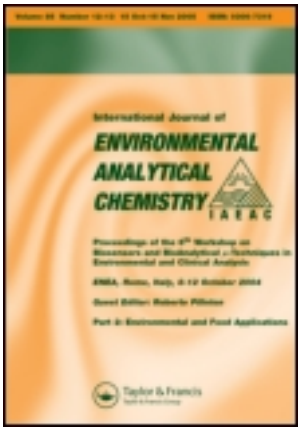

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Precise and accurate compound-specific carbon and nitrogen isotope analysis of RDX by GC-IRMS

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A new analytical method is presented for the compound-specific carbon and nitrogen isotope ratio analysis of a thermo-labile nitramine explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by gas chromatograph coupled to an isotope ratio mass spectrometer (GC-IRMS). Two main approaches were used to minimise thermal decomposition of the compound during gas chromatographic separation: programmed temperature vaporisation (PTV) as an injection technique and a high-temperature ramp rate during the GC run. $\delta^{15}N$ and $\delta^{13}C$ values of RDX measured by GC-IRMS and elemental analyser (EA)-IRMS were in good agreement within a standard deviation of 0.3‰ and 0.4‰ for nitrogen and carbon, respectively. Application of the method for the isotope analysis of RDX during alkaline hydrolysis at 50°C revealed isotope fractionation factors $\varepsilon_{carbon} = -7.8‰$ and $\varepsilon_{nitrogen} = -5.3‰$.

Keywords: RDX; thermo-labile compound; CSIA; isotope fractionation

1. Introduction

Hexahydro-1,3,5-trinitro-1,3,5-triazine, a cyclic nitramine explosive commonly known as RDX, is widely used in military munitions. Release of the explosive into the environment at military sites resulting in contamination of soils, sediments and water has been reported in different places around the world [1,2]. The seriousness of the contaminations is aggravated by the proved toxicity of this compound to many terrestrial and aquatic organisms [3,4]. Due to its toxicity, the US Environmental Protection Agency (USEPA) limited its maximum permissible concentration in drinking water down to 2 μg/L [5]. Nevertheless, the main removal mechanism of RDX from the environment is decomposition by either biotic or abiotic processes [6,7]. Biodegradation of the explosive by different micro-organisms under aerobic and anaerobic conditions has been intensively investigated and various pathways for these processes have been suggested [8–11]. However, despite the knowledge of the possible mechanisms and the end products of the degradation, assessment of RDX decomposition in the environment based on the concentration monitoring only is often ambiguous.

Recently, the use of compound-specific isotope ratio analysis (CSIA) has attracted much attention as a complementary method for distinguishing between different

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destruction pathways in the field sites and for biodegradation quantification [12]. The CSIA concept relies on the fact that lower activation energies are needed to cleave chemical bonds formed by light versus heavy isotopes and, therefore, different degradation pathways having different rate-determining steps may result in distinguishable isotope patterns. Thus, for example: different carbon and hydrogen isotope fractionation patterns were demonstrated for biotic and abiotic degradation of methyl tert-butyl ether [13–16]; pathway-dependent carbon isotope fractionation was observed during aerobic biodegradation of 1,2-dichloroethane [17]; different aerobic nitrobenzene biodegradation pathways revealed distinguishing nitrogen and carbon isotope fractionation [18]; and different nitrogen and oxygen isotope fractionation patterns were obtained for aerobic and anaerobic biodegradation of RDX [19].

Although isotope composition and fractionation of RDX have been investigated during the last decade, compound-specific isotope analysis of this compound by GC-IRMS remained problematic. The reason for this is a thermal decomposition of the explosive during the gas chromatographic separation. To avoid this, a new technique for CSIA of RDX that includes extraction of the explosive from the solution followed by its purification by thin layer chromatography (TLC) and elution from the scraped silica was developed by Bernstein *et al*. [19]. Isotope analysis of such purified RDX was performed by an elemental analyser (EA)-isotope ratio mass spectrometer (IRMS). Despite good accuracy and precision of the method, the routine use of CSIA for this purpose is limited due to its complexity.

The aim of this study was to establish a new method for compound-specific isotope ratio analysis of RDX by gas chromatograph interfaced to IRMS.

Applications of gas chromatography in combination with different highly sensitive and selective detection techniques such as electron-capture detector (ECD) [20], thermal energy analyser (TEA) detector [21], mass spectrometer [22–24], pulsed-discharge electron capture detector [25] for the trace analysis of explosives were reported. Among the approaches used for the successful analysis of RDX were programmed temperature vaporisation (PTV) as injection technique and a high-temperature ramp rate during GC run. Both of them have been applied in the present study for the development of the GC-IRMS method.

To determine accuracy of the developed method, we compared carbon and nitrogen isotope ratio values of RDX obtained by GC-IRMS to those measured for the pure compound by EA-IRMS.

We employed the GC-IRMS method for tracing isotopic composition of RDX during alkaline hydrolysis which is one of the potential ways to treat contaminated water [26] as well as a possible degradation pathway of the explosives in coastal waters [27].

2. Experimental

2.1 *Isotope analysis*

GC-IRMS carbon and nitrogen isotope analyses of RDX were performed using Trace GC Ultra (Thermo Electron Corporation, Milan, Italy) interfaced to DeltaV Plus (Thermo Fisher Scientific, Bremen, Germany) via GC Combustion III interface. Samples were injected with AI 3000 auto-injector (Thermo Electron Corporation, Milan, Italy).

A DB-5 capillary column (30m $\times$ 0.25 mm, film thickness 0.25 μm) was used for the GC separation and the run was performed under the following conditions:

A PTV injector operated in a split mode with split ratio 1 : 10, initial temperature 75°C for 0.05 min, 14.5°C/sec to 260°C (hold for 15 min), followed by the cleaning phase 14°C/sec to 270°C (hold for 2 min) with the vent flow of 50 ml/min.

GC oven temperature programme: 60°C for 1 min, 15°C/min to 180°C and 90°C/min to 290°C (hold for 5 min). Helium was used as a carrier gas in a constant flow mode with a flow rate of 3 ml/min. 2 µl of the solution was injected for the analysis.

A ceramic tube filled with CuO/NiO/Pt-wire (Thermo Fisher Scientific, Bremen, Germany) was used as a combustion reactor. The combustion reactor was operated at 980°C for nitrogen and for carbon analysis. For performing carbon isotope analysis, the oxidation reactor was reoxidised before every 100 samples at 940°C for 10 min. In contrast to carbon isotope analysis, the oxidation reactor was not reoxidised for nitrogen analysis. A standard reduction reactor containing copper wires (Thermo Fisher Scientific, Bremen, Germany) was operated at 660°C. A liquid nitrogen trap was used for the nitrogen isotope analysis to trap CO_2 produced from analyte combustion.

During isotope analysis, analyte was measured against laboratory standard gas (CO_2 for carbon and N_2 for nitrogen isotope analysis) that was introduced at the beginning and at the end of each run. All isotopic analyses were performed with a constant standard gas amplitude in the range of 3000 mV for CO_2 and for N_2. The $\delta^{13}C$ and $\delta^{15}N$ values are reported relative to Vienna PeeDee Belemnite (VPDB) and to atmospheric nitrogen respectively.

The carbon and nitrogen isotope ratios of RDX standard were also determined by an elemental analyser (EA) (Flash EA 1112; Thermo Finnigan, Milan, Italy) interfaced to Delta V Plus IRMS. The combustion reactor in EA was held at 1020°C and the reduction reactor, at 650°C.

RDX standard was prepared from solid powder (purity > 98%).

2.2 *Hydrolysis experiments*

Hydrolysis experiments were performed in a water bath. An Erlenmeyer flask with a solution of 20 mg of RDX in 250 ml of distilled water was heated with stirring until the constant temperature of 50°C was reached, which was followed by the addition of 0.18 ml of 10 M NaOH (pH = 11.5). After the desired period of time, the solution was acidified with HCl to pH < 5. Aqueous solutions were transferred into the separation funnel and extracted with dichloromethane (DCM) by extensive shaking the funnel for 5 min (3 times, 30 ml of DCM each time), followed by drying of the organic phase with anhydrous sodium sulfate (10 min) and evaporation of DCM in a rotatory evaporator (15–20 min). Dried samples were quantitatively redissolved in 0.2–1 ml of acetone and analysed. The extraction efficiency was found >95%.

Quantitative analyses of RDX in hydrolysis experiments were performed by HPLC [28]. Analysis of the reaction products- nitrite and 4-nitro-2,4-diazobutanal (4-NDAB) was performed following the previously reported methods [29,30].

It is worth noting that hydrolysis conditions (RDX concentration, pH and temperature) used in the present study are possibly very different from the conditions pertaining in real cases. However, the conditions were selected with the aim of obtaining sufficient data within a reasonable time.

2.3 *Calculations*

$\delta^{13}C$ and $\delta^{15}N$ in the investigated compounds are given in per mil units (‰) and defined as:

$$\delta(‰) = (R/R_{std} - 1) \cdot 1000 \quad (1)$$

R and R_{std} are the ratios between the heavy and the light isotopes in the investigated compound and standard, respectively.

Isotope enrichment factors (ε) for carbon and nitrogen were determined as a slope of the linear regression according to the modified Rayleigh equation (Equation 2):

$$\ln(R_t/R_0) = (\varepsilon/1000) \cdot \ln(C_t/C_0) \quad (2)$$

where R_t and R_0 are the compound–specific isotope ratios at a given time t and at the beginning of the reaction. C_0 and C_t are the concentrations of the investigated compound at the beginning and at a given time t.

3. Results and discussion

3.1 *Isotopic analysis of RDX*

In the present study we explored the use of a gas chromatograph interfaced to an isotope ratio mass spectrometer (GC-IRMS) for isotope analysis of RDX.

RDX is a thermally labile compound and may decompose during the injection and GC separation. However, the loss of the analyte may lead to isotope fractionation. To avoid this, two main approaches were employed in this study: programmed temperature vaporisation (PTV) and a high-temperature ramp rate during the GC run.

Accuracy of the developed GC-IRMS method was determined by comparison of the measured carbon and nitrogen isotope ratio values for pure RDX to those detected by EA-IRMS. Due to the quantitative transfer of the analyte into the combustion reactor of the elemental analyser and the absence of any separation process prior to analyte combustion, we assume that no isotope fractionation of RDX occurs during the EA-IRMS analysis.

Table 1 represents $\delta^{13}C$ and $\delta^{15}N$ values for RDX obtained for the pure compound by both EA-IRMS and GC-IRMS techniques. As could be seen from the table, a good agreement between $\delta^{13}C$ and $\delta^{15}N$ values of RDX measured by GC-IRMS and EA-IRMS was achieved. Although the standard deviation of the GC-IRMS measurement (0.4‰ for carbon and 0.3‰ for nitrogen) is higher than obtained by EA-IRMS (0.1‰), it is still in the range of the acceptable total uncertainty of the analysis of 0.5‰.

A high helium flow rate and two oven temperature ramps of 15°C/min and 90°C/min were applied during the GC run in the present work. The high helium flow rate and high-temperature oven ramp promoted a faster elution of the compound from

Table 1. Carbon and nitrogen isotope ratios for standard RDX compound measured by EA-IRMS and GC-IRMS. The results are based on the signals with amplitudes in the range of 2000–2500 mV ($n = 10$).

	GC-IRMS	EA-IRMS
$\delta^{13}C$, ‰	-37.8 ± 0.4	-38.3 ± 0.1
$\delta^{15}N$, ‰	-9.8 ± 0.3	-10.1 ± 0.1

the analytical column, thus also minimising its decomposition. In addition, we discovered that the high-temperature oven ramp of 90°C/min was essential for the good RDX peak shape because lower temperature ramps resulted in a significant broadening of the peaks. For example, in carbon isotope analysis, RDX peak width of 49 sec at 90°C/min ramp increases up to 58 sec at 60°C/min and to 76 sec at 30°C/min. It is worth noting, that if a mixture of organic compounds is analysed, employing the high-temperature oven ramp only during the GC run could cause poor separation of peaks, and additional lower ramp could be required. For example, a chromatogram in Figure 1 demonstrates the use of the proposed analytical method including two temperature ramps for the isotopic analysis of a mixture of possible co-contaminants at military sites – nitrobenzene (NB), trinitrotoluene (TNT) and RDX. Despite the broadness and some tailing of the RDX peaks, identical $\delta^{13}C$ and $\delta^{15}N$ values were obtained for the explosive in a mixture and as a pure compound. Of course, different combinations of organic compounds are possible at contaminated sites; therefore, the method must be adapted to each individual case.

Application of the represented GC-IRMS method for the isotopic analysis of pure RDX revealed good linearity and good precisions for nitrogen isotope values for peak amplitudes (m/z 28) down to 200 mV. However, a significant shift in carbon isotope values for peaks (m/z 44) lower than 2000 mV was observed (Figure 2). Therefore, only peaks higher than 2000 mV could be considered for the reliable carbon isotope analysis. It should be mentioned, that all above-reported analyses were performed in the split (1 : 10) mode,

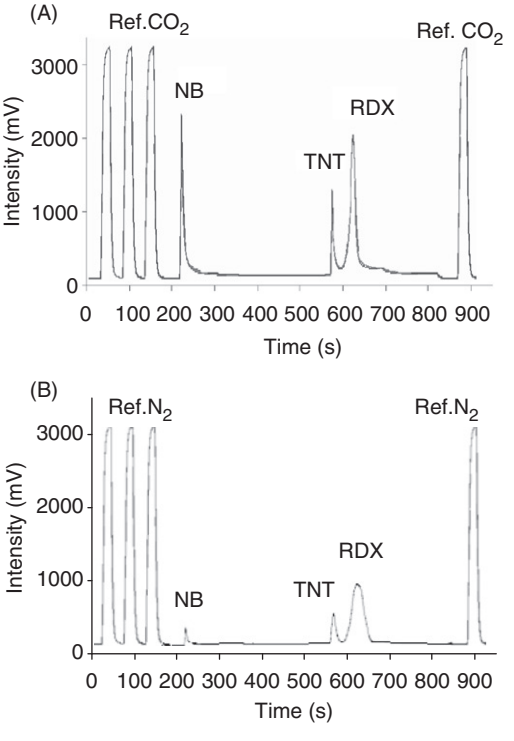

Figure 1. Typical $\delta^{13}C$ and $\delta^{15}N$ chromatograms obtained by GC-IRMS for the mixture containing 4 mg/ml of nitrobenzene (NB), 8 mg/ml of trinitrotoluene (TNT) and 20 mg/ml of RDX in acetone.

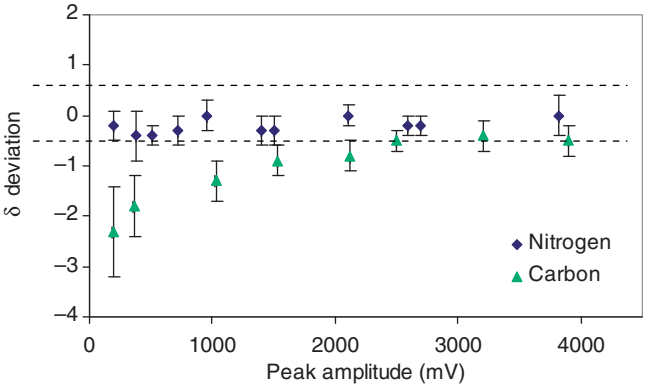

Figure 2. Deviations in $\delta^{13}C$ and $\delta^{15}N$ values of RDX for different peak amplitudes as compared to the values obtained by EA-IRMS. Error bars represent 1SD for four measurements. Dotted lines represent the maximal typical deviation of the analysis (±0.5‰).

because our preliminary results for the splitless injection revealed poorer chromatographic peak shapes and lower isotope ratio precisions. Our results revealed that in order to get peaks of 200 mV amplitude, amounts of RDX containing around 50 nmol of carbon and 120 nmol of nitrogen had to be injected into the GC-IRMS; linear dependence between the RDX peak area and the injected amount was observed.

Taking into account the limitations mentioned above, we suggest that at least 5 μg of RDX must be injected into the GC-IRMS in order to obtain reliable nitrogen isotopic results, whereas for the carbon isotope analysis at least 40 μg are required. Therefore, at some contaminated sites, where RDX concentrations in the groundwater achieve the level of hundreds μg/L [31], the method might be applied.

3.2 *Hydrolysis experiment*

Detailed investigations of RDX hydrolysis under alkaline conditions were conducted in the past by several research groups and the possible reaction mechanisms were proposed [32–34]. Among others, it was suggested that a proton abstraction from the methylene group by OH^- and a loss of NO_2^- from the adjacent ring atoms take place simultaneously in the rate-limiting step initial elimination process, followed by the fast ring cleavage and spontaneous decomposition to the final products (Figure 3). Since the initial elimination step includes the cleavage of C-H and N-NO_2 bonds and the formation of inner C=N bond, we assumed that carbon and nitrogen isotope fractionation during the reaction could be observed.

Throughout the RDX hydrolysis, formation and accumulation of the nitrite and 4-NDAB was observed. Approximately 1 mol of nitrate and 0.3 mol of 4-NDAB were produced upon the disappearance of every 1 mol of RDX. Any nitroso derivatives were not observed. Carbon and nitrogen isotopic enrichments of the remaining fraction of RDX were perceived during the process. Bulk isotope enrichment factors for carbon and nitrogen were derived from the slopes of the Rayleigh plots giving $\varepsilon_{carbon} = -7.8‰ \pm 0.2$ and $\varepsilon_{nitrogen} = -5.3‰ \pm 0.3‰$ (Figure 4).

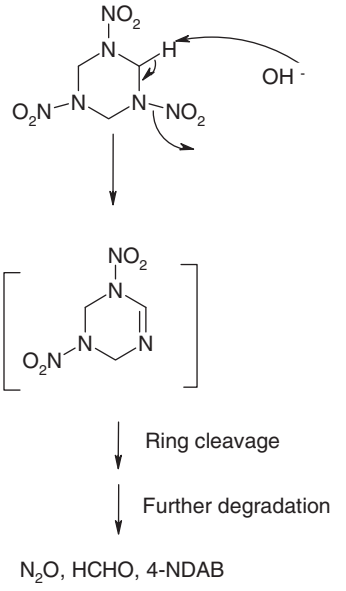

Figure 3. A possible pathway for the alkaline hydrolysis of RDX. Compound in brackets is considered an unstable intermediate.

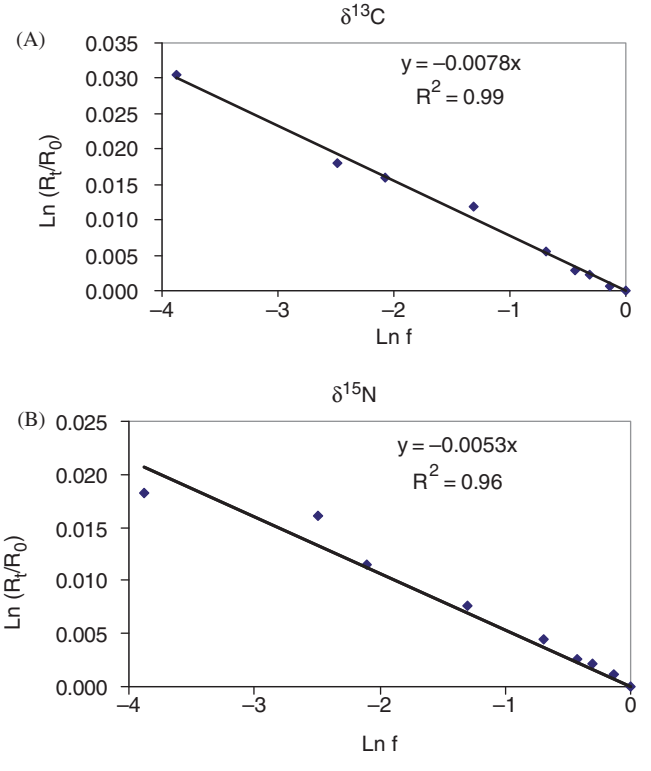

Figure 4. Enrichment in ^{13}C and ^{15}N composition of RDX during alkaline hydrolysis. Each point represents an average of three unreliable experiments.

It is interesting to note that for the aerobic biodegradation leading for the formation of 4-NDAB $\varepsilon_{\text{nitrogen}}$ of $-2.1‰ \pm 0.1‰$ was obtained [19], whereas nitrogen enrichment factor of $-5.0‰ \pm 0.3‰$ was reported for the anaerobic biodegradation of RDX accompanied by nitroso derivates [19]. To the best of our knowledge, no data on carbon isotope fractionation during RDX aerobic or anaerobic degradation have been reported in the literature yet.

4. Conclusion

A new, accurate and precise method for the compound-specific carbon and nitrogen isotope ratio analysis of thermo-labile explosive RDX by GC-IRMS was developed. Consecutive on-line separation and isotope analysis of RDX by GC-IRMS significantly simplifies the earlier proposed procedure for the compound-specific isotope analysis of this compound, which included a complex off-line separation and purification processes. However, relatively high amounts of RDX required for the reliable isotopic analysis may limit an application of the method for the sites with low levels of RDX contamination. Further adaptation and development of the proposed method for the investigation of carbon and nitrogen isotope enrichment during different RDX biotic and abiotic degradation processes is being studied.

We believe that the analytical method presented in this work will be successfully adapted and generally applied for the isotope ratio analysis of thermo-labile compounds.

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