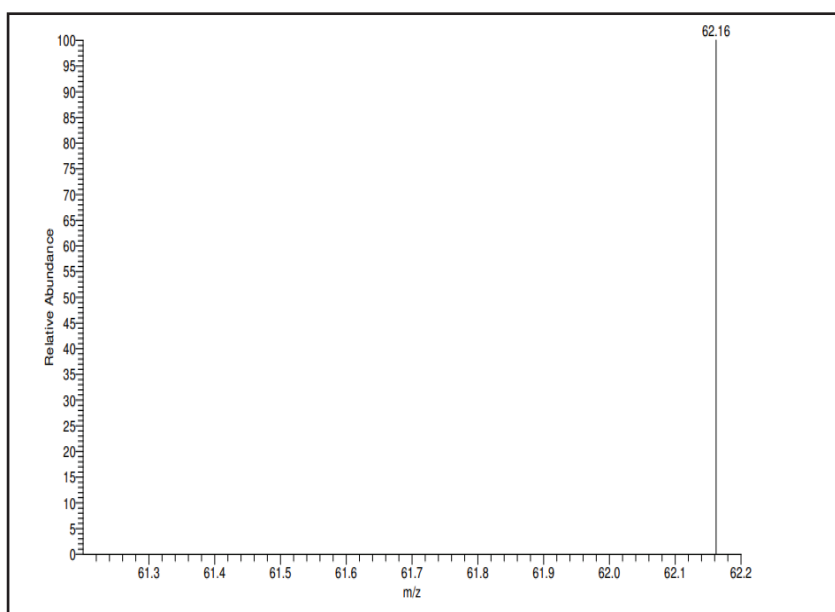


Figure 6(b)- Selected Reaction Monitoring (SRM) scan of Nitrate Ion at RT 5.40.



Table 8- Observations of the colour test performed for preliminary identification of post-blast residue.

S.No.	Tests Performed	S1	S2	S3	Control (Reagent)	Standard	Observation
1	Reagent Alc. KOH	No colour develops	No colour develops	No colour develops	Colourless	TNT- Red violet colour appeared DNT- Red colour appeared	TNT, DNT are absent
2	DPA Reagent	Blue colour appeared	Blue colour appeared	Blue colour appeared	Colourless	TNT- No colour develops DNT- No colour develops RDX- Blue colour appeared PETN- Blue colour appeared	RDX/ PETN may be present
3	Griess Reagent	Light Pink colour appeared	Light Pink colour appeared	Light Pink colour appeared	Colourless	RDX- Light Pink colour appeared PETN- Light Pink colour appeared	RDX/ PETN present
4	Thymol test	Light Green colour	Light Green colour	Light Green colour	Creamy yellow colour	RDX- Pink/Rose red colour appeared PETN- Greenish yellow colour appeared	PETN may be present

Table 9- MS/MS conditions for the identification of selected analyte in LC tandem MS.

Compound	Source	Polarity	Source Temperature	Delustering Potential	Collision Energy	Q ¹ (amu)	Q ³ (amu)	Q ¹ Ion	Q ³ Ion
PETN	HESI	Negative	2000C	-30	-28	377	62	C5H-8N4O-12Cl-	NO3-

83-96%. Repeatability was confirmed by analysing four sets of samples, with four injections for every sample. The Limit of Detection (LOD) was found based on a signal-to-noise ratio (S/N) approach. The LOD was 7.9 ng/g at 3:1 signal-to-noise ratio.

4. Conclusion

Post-blast residue analysis is the most challenging field in forensic science. Forensic scientists face

difficulties in the extraction of unburned explosives available in pico-gram level in complex soil matrices and their purification. This paper describes the extraction and purification method for the extraction of explosives from complex soil samples by the Accelerated Solvent Extraction (ASE) technique. The LC-MS/MS used in this work has proved to be an efficient technique for the confirmatory analysis of post-blast residue, due to its high accuracy, preci-



sion, resolution, selectivity, sensitivity, rapidity, and reproducibility. The high separation efficiency of UHPLC and the high specificity of MS/MS make it more reliable than other confirmatory techniques. The greatest challenge faced in using LC-MS/MS for post-blast debris analysis is the complexity in interpretation of mass spectra. Therefore, in the present study the targeted analyte was confirmed using the SRM scan mode available in the Triple Quadrupole Mass Spectrometer.

In conclusion, we herewith report that, the ASE technique is the best extraction technique for the extraction of post-blast residue from soil samples, and LC-MS/MS is applicable for the qualitative analysis of post-blast residue because of its ultra-high sensitivity.

Conflict of interest

The authors declare that there is neither any financial nor non-financial conflict of interest, nor any personal relationships that appear to influence the present work.

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