CHAPTER: 4

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

Cyclic nitramine explosives such as RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) are commonly used high explosives in armed forces munitions. These high explosives are usually dumped into the sea, burned or detonated in remote areas, constituting potentially serious and hazardous contamination problems [1]. Both RDX and HMX have low value of octanol/water partition coefficients ($K_{ow}$) and log $K_{ow}$ values for RDX and HMX are 0.90 and 0.16, respectively [2], indicating their high affinity towards aqueous phase.

![Chemical Structure of RDX and HMX](image)

**Figure 4.1**: Chemical Structure of (a) RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and (b) HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)

For soil samples, this affinity is represented in terms of organic-carbon/water partition coefficients ($K_{oc}$) which can be determined directly from $K_{ow}$ values depending on the type of sorbate [3, 4]. The $K_{oc}$ value is used as a chemical specific measure of the tendency for organics to be adsorbed by soil. It is largely independent of soil properties e.g., type and amount of clay, soil pH, cation exchange capacity (CEC) and hydrous oxide contents. For RDX and HMX, the values of log $K_{oc}$ lies in the range from 0.89 - 2.4 and 0.54 - 2.8, respectively [5]. Broadly, the value of sorption or distribution coefficient ($K_d$), which is a measure of how tightly the analyte binds or sticks to soil particles (including organic matter, clays or iron and manganese oxides) is less than unity (i.e., $K_d$
< 1) for both nitramine high explosives, indicating their high mobility in soil [6]. Therefore RDX and HMX can migrate through subsurface soil very rapidly even though both are moderately to weakly soluble in water i.e., 40.2 and 6.6 µg/mL, respectively at 25 °C [7] causing groundwater contamination very swiftly and as a consequence, can travel distances from the contamination site. It has also been shown that explosives are modestly toxic to aquatic organisms, earthworms and indigenous soil microorganisms [8, 9]. Many explosives are known toxins and carcinogens [10]. RDX has been used as a potential rat poison [11]. Also the discharge of contaminated manufacturing waste streams into rivers or ground water, burial of obsolete munitions and training exercises increase the availability of explosives for migration into water supplies [12], so they pose a threat to living species as they find their way into the food chain.

Therefore, the ecological fates of HMX, RDX and their degradation products are the subject of intensive study nowadays [13, 14]. For example, there is a growing requirement for the analysis of matrices such as soil and water in order to address environmental problems related to improper handling procedures, either in storage or in disposal of explosive products [15-17]. For analytical issues, HPLC–UV has become a widespread and powerful method which belongs among the basic tools in each modern laboratory nowadays and recommended by US Environmental Protection Agency method 8330 [18] for the analysis of explosives. Due to high thermal stability and low vapor pressure of nitro explosives, HPLC is an instrument of choice for the analysis of explosives. Application of HPLC-UV technique for the analysis of organic high explosives has been reviewed [19].

Environmental protection Agency (EPA) Method 8330 [18] provides a salting-out extraction procedure for low concentration (parts per trillion i.e., nanograms per liter) of
explosives residues present in surface or ground water with acetonitrile while direct injection of aqueous samples containing high concentrations of explosives after dilution with methanol/acetonitrile. In this method, large volumes of water sample (770 mL) and acetonitrile (aprox. 175 mL) were used for the salting out extraction process which is inconvenient. Both organic solvents are hazardous for health and environment being toxic [20-25]. Also, the method is very cumbersome, time consuming, requires much expertise, high level of accuracy and precision during analysis. This method is applicable only on miniaturized scale in the laboratory not on the large scale in the field. The developed method with the assistance of surfactants can be used on laboratory as well as on field scale. On the field scale, method can be applied for soil washing and determination of level of contamination. Soil washing is the process in which contaminants sorbed onto fine soil particles are separated from bulk soil in an aqueous based system on the basis of particle size. Wash water may be augmented with a basic leaching agents, surfactant, pH adjustment, or chelating agent to remove organics and heavy metals. The greatest advantage of soil washing with aqueous solution of surfactant is that it reduces the amount of soil needing further cleanup. This reduction lowers the cost of clean up and the cost of disposing of polluted material. This also works when the soil is much polluted. At the present time, soil washing is used extensively in Europe and has been progressively increasing use in USA. Hence the technique provides a cost effective and environmentally proactive alternative to stabilization and land filling applicable on macro scale which is not recommended with organic solvents. A lot of work has been published on the soil washing with surfactant based aqueous solutions [26-31]. Surfactants are particularly attractive for such applications as they potentially have low toxicity and favorable biodegradability in the environment than many organic-solvent based systems.
Since washing fluid can be regenerated back, increases the importance of soil washing with surfactants. The presence of SDS in either water sample or aqueous extract of soil enhances the peak area of HMX during chromatographic analysis which ultimately affect the limit of detection of HMX while the peak area for RDX remain almost same during experimental concentration range of SDS. Since the two main nitramines are present in numerous explosive compositions, so this work investigate the potential use of aqueous solution of SDS to enhance the extraction of both explosives from complex soil environment and subsequent analysis by HPLC-UV. The method could be applied successfully on other aqueous samples obtained from the environmental media.

However, Hawari et al. [32] have reported the enhanced recovery of RDX from soil (2.0 g) with the help of anionic surfactants like SDS, Ligniste 458 and Lignosol as well as cyclodextrins but at very high concentration of explosive (1000 ppm). With 1% (w/v) solution (34.7 mM) of SDS, 1.2 fold recovery was obtained as compared to water on C\textsubscript{18} column and this concentration of SDS was much higher than its own critical micelle concentration (8.2 mM). The extracted RDX was accompanied with its own hydrolyzed product \textit{i.e.,} 3,5-dinitro-1,3,5-triazacyclohex-1-ene (ca 5\%) when extracted with anionic surfactants while no RDX hydrolyzed product was obtained with the cyclodextrin assisted extraction. The recovery was much better with cyclodextrins, so major emphasis was given on it and no extraction and analytical conditions were optimized in the presence of SDS. The use of very high concentration of anionic surfactant for extraction and the methoxide anion present in solvent phase (aqueous methanol) may be the cause of the removal of acidic proton alpha to nitro group in RDX [32]. In our study reduced concentration of SDS (0.14 mM) was used for recovery of explosives and aprotic solvents were used instead as mobile phase which account for the higher stability of RDX
and HMX during analytical process. Limits of detection and other parameters were determined in the presence of SDS which were not reported earlier. 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 and in soil washing processes used for the remediation of explosives in soil.

4.2 Experimental

4.2.1 Reagents

Standard solutions of HMX and RDX in acetonitrile (1000 µg/mL) were purchased from Supelco (Bellefonte, USA). Acetonitrile (HPLC grade; UV cut off 190 nm) was purchased from J.T. Baker chemicals (Mexico, USA). Triple distilled water was used as one of the solvent for chromatographic separation. Sodium dodecyl sulphate (Qualigens fine chemicals, Bombay, India) was used as an extracting surfactant. A stock solution containing mixture of HMX and RDX, each at a concentration of 10 µg/mL was prepared in acetonitrile from the standards. Samples were wrapped in aluminum foil to prevent photodecomposition and stored at -4°C in deep freezer. All solvents were degassed on ultrasonic bath prior to use.

4.2.2 Instrumentation

The HPLC system consist of pump (Dionex P680, Dionex Softron GmbH, Germering, Germany) with four solvent chambers, a Supelco Ascentis RP-amide 150 x 4.6 mm; 5 µm reversed-phase analytical column, a Dionex UVD170U detector operated at a wavelength 230 nm connected to a computer loaded with Chromelon software for data acquisition. Separations were carried out at room temperature maintained at 20-22°C. Sample was injected directly using a rheodyne’s 20 µL loop on the valve for analysis. Aqueous and non-aqueous solvents were filtered with 0.45 µm Nylon 6,6 membrane
filters (Pall life sciences, Mumbai, India) and 0.40 µm syringe filter (Rankem, New Delhi, India). ELICO (India) double beam UV-visible spectrophotometer SL-164 was used for obtaining the absorption spectra of both explosive components.

4.3 Procedure

4.3.1 Optimization of separation conditions

Solution of mixture of HMX and RDX was prepared (100 ppb) in triply distilled water by adding 50 µL of 10,000 ppb mixture in 5 mL water. Conditions for baseline separation of HMX and RDX were optimized with respect to mobile phase and wavelength. The final optimized conditions were isocratic flow of acetonitrile:water; 70:30 (v/v) at a flow rate of 1.0 mL/min with detection at wavelength 230 nm on reverse phase amide column [Figure 4.2; Table 4.1]. A series of aqueous samples containing SDS concentrations between 0.07 and 0.21 mM were analyzed under the optimized conditions of analysis. It was found that peak area for HMX increases in the presence of surfactant and it was highest at concentration 0.14 mM of SDS and was almost constant for RDX within this concentration range of SDS [Figure 4.3]. The enhancement in peak area for HMX may be due to change in matrix properties in presence of SDS which consequently increases the sensitivity for HMX. Therefore, optimum concentration of SDS was kept constant at 0.14 mM for the extraction of explosives from soil and as an additive in groundwater sample during analysis. Calibration curves were prepared by varying the concentration from 10 to 400 ppb in the presence of SDS (0.14 mM). The regression coefficient ($R^2$) for HMX and RDX were found to be 0.996 and 0.997, respectively [Figure 4.4; Table 4.2]. The calculated limits of detections are 1.5 and 3.8 ppb (S/N = 3) for HMX and RDX standards, respectively.

4.3.2 Application to surface soil sample
Alluvial surface soil having high cation exchange capacity and rich in exchangeable cations such as Ca$^{2+}$, Mg$^{2+}$, K$^+$ was taken as sample till 20 cm depth. The soil was taken from agriculture land which was used for floriculture and contains high contents of minerals, humic substances and moisture. The soil sample was dried at 45-50°C in oven for two to three days to remove the moisture and was finely grinded in mortal pastel to 30-mesh (0.6 mm) sieve. A 500 ppb mixture of explosives in 1.0 mL acetone was added to 1.0 g of soil and mixed thoroughly. The solvent was allowed to evaporate at room temperature followed by thorough mixing of dry soil to evenly distribute HMX and RDX. Soil was equilibrated for 2-hours thoroughly for better deposition of pollutants before extraction. Similarly another soil sample was prepared by adding 200 ppb of explosive mixture in the same amount of soil following same procedural steps. Both soil samples were stirred constantly with teflon coated magnetic beads in presence of 10 mL of aqueous solution of SDS (0.14 mM) for 1 hour. Each extract was filtered on 0.45 µm Nylon - 6,6 membrane filter. The filtrates obtained were clear but to remove any particulate matter of size less than 0.45 µm, each extract was filtered with 0.4 µm syringe filtration such that the extract is completely free from all types of suspended impurities. Blank samples were also prepared and purified following the same steps. Extracts were injected directly into the injector of HPLC and were analyzed under optimized chromatographic conditions [Figure 4.5(a,b)]. Each experiment was repeated three times and the results are summarized in Table 4.3.

4.3.3 Application to ground water sample

Ground water sample was collected from a tube well located nearby an explosives testing site. A small portion of this water sample was spiked with explosive mixture, SDS solution was added and the final volume made up to 5.0 mL with water such that the final
concentration of explosive mixture was 100 ppb and that of SDS surfactant was 0.14 mM in final volume. Sample was analyzed under optimized chromatographic conditions [Figure 4.6(b)]. The experiment was repeated three times and average value was recorded. Same experiment was repeated for 50 ppb spiked sample. Blank samples were also analyzed and no traces of explosives were found [Figure 4.6(a)].

4.4 Results and Discussion

The retention time for HMX and RDX on reverse phase amide column were 1.9 and 2.1 min, respectively [Figure 4.2] which were lesser than reported in EPA method 8330, i.e., 2.4 and 3.7 min, respectively on reverse phase C\textsubscript{18} column even at lower flow rate of 1.0 mL/min as compared to EPA method (1.5 mL/min). Baseline separation of both explosives is achieved with acetonitrile/water mobile phase on RP-amide column which is not achievable on C\textsubscript{18} column with same mobile phase. In EPA method 8330, the HPLC-UV chromatogram for HMX and RDX with other nitro explosives was taken at a wavelength of 254 nm, which is very far away from the absorption maxima for both these nitramine explosives, i.e., \( \sim 234 \) nm [33-38]. So in this developed method the chromatogram was taken at \( \lambda_{\text{max}} 230 \) nm.

Recoveries were calculated for HMX and RDX from calibration curves for 500 and 200 ppb extracted samples of soil. For comparison purpose the amount of explosives recovered from soil sample with the help of water is assumed to be an integrated value 1.0 and the amount of explosives recovered with the assistance of surfactant were compared accordingly. From 500 ppb sample of spiked soil, 176 ppb (35.2%) of HMX and 325 ppb (65%) of RDX was recovered from single extraction with aqueous solution of surfactant, which is approximately 1.6 and 1.7 fold higher than water. Similarly 57.5 ppb (28.7%) of HMX and 148.8 ppb (74.3%) of RDX was extracted from 200 ppb spiked
soil sample, which is 1.4 and 1.7 times higher than recovery with water. Relative standard deviation (RSD) was calculated for each explosive [Table 4.3].

It was observed from the extraction results that the recovery of HMX is comparatively lesser than RDX, which may be accounted to lower aqueous solubility of HMX than RDX [3]. Cho et al. have reported the association of ion pair of anionic surfactant like SDS with humic acid in the aqueous environment resulting in lowering of its hydrophobic interaction with polycyclic aromatic hydrocarbons (PAH) [39] well below its critical micelle concentration (CMC). Therefore, we may consider that aqueous solution of SDS causes the lowering of intermolecular association between explosives and humic acid of contaminated soil resulting in fast release of contaminants to the aqueous media. Also in the HPLC-UV chromatogram, no peak for added SDS is observed which absorbs in UV region; $\lambda_{\text{max}}$ 222 nm (3.45 mM SDS solution, reference water) clearly proves the phenomenon. But the incorporation of higher concentration of surfactant for the extraction from soil samples causes the distortion of the chromatogram instead of increasing the peak area for explosives which may be due its effect on the surface of the packing and change of the characteristics of reverse phase column [40].

Similarly, no extra peak is observed in the chromatogram for the hydrolyzed product of RDX [32], clearly demonstrate the advantage of use of lower concentration of anionic surfactant and water/acetonitrile as mobile phase. Three to four washing cycles were needed for HMX while two to three washing cycles were needed for the complete recovery of RDX from soil sample. HPLC-UV chromatogram [Figure 4.5] is shown for the extract of washed soil spiked with 500 ppb concentration of these explosives. Similarly, HPLC-UV chromatogram after analysis of blank and spiked groundwater at 50 ppb concentration is shown [Figure 4.6 (a, b)]. Method gave satisfactory results upto 10
ppb concentration level. Experiments were repeated three times and the result of
determinations are summarized in Table 4.4. Variation in the recovered concentration of
explosives from spiked ground water may be due to interaction of SDS with ions or other
constituents present in water [41] consequently decreasing its availability for explosive
components.

The detection limits for spiked groundwater samples were found to be 1.9 and 3.4 ppb
(S/N = 3) for HMX and RDX, respectively. The detection limits for spiked soil sample
were found to be 3.0 and 5.6 ppb (S/N = 3) for HMX and RDX, respectively for first step
of extraction. The calculated quantification limits (3 x LOD) for HMX and RDX
extracted from soil were calculated to be 9.0 and 16.8 ppb after first extraction which
were much better than that reported in EPA method 8330 i.e., 2.2 and 1.0 ppm,
respectively

4.5 Conclusion

Reverse phase amide column has advantage over C_{18} column because of fast and base
line separation of explosives. The incorporation of SDS in aqueous samples has dual
advantage viz., increase in sensitivity for HMX as well as enhancement in extraction of
both nitramine explosives. The optimized concentration of SDS can be used for enhanced
recovery of explosives present in ppb range from soil samples. Limits of detection for
ground water samples and limit of quantitation for soil extract samples were found to be
much better than that reported in EPA method 8330. Hence, a sensitive and simple
method has been developed by avoiding the use of hazardous solvents like
acetonitrile/methanol for the analysis of explosives present at sub ppb level in ground
water and soil samples.
Table 4.1: Optimized conditions for HPLC-UV analysis of nitramine explosives

<table>
<thead>
<tr>
<th>HPLC</th>
<th>Pump</th>
<th>Column</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Dionex P680</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RP amide column</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 x 4.6 mm; 5 µm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isocratic flow;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acetonitrile:water;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70:30 (v/v)</td>
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<tr>
<td></td>
<td></td>
<td>1.0 mL/min</td>
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<thead>
<tr>
<th>Mobile Phase</th>
<th>Flow Rate</th>
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<table>
<thead>
<tr>
<th>UV detection</th>
<th>Wavelength (λ)</th>
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<tbody>
<tr>
<td></td>
<td>230 nm</td>
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</tbody>
</table>

Table 4.2: Calibration curve characteristics for HMX and RDX in presence of 0.14 mM SDS surfactant on RP-amide column

<table>
<thead>
<tr>
<th>Analyte</th>
<th>$y = mx + c$</th>
<th>$R^2$</th>
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</thead>
<tbody>
<tr>
<td>HMX</td>
<td>0.002x + 0.149</td>
<td>0.996</td>
</tr>
<tr>
<td>RDX</td>
<td>0.0008x - 0.001</td>
<td>0.997</td>
</tr>
</tbody>
</table>
Table 4.3: Recovery of HMX and RDX from 500 and 200 ppb soil samples

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Explosive</th>
<th>Original Concentration (ppb)</th>
<th>Solvent</th>
<th>Extracted (ppb)</th>
<th>Recovery (%)</th>
<th>Recovery Factor</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HMX</td>
<td>200</td>
<td>Water</td>
<td>39.4</td>
<td>19.8</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SDS (0.14 mM)</td>
<td>57.5</td>
<td>28.7</td>
<td>1.4</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>Water</td>
<td>109.0</td>
<td>21.8</td>
<td>1.0</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SDS (0.14 mM)</td>
<td>176.0</td>
<td>35.2</td>
<td>1.6</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>RDX</td>
<td>200</td>
<td>Water</td>
<td>87.5</td>
<td>43.5</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SDS (0.14 mM)</td>
<td>148.8</td>
<td>74.3</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>Water</td>
<td>185.0</td>
<td>37.1</td>
<td>1.0</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SDS (0.14 mM)</td>
<td>325.0</td>
<td>65.0</td>
<td>1.7</td>
<td>7.2</td>
</tr>
</tbody>
</table>

* Each experiment is repeated three times
Table 4.4: Recovery of explosives from ground water samples

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Explosive</th>
<th>Original Concentration (ppb)</th>
<th>Concentration Obtained (ppb)</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HMX</td>
<td>100</td>
<td>86.0</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>42.5</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td>RDX</td>
<td>100</td>
<td>110.0</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>55.2</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Each experiment is repeated three times
Figure 4.2: Chromatogram showing the separation of HMX and RDX in the presence of 0.14 mM SDS at 200 ppb concentration of explosives in aqueous sample with solvent ratio acetonitrile:water 70/30 at flow rate of 1.0 mL/min; $\lambda_{\text{max}}=230$ nm
Figure 4.3: Optimization of concentration of SDS added in aqueous sample containing 100 ppb mixture of HMX and RDX on RP amide column with mobile phase acetonitrile:water; 70/30 (v/v) at flow rate of 1.0 mL/min; $\lambda_{\text{max}}=230$ nm
Figure 4.4: Calibration curves of RDX and HMX in presence of 0.14 mM concentration of SDS
**Figure 4.5:** (a) HPLC-UV chromatogram soil washed with SDS solution (0.14 mM) (b) HPLC-UV chromatogram of spiked soil (500 ppb) washed with aqueous solution of SDS (0.14 mM) under optimized chromatographic conditions
Figure 4.6: (a) HPLC-UV Chromatogram showing blank peak in groundwater sample containing 0.14 mM SDS (b) chromatogram of spiked groundwater with explosive mixture (50 ppb) containing 0.14 mM SDS in the sample under optimized chromatographic conditions with solvent ratio acetonitrile:water 70/30 at flow rate of 1.0 mL/min; $\lambda_{max}=230$ nm
References


