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Determination of cyclonite (RDX) in human plasma by high-performance liquid chromatography

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Abstract

A simple and sensitive HPLC method has been developed for the assay of cyclonite (RDX) in human plasma. The assay involves solid-phase extraction on Tox-clean RC SPE cartridges and isocratic reversed-phase chromatography with diode-array detection. The assay was linear over the concentration range of 0.01–2.0 μg/ml for plasma with a lower limit of detection of 0.005 μg/ml. Both the within-day and day-to-day reproducibilities and accuracies were less than 10.15 and 1.9%, respectively. The method was applied to evaluate RDX concentration in plasma samples obtained from soldiers exposed RDX.

Keywords: RDX; Cyclonite; Explosive

1. Introduction

1,3,5-Trinitrotriazacyclohexane (Fig. 1) is a heterocyclic nitramine compound commonly referred to as RDX, which is an acronym for Research Department Explosive. It is also known as cyclonite or hexogen.

RDX is a nitrate compound that has been widely used as plastic explosive in Europe and United States since the early 1900s. The plasticized form, called composition C-4, contains 91% RDX, 2.1% polyisobutylene, 1.6% motor oil and 5.3% 2-ethylhexyl sebacate. RDX has also civilian applications, where it is used in fireworks and as a heating fuel for food rations. RDX is classified as an Environmental Protection Agency (EPA) Group C compound, which labels it a possible human carcinogen. However, carcinogenic effects were not noted in rat studies and no human data are available. RDX can cause seizures, methemoglobinemia, anemia, liver and kidney damage in humans and animals when large amounts are eaten or inhaled.

Reported cases of human RDX intoxications and its human pharmacokinetic data are very limited. Occupationally related cases of human RDX intoxication have been reported in Russia [1], Hungary [2], Italy [3], Germany [4] and the United States [5]. RDX intoxication has also been demonstrated in non-occupational circumstances; for example, chewing by a 19-year-old soldier [6], accidentally ingestion by a 3-year-old boy [7]. Soldiers in the Vietnam War were known to ingest composition C-4 intentionally because of presumed psychotropic properties similar to that obtained with cannabis [8].

As RDX and its degradation products can be found in the environment, most of the methods developed are for its detection and determination in water, soil and forensic specimens. The analytical methods used are high-performance liquid chromatography combined with UV detection (HPLC-UV) [9], thermal energy analyzer (HPLC-TEA) [10], mass spectrometry (HPLC-MS) [11]; gas chromatography with either electron-capture detection (GC-ECD) [12] or mass spectrometry (GC-MS) [13]; capillary liquid chromatography [14]; micellar electrokinetic chromatography (MEKC) [15]; high-performance thin-layer chromatography (HPTLC) [16]; supercritical fluid chromatography (SFC) [17]. Other methods have also been proposed, including liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS) [18]; electrospray ionization/ion mobility spectrometry (ESI/IMS) [19]; derivative-UV spectrophotometry [20];

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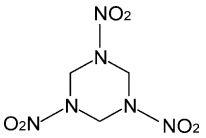

Fig. 1. RDX (cyclonite, hexogen).

liquid chromatography-amperometric detection [21]. Analytical methods specifically used for the determination of RDX in plasma are limited [7,10,22].

In this study, a simple and a rapid method for detecting RDX exposure in a clinical setting was developed. The method was applied to evaluate RDX concentration in plasma samples obtained from 12 soldiers exposed RDX by dermal and inhalation accidentally. The soldiers presented to the emergency department with neurological symptoms from various degrees. After necessary medical treatment they were discharged from hospital. In plasma RDX was determined by HPLC with a reversed-phase C_{18} column and UV-DAD using Tox-clean RC cartridge for SPE.

2. Materials and methods

2.1. Chemicals

RDX (C-4) was acquired from Gulhane Military Medical Academy (GATA, Istanbul, Turkey). All solvents (HPLC grade) and all other chemicals (Analytical reagent grade) were obtained from Merck (Darmsdat, Germany). Tox-clean RC (C_{18}) SPE cartridges (225 mg/ml) were from Alltech (Germany). Ultra-pure water was prepared by ultrafiltration with a Mili-Q water purification system from Millipore (Bedford, MA). Mobile phase was filtered through 0.2 μm membrane filters (Phenomenex, CA). Fresh mobile phase was prepared daily.

2.2. Solutions

The stock standard solution of RDX (15 μg/ml) was prepared in acetonitrile. Working standard solution of this compound, at concentrations range of 0.01–2.0 μg/ml, were obtained by dilution with mobile phase (acetonitrile–water; 35/65; v/v). The stock solutions were stored at 4 °C.

2.3. Instrumental conditions

Liquid chromatographic analyses were performed with a thermo separation products liquid chromatograph (model spectra system®, TSP, CA), equipped with HPLC pump (spectra series pump P4000, TSP, CA), vacuum degasser for liquid chromatography (solvent degasser SCM 1000, TSP, CA), Rheodyne and injection valve (TSP, CA and injection volume: 50 μl). System parameters were controlled with system controller (SN 4000, TSP, CA) and chromatographic data were collected and recorded using the PC 1000 system software. The separation was carried out using a C_{18}, 5 μm Luna column (150 mm × 4.6 mm I.D., Phenomenex, CA) fitted with guard column (4 mm L × 3 mm I.D., Phenomenex, CA) packed with same material. The column eluate was monitored with an UV 6000LP photo-diode array detector.

The analyses were performed at 240 nm with a band of 4 nm and column temperature was ambient. The mobile phase was acetonitrile–water (35:65, v/v) at a flow-rate of 1.0 ml/min.

2.4. Extraction procedures

Plasma (0.1 ml) was deproteinized by adding 0.9 ml methanol then vortex-mixing for 1 min and centrifugation for 10 min at 4500 × g, 0.5 ml of the supernatant was evaporated until its half volume under nitrogen. SPE cartridge was conditioned with 2 ml of methanol and 2 ml of water. The sample was passed through conditioned Tox-clean RC SPE cartridges under vacuum. After washing with 0.5 ml of water, the sorbent bed was dried under vacuum for 10 min. RDX was eluted with 0.5 ml methanol and the eluate evaporated under a gentle stream of nitrogen at 40 °C. The residue dissolved in the mobile phase to a final volume of 0.1 ml.

2.5. Recovery studies

The recovery was assessed by using the samples spiked with RDX at concentrations of 0.01, 0.2 and 1.0 μg/ml in plasma. Spiked samples were extracted on C_{18} columns and chromatographed using the procedure outlined above for each concentration in four replicates.

3. Result and discussion

RDX was separated chromatographically from endogenous compounds of plasma using reversed-phase HPLC with DAD. A C_{18} column with isocratic elution of acetonitrile-water was chosen. This afforded good resolution in a reasonable time. The retention time of RDX was 8.2 min and the run time was 10 min. The analyses were performed at 240 nm. RDX was identified and quantified by comparing its retention value, spectral data and integrated peak area with its known external standard. Blank plasma samples from unexposed human were used as references for the plasma collections. The chromatogram of blank plasma showed no interfering peaks having the same retention times as RDX. Typical chromatograms of the plasma sample spiked with RDX,

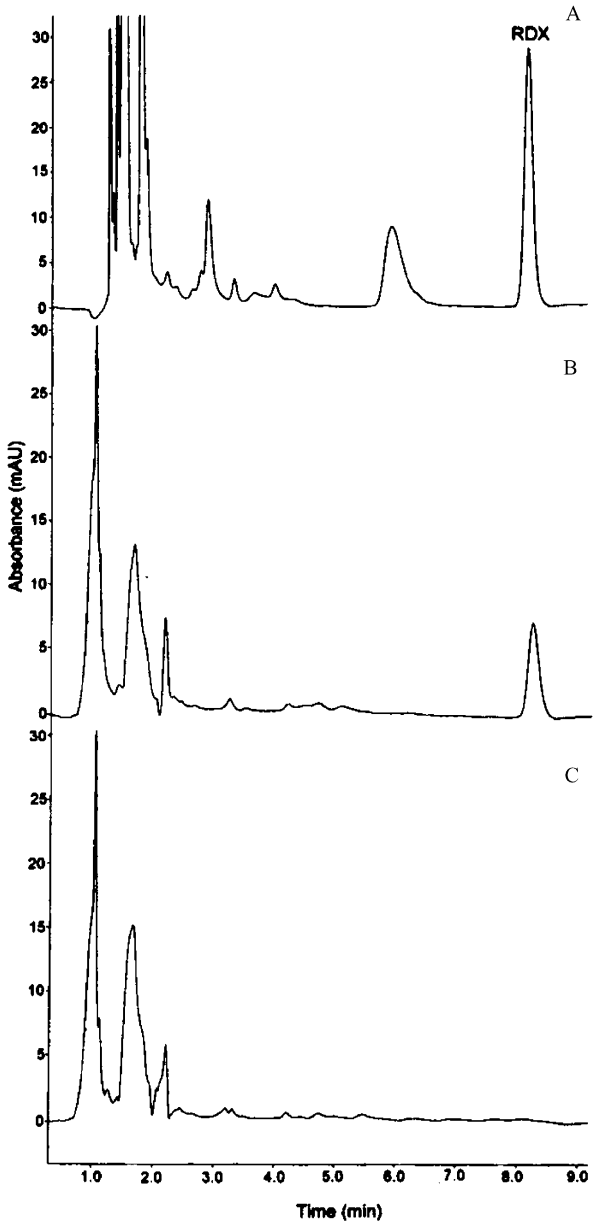

Fig. 2. Chromatograms of patient plasma (A), plasma containing 0.1 μg/ml RDX (B), and blank human plasma (C) with acetonitrile–water (35/65) as mobile phase and detection at 240nm.

patient plasma and blank human plasma are shown in Fig. 2.

The extraction recoveries of RDX were in the range 92.0–98.8% for plasma (Table 1). The linearity was observed in two concentration ranges between 0.01–0.20 μg/ml and 0.1–2.0 μg/ml in plasma with an excellent correlation coefficient of 0.9993 and 09997, respectively. Under the experimental conditions used, the lower limit of detection (LOD) was 0.005 μg/ml for RDX at a signal-to-noise ratio of 3. The lower limit of quantification (LOQ) was found to be 0.01 μg/ml in plasma.

The results of the assay validation study are presented in Table 2. The within-day and day-to-day reproducibilities expressed as relative standard deviation (RSD) were found to be 7.72–10.15 and 8.42–9.15% respectively, indicating good precision. The accuracy of the method expressed as relative mean error (RME) below 1.9%, which was shown to be satisfactory.

Compared with the previously described HPLC methods, the sensitivity of the present study was less than that of HPLC/TEA (LOD 10 ng/l), but better than that of the HPLC/UV method reported by Turley and Brewster [22]. In another HPLC/UV method [7] the data reported are not sufficient to make comparisons of sensitivity.

The proposed analytical methodology was applied to evaluate RDX concentration in plasma samples obtained from 12 soldiers exposed RDX in an ammunition plant. Concentrations of RDX in plasma samples obtained 24, 48 and 96 h following exposure from five people are given in Table 3. Blood samples of another seven people exposed to RDX could be only collected after 24 h. The concentrations of RDX in these samples were ranged from 0.01 to 0.15 μg/l.

As seen in Table 3, it was possible only from one patient to obtain sample at 24, 48 and 96 h after exposition with a peak level at 24 h. The kinetic data of RDX are scare. Only Woody et al. [7] reported some data about its pharmacokinetic. They detected RDX in the serum for 120 h with a peak level at 24 h.

It can be concluded from the study that the purpose. HPLC method, which combines with solid-phase extraction and diode-array detection, reaches the optimum performance in terms of sensitivity, precision and accuracy for pharmacokinetic studies of RDX. On the

Table 1
Extraction recovery for the assay of RDX in plasma ($n = 4$)

Sample	Concentration (μg/ml)		Recovery (%)	RSD (%)
	Added	Found (mean ± SD)		
Plasma	0.01	0.0092 ± 0.001	92.0	10.87
	0.2	0.1960 ± 0.013	98.0	6.63
	1.0	0.9881 ± 0.044	98.8	4.45

Table 2
Within-day and day-to-day precision and accuracy of RDX in plasma

Sample	Concentration (μg/ml)		RSD (%)	RME (%)
	Added	Found (mean ± SD)		
Plasma	Within-day ($n = 6$)			
	0.1	0.0985 ± 0.010	10.15	1.5
	0.5	0.4920 ± 0.038	7.72	1.6
	1.5	1.4715 ± 0.130	8.84	1.9
	Day-to-day ($n = 6$)			
	0.1	0.0984 ± 0.009	9.15	1.6
	0.5	0.4941 ± 0.042	8.50	1.2
	1.5	1.4735 ± 0.124	8.42	1.8

Table 3
RDX concentrations (μg/ml) in plasma samples

Patient no.	RDX concentration (μg/ml)		
	24 [a]	48 [a]	96 [a]
1	0.66	0.52	
2	0.27		0.14
3	0.38		0.02
4	0.92	0.86	
5	0.97	0.36	0.16

[a] Time following exposure (h).

other hand, the data, obtained in this study about the plasma level after RDX human toxication, may be useful when added to previous reports for the evaluate of its human pharmacokinetics.

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