SUMMARY

The present work is devoted to the development of standard conditions and methods for the analysis of nitroexplosives viz., nitramine (e.g., HMX and RDX) and nitro aromatic explosives (e.g., TNT, Tetryl and metabolites of TNT) using high performance liquid chromatography (HPLC) with ultraviolet (UV) detection from various environmental samples. Many classes of organic explosives are semi or non-volatile and are best analyzed by HPLC. Along with method development on these instruments, various chromatographic parameters which are the characteristics of the separation of these compounds are also considered for the study. Solid phase microextraction (SPME) has evolved rapidly as a major sample pre-concentration technique with a wide application area. The number of SPME-HPLC applications is substantially less despite its potential. The present studies describes analytical methods that integrates the adsorption of HMX and RDX on SPME fiber for very sensitive and direct determination in aqueous matrices. In SPME-HPLC technique, desorption is carried out in an appropriate interface. New SPME-HPLC methods for the separation and determination of these explosives from soil and various aqueous samples have been developed. The separation of HMX and RDX was studied using direct injection of samples to HPLC and different chromatographic conditions like selection of suitable wavelength, effect of flow rate and composition of mobile phase were optimized. Aqueous solution of anionic surfactant sodium dodecyl sulphate (SDS) was used for the extraction of both nitramine high explosives viz., HMX and RDX from soil samples, which were subsequently sorbed on SPME fiber for analysis. Similarly, SPME-HPLC analysis was done for the study of TNT and its metabolites with applications on aqueous environmental and industrial effluent samples. The chapter wise summary of the work done is presented here.
CHAPTER 1: General Introduction

This chapter deals with general introduction to nitroexplosives selected for the study, their properties and environmental fate and toxicity in brief. The first part deals with the introduction to explosives. In the second and third parts of chapter, history and development of explosives and their classification based on different parameters are discussed, respectively. A brief introduction is presented on the polluting behaviour of explosives in the environment in next part. Special emphasis is given on considerable understanding the environmental fate, transport and toxicity of main organo-explosives like HMX, RDX, TNT and metabolites of TNT in fifth part. Major incidents of terrorist activities are tabulated in the subsequent chapter as menace of explosives and need of determination of explosives in forensic applications. A brief introduction is presented on methods for soil decontamination under use these days in seventh part of the chapter.

CHAPTER 2: Instrumentation and Sample Preparation

This chapter deals with analytical instrumentation and parameters involved during the analysis of explosives. Due to high polarity and low vapour pressure, detection of explosives in complex environmental matrices like soil, water and air is highly difficult and challenging and is discussed in first part. Next part is designed to cover the essentials of HPLC technique used to separate the organic explosives. An effort is made to summarize introduction, principle, types of liquid chromatography and instrumentation with proper figures and pictures. The third part provides an overview of the different physical parameters like partition coefficient for the distribution of the analytes in different phases, retention time, retention factor, separation factors and efficiency factor dealing with the separation of the analytes. The subsequent fourth part deals with the
need of sample preparation for efficient and fast analysis by the chromatographic
ingredients and comparative criticism of various sample preparation techniques.
The last fifth part is concerned with the use of solid phase microextraction (SPME) as
preconcentration technique which emphasizes complete description of the principle,
working instrumentation and application of SPME for explosive analysis. It is
concentrated on the factors required to improve the extraction of different analytes. The
advantages of SPME using a specially designed interface for integrating sampling,
extraction, preconcentration and sample injection into a single solvent free step over
classical techniques is presented in this part.

CHAPTER 3: Review of Literature

This chapter provides an overview of different applications of HPLC for the analysis of
organo nitro explosives compounds reported in literature. The first part dealt with the
summary of different methods used for the HPLC analysis of nitro explosives in the
literature. Since HPLC has been used extensively for the analysis of the explosives
therefore, all methods of extractions, analysis and detection are summarized. A review of
the data has been taken on developments in the field of analysis of explosives by HPLC
for forensic and environmental applications. The review covers almost all aspects of
analysis like analyte’s category, matrix involved, technique and conditions used for
preconcentration, column and mobile phase used and subsequent detection, in last thirty
years.

From the thorough review of literature, it was found that SPME has an over edge in the
preconcentration methods over the normal SPE methods in terms of detection limits and
other parameters like simplifying sample preparation, reliability, selectivity and
sensitivity. Keeping in view the advantages of SPME, a special collection of data was
created for HPLC method for analysis of explosives in hyphenation of SPME and was discussed thoroughly as separate section in last part of this chapter.

CHAPTER 4: Enhanced Extraction of HMX and RDX in the Presence of Sodium Dodecyl Sulphate (SDS) and its Application to Environmental Samples

In this chapter, HPLC-UV determination of cyclic nitramine explosives such as RDX and HMX which are commonly used high explosives in armed forces munitions is discussed. These high explosives are usually dumped into the sea, burned, or detonated in remote areas, constituting potentially serious and hazardous environmental contamination problems. Therefore, method for enhanced extraction of HMX and RDX from environmental samples is developed with the assistance of aqueous sodium dodecyl sulphate (SDS) surfactant. In this study, the concentration of sodium dodecyl sulphate (SDS) surfactant and other analytical parameters are optimized on HPLC-UV system. Chromatographic parameters like composition of mobile phase, flow rate and wavelength were optimized on reverse phase amide column (5 μm) for baseline separation of explosives and making calibration curves. The optimized conditions are isocratic flow of 1.0 mL/min with mobile phase acetonitrile:water; 70:30 (v/v) at 230 nm wavelength. The amount of recovered explosives from spiked soil and water samples are calculated. Limits of detection and other parameters were determined in the presence of SDS which were not reported earlier. The limits of detection obtained for HMX and RDX standards were much better than obtained by Environmental Protection Agency (EPA) method 8330. The recoveries are found to be enhanced by 1.7 and 1.6 fold with SDS solution as compared to water for HMX and RDX, respectively from soil samples. So this study can be used to monitor the concentration of explosives present in soil and water samples obtained from munitions manufacturing sites or other military related activities by
avoiding the use of hazardous solvents and in soil washing processes used for the bioremediation of explosives in soil. Hence, a sensitive and simple method has been developed for the analysis of explosives present at sub ppb level in environmental samples.

CHAPTER 5: SPME-HPLC Determination of HMX and RDX in the Presence of Sodium Dodecyl Sulphate (SDS) Surfactant

In this chapter, SPME-HPLC-UV determination of nitramine explosives like HMX and RDX in presence of anionic surfactant is discussed. Aqueous solution of anionic surfactant sodium dodecyl sulphate (SDS) was used for the extraction of both nitramine high explosives, *viz.*, HMX and RDX from soil samples, which were subsequently sorbed on SPME fiber. Concentration of SDS and chromatographic parameters like composition of mobile phase, flow rate and wavelength were optimized for baseline separation of explosives. The optimized conditions were 0.5 mM concentration of SDS and ratio of acetonitrile:methanol:water (30:35:35; v/v) as mobile phase at a flow rate of 0.5 mL/min and detection at 230 nm. For this purpose, a C\textsubscript{18}, 5 \textmu m reverse phase analytical column was used as a separation medium in the method. Several parameters relating to SPME, *e.g.*, adsorption/desorption time, concentration of salt, stirring rate, *etc.*, were optimized on CW/TPR fiber. The method was linear over the range of 20-400 ng/mL for HMX and RDX standards in the presence of surfactant in aqueous phase. With SPME, the detection limits were calculated for HMX and RDX, respectively in the presence of the SDS surfactant. The developed method has been applied successfully to the analysis of real environmental samples like bore well water, river water and ground alluvial soil. The method proved to be a better option for extraction of analytes instead of costly and hazardous organic solvents.
CHAPTER 6: Development of SPME-HPLC-UV Method for the Analysis of TNT and its Metabolites on Reverse Phase Amide Column and Application to Analysis of Aqueous Samples

In this chapter, SPME-HPLC-UV determination of nitroaromatic explosive TNT and its metabolites are conferred. A rapid, simple, sensitive and accurate quantitative method has been developed for the determination of eleven nitroaromatic components by solid phase microextraction (SPME) coupled to high performance liquid chromatography (HPLC) with UV detection from aqueous samples. PDMS/DVB resin fiber (60 µm) was used for concurrent extraction of all the analytes from aqueous matrix. Several parameters related to SPME e.g., adsorption/desorption time, concentration of salt, stirring rate, etc., were optimized on PDMS/DVB fiber. Static desorption was carried out in the desorption chamber of SPME-HPLC interface containing mobile phase. Chromatographic parameters like composition of mobile phase, flow rate and wavelength were optimized for baseline separation of explosives and making calibration curves. The optimized conditions were ratio of solvents methanol:water 43:57 (v/v) at isocratic flow rate of 1.3 mL/min and detection at 254 nm. A reverse phase amide column (5 µm) was used as a separation medium. The limits of detection were calculated. Developed method has been applied successfully to the analysis of aqueous samples obtained from environmental and industrial sources like river water, ground water, drinking water and industrial waste water. With the help of developed method, concentration of TNT, Tetryl and their metabolites, much below the USEPA toxicity level (e.g., 2 µg/mL for TNT) could be determined and lower detection limits over other methods could be achieved. Therefore, the above advantages make this method a good choice amongst the existing methods for the analysis of explosives.