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Optimizing Forensic Detection of Explosive Substances: Extended Column Analysis of TNT

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Abstract: ***Trinitrotoluene (TNT) is a secondary high explosive substance which is found in many explosive compositions. It is used for military and industrial purposes; however, it is also misused for illegal and terrorist activities. Forensic analysis for detection of TNT in pre and post blast explosion samples is important for identifying the antisocial elements and tracking the source of their illegal activities. Traditional Gas Chromatography mass spectrometer instruments use short column having length of 15-meter for analysis of explosive substances due to their thermolabile nature. Our work presents multiple methods using longer column of 30-meter length for analysis of TNT. The same instrument can be used for multiple applications thus precluding the need of changing the GC column every time for analysis work of different types of the samples leading cost effective of the organisation.***
Keywords: Trinitrotoluene, GC-MS, Post-Blast, 30m Column, Cost effective.

I. INTRODUCTION

Trinitrotoluene (TNT) is the widely used high explosive with various military and certain industrial or commercial applications in the form of melt cast explosives for mortars, grenades, artillery rounds, warheads, and antipersonnel mines [1]. Apart from legal applications, TNT is also used for destructive applications like terrorist activities and illegal activities. Explosion debris from unidentified bombings and suspected explosive materials are forwarded to the Central Forensic Science Laboratories from various central law enforcement agencies and state agencies as well. This necessitates analysis of exhibits for detecting presence of various explosives in them [2]. Bombings suspected of using TNT explosive compositions require targeted analysis of this high explosive compound using various methods like colour tests, Thin Layer Chromatography (TLC) and instrumental methods like Fourier Transform Infrared Spectroscopy (FTIR), High Performance Liquid Chromatography (HPLC)[3]. Using these methods required simultaneous analysis of a traceable certified standard material which may not be available at each and every forensic establishment. Hence, in such a scenario use of a Mass Spectrometer (MS) can serve as a better alternative.

MS analysis is based on mass to charge (m/z) ratio of the charged molecular entity. Hence, it is a definite identification method. Combining chromatography method with MS gives an added advantage to analyse complex matrices such as that found on the explosion site. A chromatographic method helps to separate the complex mixture and the MS allows to identify the individual components of the mixture [4]. By hyphenating Chromatography with MS, two such methods can be used for explosive analysis: Gas Chromatography-Mass spectrometry (GC-MS) and Liquid Chromatography – Mass Spectrometry (LC-MS). The methods for analysis of forensic exhibits are based on GC-MS and LC-MS, however due to thermolabile nature of explosive compounds, LC-MS has an obvious advantage over GC-MS based methods [5]. However, LC-MS may not be available at all forensic facilities and in such places reliance may have to be made on GC-MS methods equipped with strong and latest library databases due to its wide applications in various forensic chemical analysis. Hence, GC-MS has also proved to have several benefits over other methods [6].

GC-MS has been used in different ionization modes such as Electron Ionisation (EI), Chemical Ionisation (CI) and Negative ion Chemical Ionisation (NCI), but the basic form is EI which is a hard ionisation method resulting in fragmentation of the analyte molecule. The fragment ions and the molecular ion results in different peaks on the mass spectrum which is like a fingerprint pattern of the analyte molecule. Thus, EI mass spectra can be used for definite identification of the analyte [4]. Many methods in past have relied on the usage of a short GC column for quick separation and elution of thermally labile compounds like high explosives [7,8,9,10,11,12].

However, a GC-MS system in the forensic labs is used for multiple other forensic applications most of which make use of a standard 30m column. Using a 15 m and 30 m column on the same GC-MS instrument will force the analyst to change the GC column depending on the type of sample used. This is a time consuming, tedious and costly exercise which may be resorted to only in unavoidable circumstances.

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Purchasing a dedicated GC-MS instrument for analysis of explosive substances may not be feasible. Hence a better option is to explore methods using the standard 30m column for explosives analysis to allow cost efficient usage of the available instrument.
The present work is one such attempt to develop a method for detection of TNT in forensic exhibits using the 30m column. In past such GC-MS methods have been used to detect TNT and its degradation products at trace levels in various matrices such as marine samples, surface water, soil and ground water from environmental pollution and remediation perspective. [11,13,14,15,16,17,18,19]. An ,attempt to apply such methods to TNT detection in forensic analysis of pre and post blast debris is essential from the explosion investigation perspective.

II. MATERIALS AND METHODS

A. Chemicals and Preparation of Solutions

Known TNT sample has been maintained in our laboratory for qualitative purposes. TNT solution was prepared by dissolving 5 mg known TNT sample in 10ml Acetone solvent. Acetone (AR) solvent was obtained from Advent Chembio Pvt. Ltd., Navi Mumbai. 10% Alcoholic KOH solution was prepared by dissolving 10g potassium hydroxide (KOH) in 100ml absolute alcohol. Potassium Hydroxide (Extrapure AR) was obtained from Sisco Research Laboratories Pvt. Ltd., Taloja and Absolute alcohol (Ethanol 99.9% v/v) was obtained from Changshu Hongsheng Fine Chemical Co. Ltd., Jiangshu province. Filter Paper with 0.2mm thickness, 2–3-micron particle size was obtained from Clairofilt (India), Bhiwandi.

B. Extraction of TNT from forensic case sample

Soil Sample from post blast site was forwarded to CFSL Pune for forensic analysis. The sample was taken in a glass beaker. It was extracted with two portions of 50ml acetone by continuous stirring with a glass rod. The acetone extracts were passed through the Whatman-42 filter paper and combined. The combined acetone extract was dried at room temperature. The residue obtained was reconstituted in 5ml acetone and used for colour test and GC-MS analysis.

C. Colour test for detection of TNT

Alcoholic KOH solution was used as reagent for colour test. It was performed on a ceramic spot plate by adding one drop of reagent to a drop of extract which was first dried on the spot plate. A blank and known TNT solution was tested in similar manner. The colour produced by the known TNT sample and extract were noted immediately and compared. Blank test is also conducted simultaneously. Alcoholic KOH solution gives purple red colouration in presence of TNT.

D. Fourier transform infrared spectroscopy (FTIR) of Known TNT sample

Known TNT sample was analysed on a Thermo Fisher Scientific Nicolet iS20 FTIR spectrometer instrument equipped with an IR source, an attenuated total reflectance (ATR) accessory, a DTGS detector and KBr beam splitter from Thermo Fisher Scientific. The instrument was operated at resolution of 4.000 between wavenumber 4000.12 cm-1 to 525.03 cm-1. The analysis was done by scanning background and sample using Thermo Scientific OMNIC software. The sample was scanned for 64 times and a characteristic spectrum was obtained. The spectrum was searched using correlation search type in the libraries of the instrument to identify the sample.

E. GC-MS Analysis of Known TNT sample and Extract

GCMS analysis was performed on Thermo Scientific trace 1600 series Gas Chromatograph coupled with Thermo Scientific ISQ 7610 Single Quadrupole mass spectrometer as detector. Sample Injection was performed using Thermo Scientific AI/AS 1610 Autosampler and split-splitless injector (SSL) in split mode. Chromatographic separation was carried out on a Thermo Scientific TG-5MS column of 30 metres length, I.D. 0.25mm and film thickness 0.25 micron. Helium with 99.999% purity was used as carrier gas. Thermo Scientific Standard Instrument Integration (SII) for X calibur Software was used to control the instrument, acquire data and perform integration of the data. The instrument is equipped with NIST Library for identification. The MS was operated in Electron Ionisation (EI) mode in Positive ion polarity at 70eV. The data was obtained in full scan mode from 40 to 500 amu with 0.2 sec scan time. Solvent delay of 3.5 min was used in all the methods.
Six different Instrument Methods (HE-1, HE-2, HE-3, HE-4, HE-5 and HE-6) were adapted from earlier works reported in environmental pollution and remediation field for detection of TNT and its degradation products [11,14,15,16,18,19]. These

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Instrument Methods were applied in the present work after making a few modifications in various parameters for analysis of TNT for post blast forensic case sample. Minor modification was necessary to suit the feasibility of analysis using the above instrument. The Total Ion Chromatogram (TIC) and mass spectra of the blank (Acetone), known TNT sample and the extract of the forensic case sample were obtained using all six methods to check their applicability to the forensic samples. The details of the instrument methods used are given in Table-1.

Table-1. Details of the instrument methods (HE-1, HE-2, HE-3, HE-4, HE-5 and HE-6)

PARAMETERS	INSTRUMENT METHODS					
	HE-1	HE-2	HE-3	HE-4	HE-5	HE-6
MS transfer line temperature [°C]	250	300	260	250	240	250
Ion source temperature [°C]	250	194	230	230	240	200
Total Run time [min]	6.250	30.000	25.400	11.000	9.333	19.500
Inlet Temperature [°C]	230	300	250	270	250	250
Carrier Flow [ml/min]	1.000	1.000	1.000	1.000	1.000	0.900
GC Oven Temperature Nominal	Start at 100.0 °C hold for 0.2 min	Start at 60.0 °C hold for 1.0 min	Start at 40.0 °C hold for 2.0 min	Start at 70.0 °C hold for 1.0 min	Start at 190.0 °C hold for 1.0 min	Start at 100.0 °C hold for 2.0 min
	Ramp to 220 °C at 30 °C/min	Ramp to 300°C at 10 °C/min	Ramp to 200 °C at 10 °C/min	Ramp to 250 °C at 20 °C/min	Ramp to 240 °C at 6 °C/min	Ramp to 200 °C at 10 °C/min
	Hold for 0.3 min at 220 °C	Hold for 5 min at 300 °C	Ramp to 260 °C at 25°C/min	Hold for 2 min at 250 °C		Ramp to 250 °C at 10 °C/min
	Ramp to 280 °C at 80°C/min		Hold for 5 min at 260°C			Hold for 5 min at 250°C
	Hold for 1 min at 280°C					
°C- degree Celsius, min-minutes						

III. RESULTS AND DISCUSSION

A. Characterisation of known TNT sample using FTIR Spectroscopy

The characteristic FTIR spectrum of known TNT sample obtained using the ATR-FTIR. The library search utility in the software was used to find the match for the tested sample. The IR spectrum of the known TNT and its library match is shown in Fig-1. A match value of 91.59% was obtained with 2,4,6-TNT (Index 4220 in HR Comprehensive Forensic FT-IR Collection Library), thus confirming the presence of TNT in the sample.

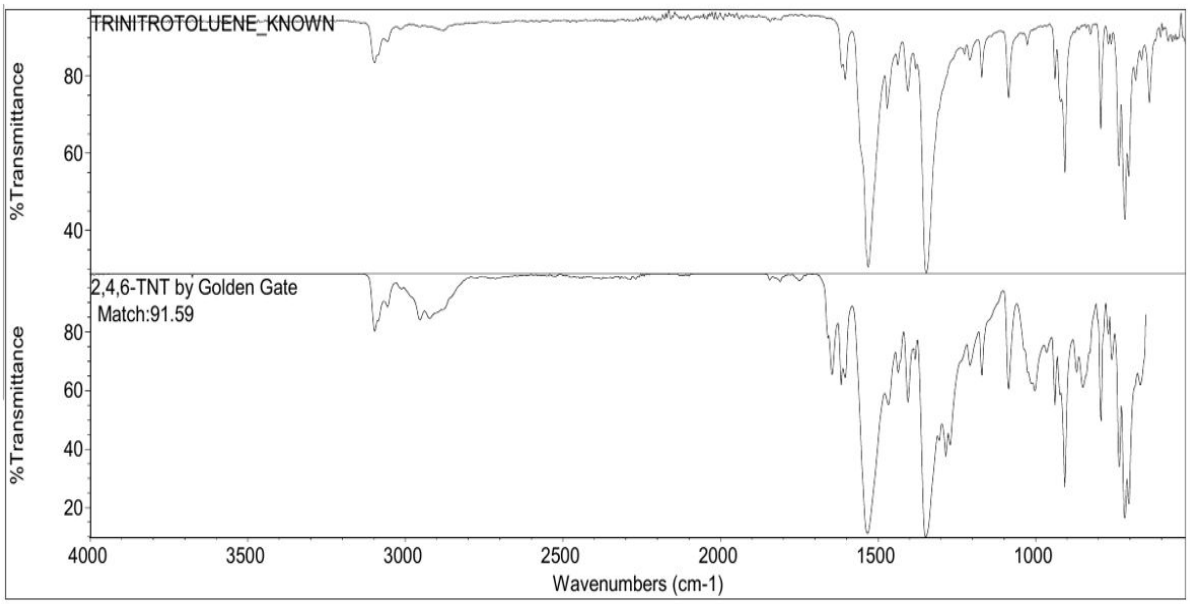

Fig-1. IR spectrum of the known TNT sample (top) and its library match (bottom)

B. Colour test of known TNT sample and Forensic Case sample

Polynitroaromatic compounds give coloured products in presence of the alkalis. Hence alkaline aqueous or alcoholic solutions can be used for detection of polynitroaromatic explosive substances such as Trinitrotoluene. Alcoholic KOH solution gives a purple red colouration indicating presence of TNT in samples. [20,21] The colour test gave positive reaction for both, the known TNT sample and the extract of forensic case sample indicating presence of TNT.

C. GC-MS Analysis of samples.

The known TNT sample and extract of the forensic case sample was analysed using the six different GC-MS methods (HE-1, HE-2, HE-3, HE-4, HE-5 and HE-6) entailed above. Same volumes of known TNT sample and extract of forensic sample were injected for analysis in each of the six methods, in order to obtain comparable results. The raw data obtained was subjected to processing method. The peaks were assigned retention time (RT) values and subjected to mass spectral library search to ascertain the identity of the compounds using their RT values mass spectral data.

The RT values of the peaks detected in the forensic case sample and the known TNT sample were compared (Fig-2 to 7). The RT values of the TNT peak in the known TNT sample and the forensic case sample matched in all GC-MS methods (HE-1, HE-2, HE-3, HE-4, HE-5 and HE-6).

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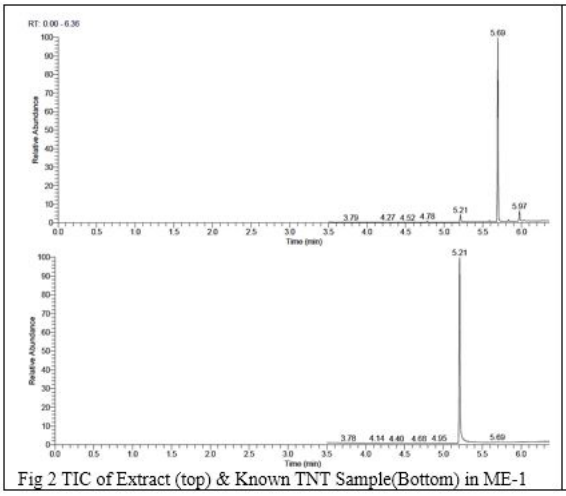

Fig 2 TIC of Extract (top) & Known TNT Sample(Bottom) in ME-1

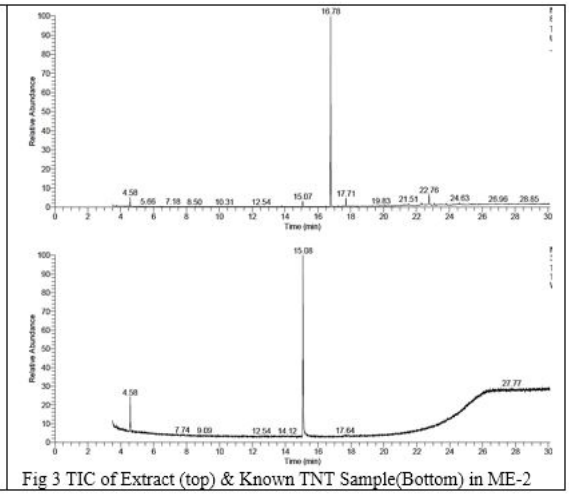

Fig 3 TIC of Extract (top) & Known TNT Sample(Bottom) in ME-2

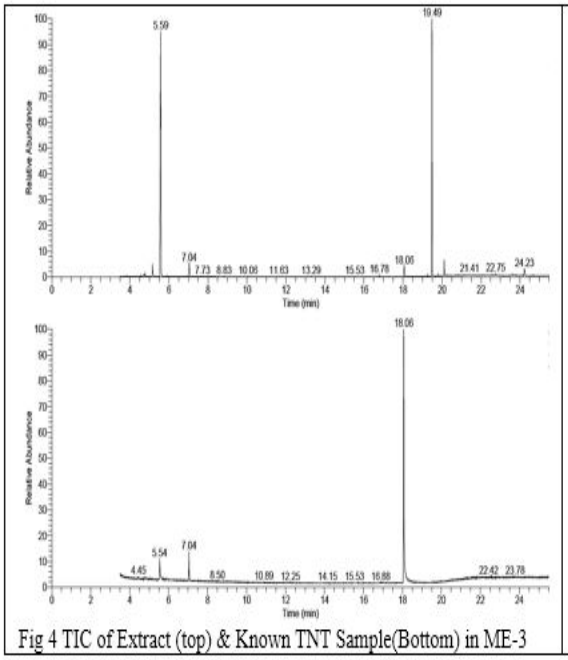

Fig 4 TIC of Extract (top) & Known TNT Sample(Bottom) in ME-3

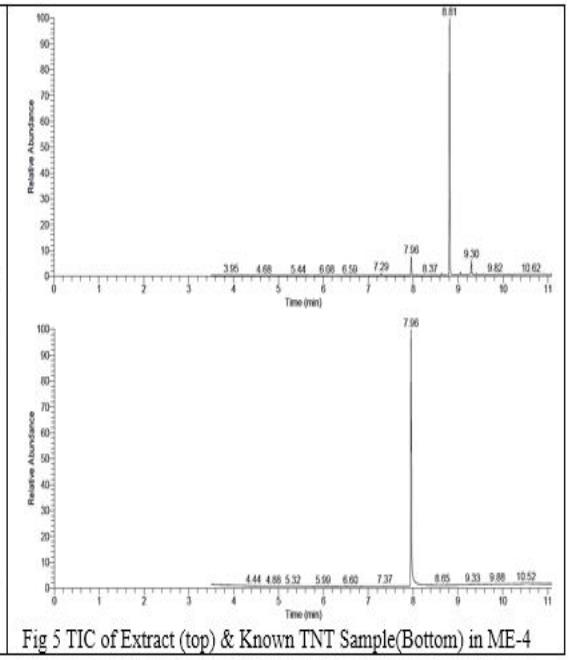

Fig 5 TIC of Extract (top) & Known TNT Sample(Bottom) in ME-4

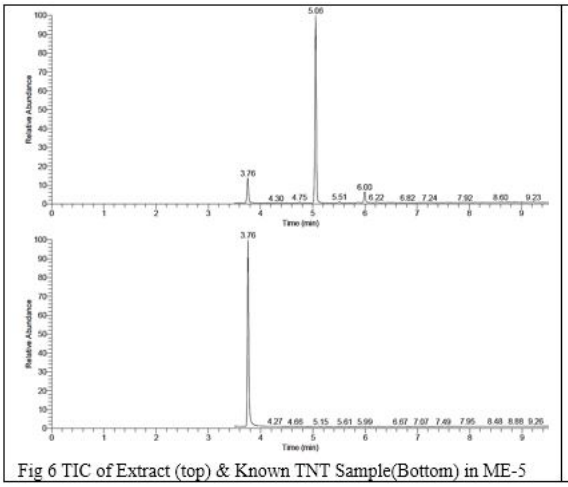

Fig 6 TIC of Extract (top) & Known TNT Sample(Bottom) in ME-5

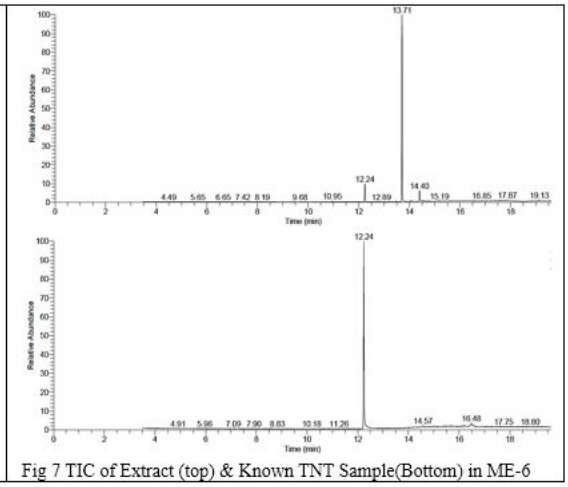

Fig 7 TIC of Extract (top) & Known TNT Sample(Bottom) in ME-6

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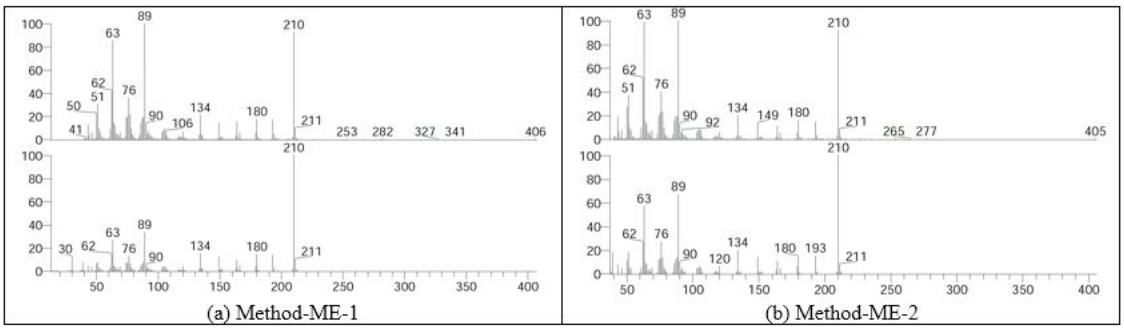
The study of the bottom TIC in Figs. 3 and 4 shows that additional peaks apart from that of TNT can be seen in case of methods ME-2 and ME-3, the known TNT sample did not give one single peak. The base line is not flat for these two methods in case of known TNT sample.

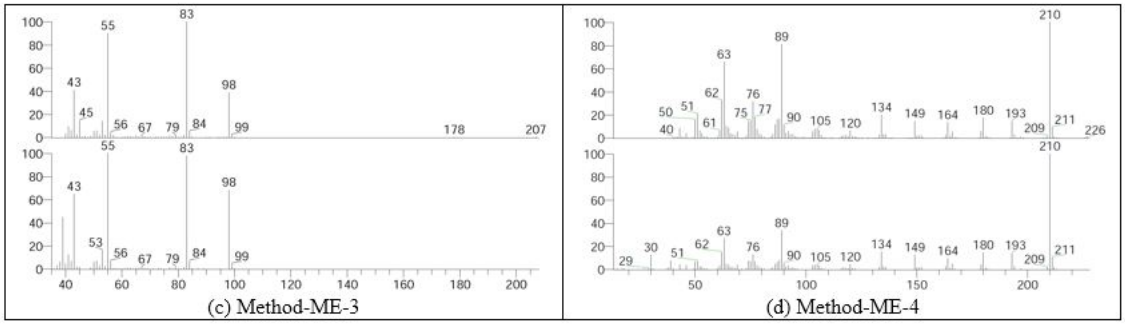
The further analysis involved submitting the obtained mass spectra of the known TNT sample and the forensic sample to a library spectrum search. The obtained mass spectral data was compared against, the library spectrum data and two values of match factor (SI) and Reverse match factor (RSI) were obtained. Based on these factors a hit list of compounds was generated which were identified by the library to be the possible matching chemical structures to the analyte in this study that is TNT. Higher the score of SI (Search Index) and RSI (Reverse Search Index) better the fitness of the obtained spectrum and the library reference spectrum. NIST uses a scale of 0 to 1000, however, no compound can exceed 999 fitness. As per the NIST library guidelines, score of 900 and above is an excellent mass spectral match, score of 800-900 is good mass spectral match, score of 700-800 is a fair mass spectral match and lastly score of less than 600 is a poor mass spectral match [22,23]. The library matches of the forensic case sample resulted in good match values of SI (Search Index) and reverse match value of RSI (Reverse Search Index) for the chemical compound Trinitrotoluene. The values of SI and RSI of library search for all the six GC-MS methods was above 800 as can be seen from table 2. Trinitrotoluene was the first hit for all the library spectrum search conducted in our study. Thus, the spectral library search confirmed presence of TNT in the forensic case sample at good level of confidence based on SI and RSI values for the Trinitrotoluene. The RT values of the known TNT sample and extract of forensic case sample in all six GC-MS methods, SI and RSI values for extract are tabulated in Table-2.

Table-2. RT values, SI & RSI values in methods HE-1, HE-2, HE-3, HE-4, HE-5 and HE-6

METHOD	Run Time [min]	RT VALUE OF KNOWN TNT SAMPLE[min]	RT VALUE OF TNT DETECTED IN EXTRACT[min]	SI VALUE OF EXTRACT	RSI VALUE OF EXTRACT
HE-1	6.25	5.21	5.21	912	924
HE-2	30.00	15.08	15.07	918	942
HE-3	25.40	18.06	18.06	930	937
HE-4	11.00	7.96	7.96	935	941
HE-5	9.33	3.76	3.76	940	947
HE-6	19.50	12.24	12.24	939	956

The mass spectra of the forensic case sample obtained by analysis using the six GC-MS methods (HE-1, HE-2, HE-3, HE-4, HE-5 and HE-6) along with the TNT reference library mass spectra are shown in Fig-8.(a) to (f). The x axis presents abundance and y axis presents m/z values.





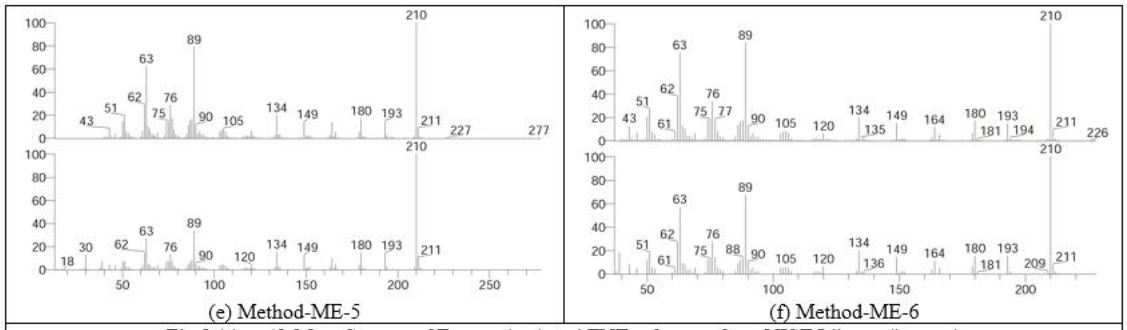


Fig 8 (a) to (f) Mass Spectra of Extract (top) and TNT reference from NIST Library (bottom)

Mass spectra of the forensic case sample and reference library mass spectra showed identical base peak and other major fragment ions peaks. The study of the reference mass spectra showed a very small molecular ion peak around 227 m/z and the base peak of 210 m/z. Other significant mass ion peaks were observed around 193, 134, 89, 63 and 30 m/z along with other minor mass ion peaks. The study of mass spectra of the extract from forensic case sample showed all these mass ion peaks close to these values thus confirming the presence of TNT in this sample. This is supported by the excellent SI and RSI values above 900 for all the methods.

IV. CONCLUSION

The known TNT sample was characterised using FTIR spectroscopy and it was confirmed to contain TNT. This TNT sample and unknown Forensic Case sample were simultaneously analysed with colour test and GC-MS methods. The results of colour test indicated presence of TNT in the forensic case sample. The GC retention times obtained using the six methods for known TNT sample and the unknown extract of forensic case sample matched thus identifying the presence of TNT in forensic sample. Further the library match of the mass spectra of the forensic sample resulted in hit list of compounds, first of which was Trinitrotoluene hence confirming the presence of TNT in forensic sample in all the GC-MS methods (HE-1, HE-2, HE-3, HE-4, HE-5 and HE-6).

The main aim of this study was to perform qualitative analysis of TNT using an extended GC column of 30 m length and optimization of GC-MS methods previously used in environmental and bio-remediation sample analysis to the forensic scenario. The detector employed was MS which itself is an identification technique.

Thus we used a hyphenated technique for identification of the detected analyte TNT in the forensic case sample. In case of qualitative analysis employing GC, identification of analyte (here TNT) is based on retention time (RT) and if the GC is coupled with MS, it is based on the spectral data. While using a hyphenated technique like GC-MS along with TNT known sample and comparing it with the unknown such as the forensic case sample, we actually confirmed the presence of TNT by using two analytical methods, not just one.

Here we have not only employed GC for separation, but also for identification on the basis matching of RT values using a known TNT sample. Further the mass spectral data at this RT value was subjected to library spectral matching using the NIST Library. Library search also resulted in identification of trinitrotoluene with excellent match as all the values of SI and RSI obtained were above 900.

This comparison of RT values and the library match and reverse match values in table 2 shows that TNT could be detected in forensic case sample by using all the six GC-MS methods (HE-1, HE-2, HE-3, HE-4, HE-5 and HE-6) studied in this work. Although various methods were used in previous works for detection of TNT in case of environmental and bio-remediation samples,

our work has optimized these six methods for detection of TNT in forensic post blast samples. Thus, qualitative identification of TNT could be achieved on the basis of studying the gas chromatography RT values and further confirmed on the basis of interpretation of the mass spectra library search results using all the six instrumental methods.

V. CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

VI. ABBREVIATIONS

TNT-Trinitrotoluene, FTIR- Fourier Transform Infrared, CFSL-Central Forensic Science Laboratory, TLC-Thin Layer Chromatography, HPLC- High Performance Liquid Chromatography, MS- Mass Spectrometry, m/z- mass to charge, GC- Gas Chromatography, LC- Liquid Chromatography, EI- Electron Ionisation, NCI- Negative Chemical Ionisation, mg- milli gram, mm- milli meter, pvt- Private, ltd- limited, AR- Analytical Reagent, v/v- volume / volume, ATR- Attenuated Total Reflectance, DTGS- Deuterated Triglycine Sulfate, HE- High Explosive, ID- Inner Diameter, TIC- Total Ion Chromatogram, MW- Molecular Weight, RT- Retention Time, EI- Electron Ionization, KBr- Potassium Bromide, cm- centi meter, AI- Automatic Injector, AS- Auto Sampler, min- minute, SI- Search Index, RSI- Reverse Search Index, NIST- National Institute of Standards and Technology, SII- Standard Instrument Integration, amu- atomic mass unit, TIC- Total Ion Chromatogram, eV- electron volt.

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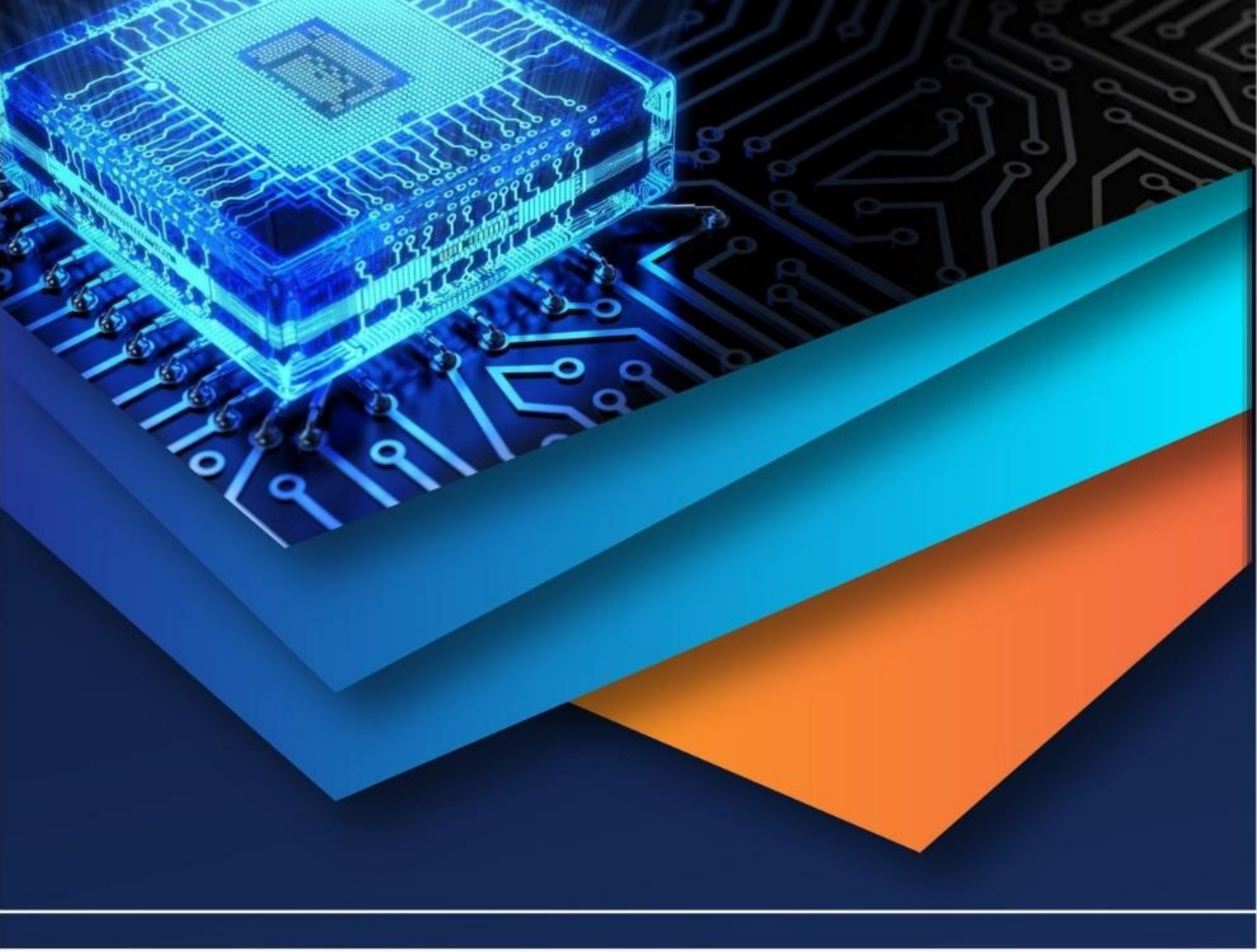

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