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Note

Liquid chromatographic analysis of cyclotrimethylenetrinitramine in biological fluids using solid-phase extraction

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Cyclotrimethylenetrinitramine (RDX) is a nitrate explosive compound found in "plastic" explosives. The plasticized form, called composition C4, contains 91% RDX, 2.1% polyisobutylene, 1.6% motor oil and 5.3% 2-ethylhexyl sebacate. RDX has both military and civilian applications, where it is used in demolition blocks, fireworks and as a heating fuel for food rations. It has lately become a popular tool for terrorists.

Human ingestion and inhalation has been documented on numerous occasions. Soldiers in Vietnam were known to ingest composition C4 accidently or intentionally, the latter in an attempt to produce a "high" similar to that obtained with cannabis [1]. Tsa and Lee [2] reported a case of accidental ingestion of RDX that was present in cooking utensils. Woody et al. [3] recently described a case of a child who developed seizures after ingesting composition C4.

Analytical methods to determine RDX in biological fluids employ liquid–liquid extraction followed by gas chromatography [4] or high-performance liquid chromatography (HPLC) using an external standard approach [3]. In this paper, an HPLC method with ultraviolet detection at a specific wavelength is used to identify RDX by an internal standard approach. The explosive is separated from interfering components in serum and urine using a C_{18} bonded-phase extraction column.

EXPERIMENTAL

Materials

RDX in ethanol was obtained as a gift from Oak Ridge National Labs. (U.S.A.). Drug-free human serum and urine were collected from laboratory employees. Dis-

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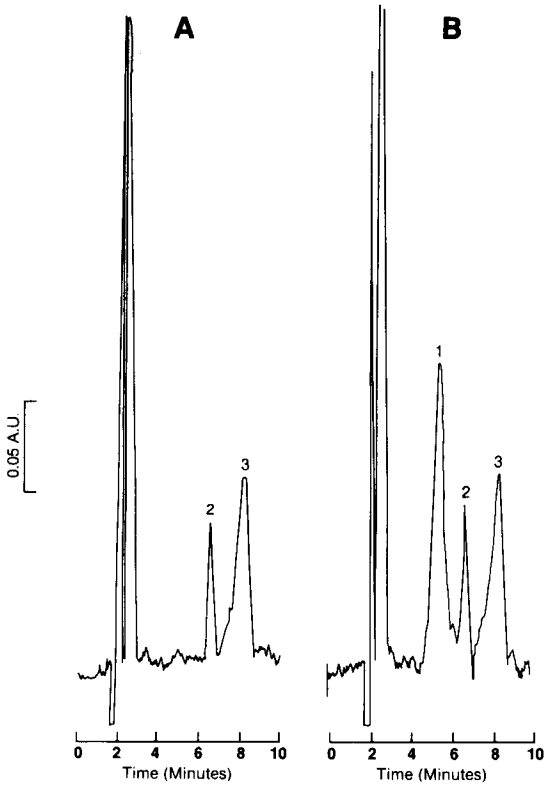

Fig. 1. Chromatograms of urine containing 5.0 mg/l RDX (peak 1), 20 mg/l phenobarbitol (peak 2) and the internal standard, PNA (peak 3). (A) Urine containing no RDX; (B) urine containing RDX.

posable 1-ml C_{18} solid-phase extraction columns (Bond-Elut, Analytichem International, Harbor City, CA, U.S.A.) were used to purify the samples before injection. *p*-Nitroacetanilide (PNA) and phenobarbital were from Sigma (St.

TABLE I

RECOVERY OF RDX IN SERUM AND URINE

RDX at the concentrations indicated was added to serum or urine and measured by HPLC as described. Results are expressed as the mean ± S.D. for three determinations at each concentration.

Matrix	Concentration added (mg/l)	Concentration measured (mg/l)	Recovery (%)
Serum	1.0	0.9 ± 0.02	90 ± 2.0
	5.0	5.0 ± 0.09	100 ± 1.8
	10.0	10.1 ± 0.11	101 ± 1.1
Urine	1.0	1.0 ± 0.01	100 ± 1.0
	5.0	4.9 ± 0.08	98 ± 1.6
	10.0	10.1 ± 0.13	101 ± 1.3

Louis, MO, U.S.A.).

Apparatus

A Tracor liquid chromatograph with Model 995 isochromatographic pump and Model 970 variable-wavelength detector was used in a reversed-phase system with a 30 cm × 3.9 mm μBondapak C_{18} column, 10 μm particle size, (Waters, Milford, MA, U.S.A.) and methanol–water (36:65, v/v) as the mobile phase. Assays were performed at ambient temperature with a flow-rate of 1.8 ml/min. A 0.05-ml sample was injected (Rheodyne, Berkely, CA, U.S.A.) and the effluent monitored at 240 nm.

Sample preparation

Serum or urine (0.1 ml) was added to 0.01 ml of the internal standard consisting of 1 mg/ml PNA in water and vortex-mixed for approximately 30 s. The specimens were then applied to water-conditioned C_{18} bonded-phase extraction columns and eluted at 30–40 kPa pressure. A 0.5-ml volume of water was washed through the extraction column to remove interfering materials. RDX and the internal standard were eluted with 0.5 ml of methanol and evaporated to a volume of approximately 0.1 ml for injection into the HPLC system.

RESULTS AND DISCUSSION

A chromatogram of urine containing RDX, the internal standard and phenobarbital is shown in Fig. 1. There was clear resolution of each component, with retention times of 5.8 min (RDX), 6.4 min (phenobarbital) and 8.4 min (PNA). Use of the internal standard PNA should improve the accuracy and recovery of the analysis. Phenobarbital is the most commonly used anticonvulsant for seizures of the type produced in RDX intoxication, and its distinct resolution from RDX and PNA should not confound analysis. Linearity was established between 0.2 and 10.0 mg/l, and recovery was excellent (Table I). The limit of detection was 0.1 mg/l. Between-day precision ($n=7$) at 5.0 mg/l was ±0.1 mg/l (coefficient of variation = 2%). Specimen clean-up through the C_{18} bonded-phase extraction columns removed numerous extraneous compounds from the urine specimen that were found to elute in close proximity to RDX in the absence of sample prepurification.

HPLC is one of the few methods suitable for detecting RDX exposure in a clinical setting. Although its structure is that of a nitrate compound, it does not appear to produce methemoglobinemia. The potential for exposure in munitions workers, military personnel and others is considerable, and may lead to serious medical complications or fatalities (although by a mechanism different from which it was designed). In a case recently reported from our institution, a three-year-old child accidently chewed on "clumps" of composition C4 and, with the use of HPLC, we were able to identify RDX in body fluids and conduct a pharmacokinetic evaluation [3]. Serum concentrations at 24 and 48 h after ingestion were 10.74 and 3.56 mg/l, respectively. Urine concentrations at 24 and 48 h following ingestion were 4.68 and 38.41 mg/l, respectively.

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