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APPLICATIONS OF LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY IN METABOLIC STUDIES OF EXPLOSIVES

J. YINON* and D.-G. HWANG
Department of Isotope Research, Weizmann Institute of Science, Rehovot (Israel)

SUMMARY

Liquid chromatography–mass spectrometry was used for the detection and identification of metabolites of 2,4,6-trinitrotoluene (TNT) in urine and blood. The metabolites were found in the urine of rats and in the blood of rabbits fed with TNT, in the urine of rats exposed to TNT by skin absorption and in the urine of TNT munition workers. The detected metabolites, formed by reduction processes, included 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene, in addition to untransformed TNT.

INTRODUCTION

The metabolism of explosives in the human body and the analysis of explosives and their metabolites in body fluids have important applications in preventive medicine and in environmental analysis. In the medical field, because of the toxicity of many explosives[1], contact with and inhalation of their dust or vapour represents a major health hazard for munition workers. Periodical analysis of the body fluids of personnel working in explosives manufacturing plants could reveal traces of explosives and their metabolites. In the environmental field, improper disposal of obsolete explosives may cause serious contamination problems[2]. Trace detection of explosives and their metabolites in the blood and urine of animals and humans in the disposal area may reveal the extent of contamination.

We have focused our attention on the metabolites of 2,4,6-trinitrotoluene (TNT) because it is one of the most widely used explosives. The purpose of this study was to detect and identify metabolites of TNT in the body fluids of laboratory animals and in occupationally exposed humans. Liquid chromatography–mass spectrometry (LC–MS) was chosen because it incorporates good separation characteristics with highly specific detection. LC–MS has the advantage over gas chromatography–mass spectrometry (GC–MS) that it is suitable for thermally sensitive and involatile compounds.

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EXPERIMENTAL

Equipment

The LC–MS system consisted of a high-performance liquid chromatographic (HPLC) system interfaced to a laboratory-built 90°, 4-in. radius magnetic sector mass spectrometer equipped with a high-speed differential pumping system[3]. Two HPLC systems and LC–MS interfaces were used:

(1) An Eldex A-30-S pump (Eldex Labs., Menlo Park, CA, U.S.A.), a Rheodyne 7125 sample injector (Rheodyne, Cotati, CA, U.S.A.) and a Waters 441 UV detector (Waters Assoc., Milford, MA, U.S.A.), operated at 214 nm. The HPLC column used was an RP-8 (C_8) reversed-phase column containing LiChrosorb, particle size 5 μm (10 cm × 4.6 mm I.D.) (Brownlee Labs., Santa Clara, CA, U.S.A.). The mobile phases were acetonitrile–water in various proportions at a flow-rate of 1 ml/min. The LC–MS interface was a Hewlett-Packard variable split-type direct liquid introduction (DLI) probe (Hewlett-Packard, Palo Alto, CA, U.S.A.).

(2) A Gilson 302 pump (with 5S head) (Gilson, Villiers Le Bel, France), a Rheodyne 7520 micro sample injector and a Waters 441 UV detector with a microbore cell, operated at 214 nm. The HPLC column used was an RP-8 (C_8) reversed-phase column containing LiChrosorb, particle size 5 μm (10 cm × 2.1 mm I.D.) (Brownlee Labs.). The mobile phases were acetonitrile–water and methanol–acetonitrile–water in various proportions at flow-rates of 120–130 μl/min. The LC–MS interface was a laboratory built Henion-type[4] direct liquid introduction probe.

Samples and solvents

TNT was obtained in pure form from the Israeli Police Analytical Laboratory. Toluene (Aldrich, Milwaukee, WI, U.S.A.) and methylene chloride (Merck, Darmstadt, F.R.G.) were of UV grade. The HPLC solvents used were UV-grade acetonitrile and methanol (Fluka, Buchs, Switzerland) and triply distilled water. The solvents were filtered through a 1.0 μm filter (Whatman, Maidstone, U.K.) and the sample solutions through a 0.5 μm filter (Millipore, Bedford, MA, U.S.A.).

Animal experiments

Male SPD rats weighing 200–250 g were fed with single doses of 20 mg of TNT dissolved in 1 ml of corn oil, using a stomach tube. Each rat was housed in a metabolic cage for urine collection. Urine extracts (with toluene) were analysed by LC–MS[5].

Male rabbits weighing *ca.* 2 kg were fed with doses of 100 mg of TNT dissolved in 3.5 ml of corn oil, using a stomach tube. Blood samples (3 ml) were taken from the rabbits' ears 1, 3, 6 and 12 h after feeding. Serum extracts (with methylene chloride) were analysed by LC–MS[6].

Patches containing a mixture of 20 mg of TNT with five drops of glycerol were applied on a shaved surface of 4 cm^2 of the skin of male SPD rats for 2 h. The patches were then removed and the rats were housed in metabolic cages for urine collection. Urine extracts were analyzed by micro-LC–MS.

In all animal experiments blank urine samples were taken for comparison from animals that had not been fed with or had skin contact with TNT.

Analysis of human urine

The toluene extracts of seven urine samples from TNT munition workers were analysed by micro-LC–MS[7]. The full extraction procedure is given in detail elsewhere[5]. As no information was available regarding the origin of the urine samples (apart from the fact that they were from TNT workers), TNT could have been absorbed by inhalation, digestion or skin absorption.

The recovery efficiency of TNT, 2-amino-4,6-dinitrotoluene (2-A) and 4-amino-2,6-dinitrotoluene (4-A) was 90%, whereas that of 2,4-diamino-6-nitrotoluene (2,4-DA) and 2,6-diamino-4-nitrotoluene (2,6-DA) was only 30%[5].

LC–MS analysis

Standard metabolites of TNT, which had been previously synthesized[8], were used for quantitative calibration. This was done by plotting HPLC peak heights against the amounts of standard metabolite injected, giving calibration graphs that could be used for the quantitation of sample metabolites[7]. No internal standard was used. The measurement reproducibility was therefore ± 10%. Higher accuracy was not necessary because of the wide range of the amounts of metabolites obtained from animals of the same type and weight.

RESULTS AND DISCUSSION

In rats fed with 20 mg of TNT, amounts of 50–500 ng/ml of TNT, 100–1200 ng/ml of 2-A, 50–1350 ng/ml of 4-A and 1–12 ng/ml of 2,4-DA were found in the urine between 12 and 48 h after feeding[5].

In rabbits fed with 100 mg of TNT, amounts of 4–306 ng/ml of TNT 2–161

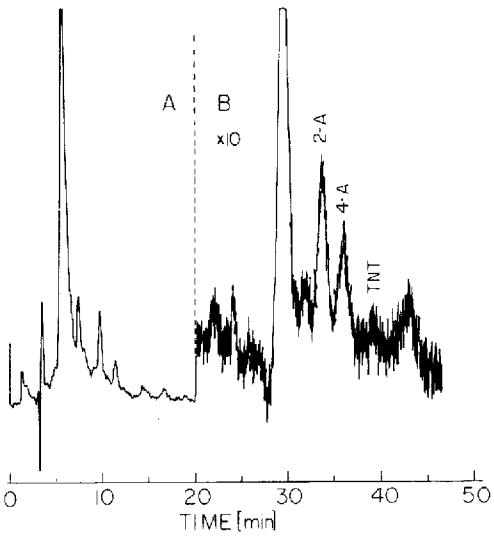

Fig. 1. Micro-HPLC UV trace for a rat urine sample containing metabolites of TNT in the skin absorption experiment. Column, RP-8 (10 cm × 2.1 mm I.D.); mobile phase, methanol–acetonitrile–water (4:23:73); flow-rate, 125 μl/min; UV wavelength, 214 nm.

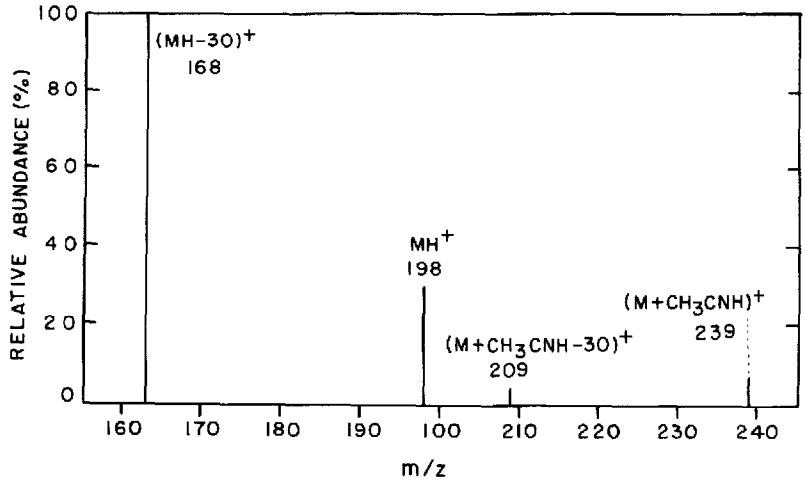

Fig. 2. Mass spectrum of separated 2-A fraction from the same urine sample as in Fig. 1.

ng/ml of 2-A and 1–96 ng/ml of 4-A were found in serum samples between 1 and 12 h after feeding[6].

In the skin absorption experiment with rats, amounts of 0–12 ng/ml of TNT, 0–34 ng/ml of 2-A and 0–24 ng/ml of 4-A were found in the urine between 6 and 12 h after removal of the applied TNT from the skin.

Fig. 1 shows the UV trace of a urine extract from a rat in the skin absorption experiment. Several large peaks originating from urine components that were not separated during the extraction procedure appear in the chromatogram, but they do not interfere with the metabolites of interest.

Fig. 2 shows the mass spectrum of 2-A. This is a chemical ionization (CI) mass spectrum using the mobile phase as CI reagent. The peak at m/z 239 is due to the adduct ion $(M+CH_3CNH)^+$. The loss of 30 mass units from two ions was mainly due to a reduction effect in the CI ion source in the presence of water[9]. These two ions are $(MH-30)^+$ at m/z 168 and $(M+CH_3CNH-30)^+$ at m/z 209. The mass spectrum of 4-A is very similar. 2-A and 4-A can be differentiated by mass spectrometry only in the electron-impact mode[10].

CONCLUSIONS

The results of these laboratory animal experiments indicate that TNT absorbed by ingestion and skin penetration, as reflected in the urine, and by ingestion, as reflected in the blood, is transformed into metabolites formed by reduction processes.

Similar reduction metabolites were found in the urine of TNT munition workers, where TNT could have been absorbed through inhalation, digestion or skin absorption. The detection limit of the metabolites in urine was about 0.1 ng/ml, and was obtained in the single ion monitoring mode.

Micro-LC–MS seems to be an adequate screening method for the early detection of TNT metabolites in the urine of munition workers.

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