CASE REPORT

R.-L. Lin,[1] *Ph.D., R. J. Stein,*[1] *M.D., and M. I. Schaffer,*[1] *Ph.D.*

A Purinethol® (6-Mercaptopurine) Fatality in a Case of Prescription Negligence: A Gas Chromatographic Determination of 6-Mercaptopurine

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ABSTRACT: A 64-year-old white female was given Purinethol® (6-mercaptopurine) in place of propylthiouracil. A gas chromatographic method for the determination of 6-mercaptopurine in blood and other biological tissues has been developed. Samples were extracted with chloroform/isopropanol (4:1) at pH 7.0 and back-extracted into 0.5*N* sodium hydroxide (NaOH). The NaOH fraction was neutralized and buffered at pH 7.0 and extracted with chloroform/isopropanol (4:1). Quantitation was made by gas chromatography following methylation of the drug with trimethylanilinum hydroxide on an OV-101 or OV-225 column, using an internal standard. 6-Mercaptopurine was identified in all tissues by gas chromatography/mass spectrometry. The derivatized drug was identified by its electron impact mass spectrum as a dimethylated compound that has a molecular ion at *m/e* 180, which is also the base peak. The highest concentration of Purinethol was found in blood (110 mg/L). Concentrations in other tissues have been given. This is probably the first reported death by Purinethol.

KEYWORDS: toxicology, 6-mercaptopurine, chromatographic analysis

Purinethol® (6-mercaptopurine) was first described by Elion and her co-workers in 1952 and shortly thereafter was found to have significant activity against human leukemias. Today, 6-mercaptopurine remains the most valuable purine analog for the treatment of acute leukemia [*1*].

Chemically, 6-mercaptopurine is the 6-thio analog of the purine base hypoxanthine and adenine, a constituent of nucleic acid. It is an antimetabolite for adenine in the synthesis of nucleotides by living cells. Therefore, it can repress cell division and cause a temporary remission of leukemia [*1,2*].

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[1]Assistant chief toxicologist, chief medical examiner, and chief toxicologist, respectively, Cook County Institute of Forensic Medicine, Office of the Medical Examiner, Chicago, IL 60612.

The first signs of toxicity of 6-mercaptopurine are a dramatic decrease in the white cell (or leukocyte) count and a depression of the bone marrow [*1,3*]. The toxic effects include leukopenia, hemorrhage, jaundice, liver damage, and impairment of renal function.

After oral ingestion, 6-mercaptopurine is readily absorbed. It is rapidly metabolized by the enzyme xanthine oxidase to 6-thiouric acid [*1*]. The usual dosage for adults is 100 to 200 mg daily [*1,3*]. The half-life of the drug is relatively short (about 90 min).

There is no information available on methods for determining the presence of 6-mercaptopurine in postmortem tissues. Fluorometric procedures have been reported [*4,5*] in patients. High-performance liquid chromatography has also been used to determine 6-mercaptopurine [*6,7*] in renal transplant patients. A microbiological assay [*8*] and a mass spectrometric determination of 6-mercaptopurine [*9*] have also been reported. This study will describe a gas chromatographic determination of 6-mercaptopurine and its tissue distribution in a fatal case.

Case Report

A 64-year-old white female had been in the hospital for a week and was then released. The patient had been treated for pulmonary edema and hyperthyroidism. When the patient left the hospital, she was given prescriptions for propranolol, potassium iodide solution, and propylthiouracil. The patient had these prescriptions filled at a local pharmacy. After two weeks, the patient was readmitted to the emergency room at the same hospital with an admission diagnosis of multiple cardiac arrests. The doctor at the hospital reviewed the patient's prescriptions and discovered that the patient was taking 200 mg of Purinethol every 8 h. This compound had been dispensed in place of propylthiouracil. When admitted, the patient was in hepatic failure and anemic. The day after admission she had the first of three seizures. She died on the third day. An investigator from the medical examiner's office recovered four prescription containers. One of the containers held propranolol, one held potassium iodide, and two held 6-mercaptopurine.

Materials and Methods

A gas chromatographic method for the determination of 6-mercaptopurine was developed and used to measure the amount present in the tissues of the deceased. The extraction procedure was based on the solubility of 6-mercaptopurine in dilute alkaline solution. 6-Mercaptopurine was converted into its dimethylated derivative, which was then determined by gas chromatography.

Extraction Procedure

One millilitre of blood, bile, or tissue homogenate was obtained and 20 μL of the internal standard, theophylline (1.0 mg/mL), was added; it was then made neutral with 2 mL of pH 7.0 phosphate buffer and extracted with 10 mL of a 4:1 mixture of chloroform to isopropanol. After centrifugation, the organic layer was pipetted into a clean glass tube. Two millilitres of 0.5*N* sodium hydroxide was added, and the mixture was shaken on a mechanical shaker for 5 min. After centrifugation, the sodium hydroxide fraction was transferred to a clean glass tube and neutralized with 0.5 mL of 2*N* hydrochloric acid and 1.5 mL of pH 7.0 phosphate buffer. Ten millilitres of a 4:1 mixture of chloroform to isopropanol were added and the mixture was shaken for 5 min. After centrifugation, the solvent fraction was transferred to a clean concentrator cup and evaporated to dryness at 50°C on a concentrator with the aid of a gentle stream of nitrogen.

Gas Chromatography/Mass Spectrometry

A Finnigan 3200 gas chromatograph/mass spectrometer equipped with an Incos data system was used for the qualitative identification of 6-mercaptopurine in all tissues of this case. The gas chromatographic conditions used were similar to those described in the section on gas chromatography below, except that helium served as the carrier gas. All electron impact (EI) spectra were obtained at 70 eV.

Gas Chromatography

The dry residue was dissolved into 20 μL of methanol. Twenty microlitres of trimethylanilinum hydroxide (TMAH) were added to the cup and 1 μL of the solution was then injected into a gas chromatograph equipped with a flame ionization detector (FID). When a nitrogen-phosphorus detector (NPD) was used, the mixture was further diluted before injection.

All gas chromatographic determinations were performed on a Hewlett-Packard 5840A computerized gas chromatograph. A 1.2-m (4-ft) glass column packed with either 3% OV-101 or 5% OV-225 was used with the column temperature at 180°C isothermal, injection temperature at 275°C, detector temperature at 275°C, and carrier gas (nitrogen) flow rate at 30 mL/min.

Quantitation was based on a least-squares plot of peak area ratio of dimethylated 6-mercaptopurine to the internal standard, methylated theophylline.

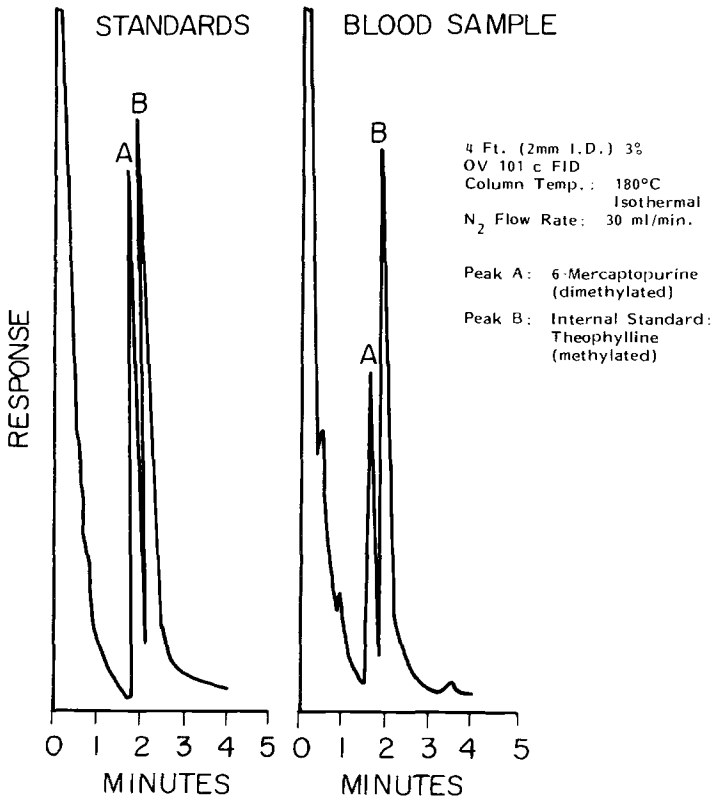

FIG. 1—*Gas chromatogram of dimethylated 6-mercaptopurine and methylated theophylline (internal standard).*

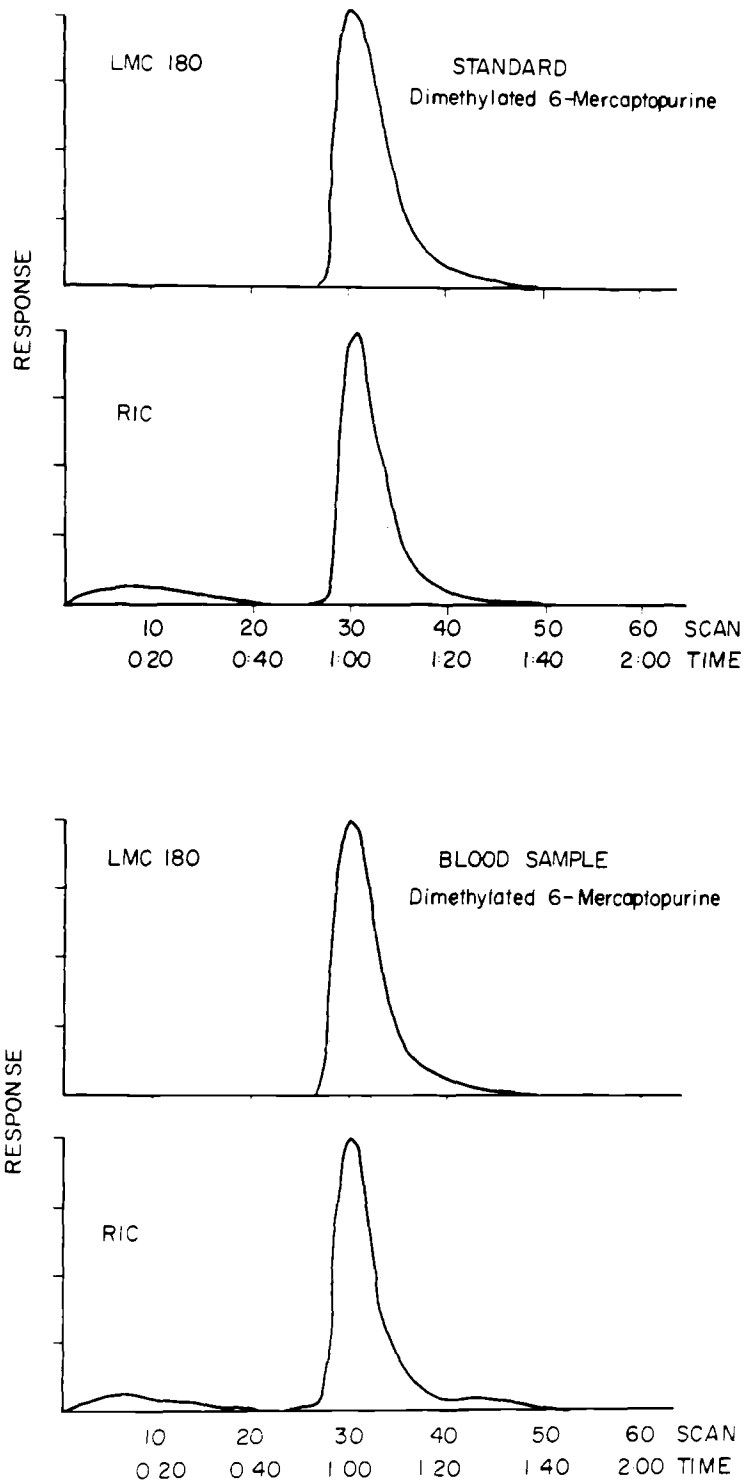

FIG. 2—*Limited mass chromatogram and reconstructed ion chromatogram of dimethylated 6-mercaptopurine.*

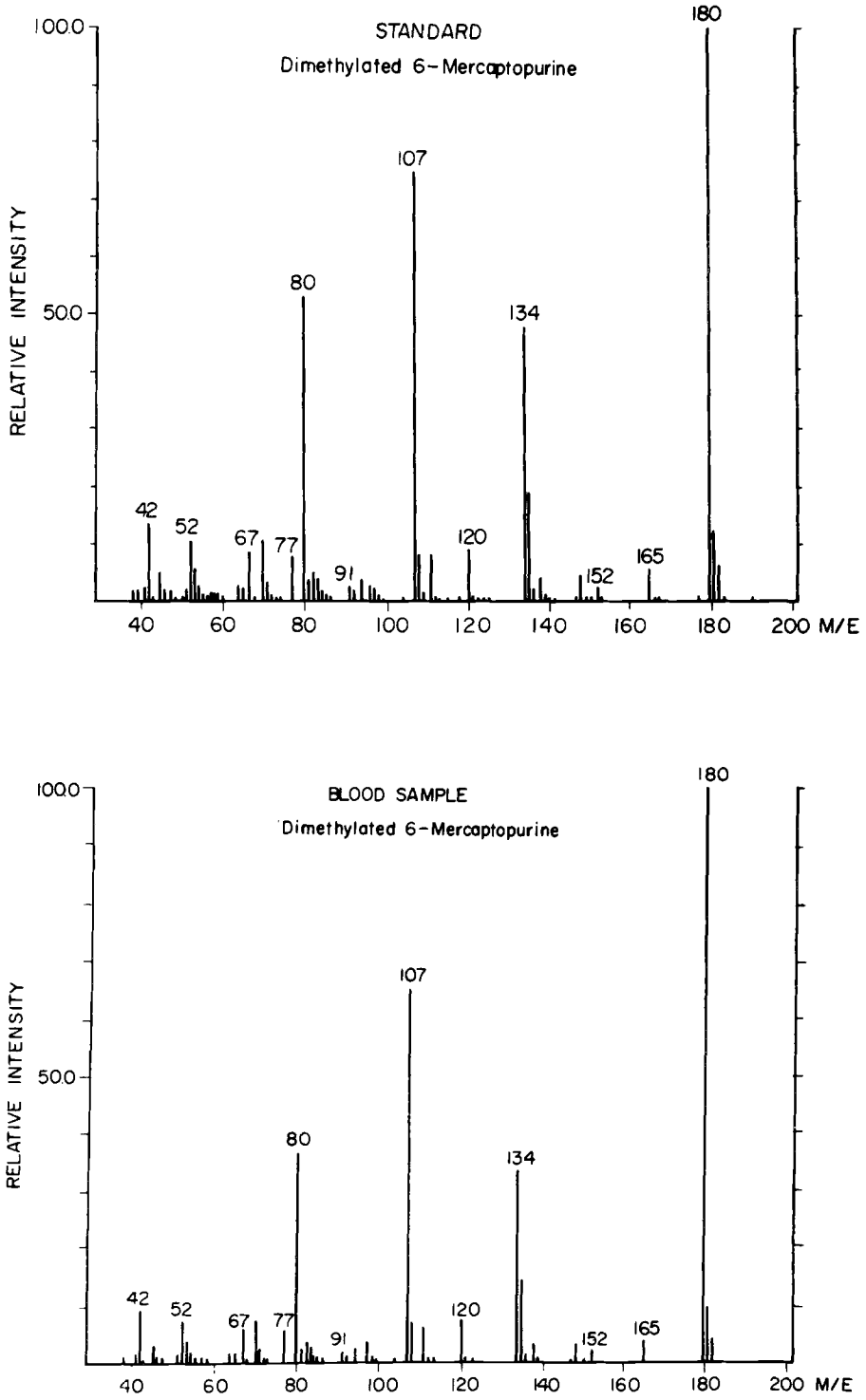

FIG. 3—*Electron impact mass spectrum of dimethylated 6-mercaptopurine.*

Results and Discussion

A typical gas chromatogram obtained under the experimental conditions described in the methods is shown in Fig. 1. A plot of concentration versus gas chromatographic response was linear for the drug over the range between 2 and 20 mg/L. All tissue homogenates were properly diluted to fall within these concentrations. Theophylline served as a good internal standard. Allopurinol, which is chemically closer to 6-mercaptopurine, can also be used as an internal standard. However, allopurinol, a xanthine oxidase inhibitor, is usually given concurrently with 6-mercaptopurine to slow the metabolism of the 6-mercaptopurine [*3*]. A specimen analyzed for 6-mercaptopurine, therefore, might also contain allopurinol.

The presence of 6-mercaptopurine was identified in all tissues by gas chromatography/mass spectrometry. Figure 2 shows the mass chromatograms of the standard dimethylated 6-mercaptopurine and the blood sample. The limited mass chromatogram (LMC) of the base peak at *m/e* 180 of dimethylated 6-mercaptopurine and the reconstructed ion chromatogram (RIC) gave a single peak at Scan 30. The LMC 180 and RIC of the blood sample showed very similar results. The mass spectra of the corresponding peaks (Scan 30) obtained from the standard and the blood sample are compared in Fig. 3. Both the standard and the blood sample gave almost identical mass spectra. The derivatized drug was identified as a dimethylated 6-mercaptopurine. It has a molecular ion at *m/e* 180 as a base peak. The other predominant fragmentation ions were at *m/e* 80, 107, and 134. Similar gas chromatographic/mass spectrometric identification of 6-mercaptopurine in such other tissues as urine, bile, kidney, brain, and gastric contents was successful.

The concentrations of 6-mercaptopurine in the tissues were examined, and the highest concentration was found in the blood (110 mg/L). The concentrations in the other tissues tested were 16 mg/L, 33 mg/kg, and 10 mg/kg in bile, kidney, and brain, respectively. The therapeutic peak concentrations of 6-mercaptopurine in plasma, reported for four renal transplant patients who received oral doses of 75 to 100 mg, were 30 to 50 ng/mL after 1 to 2 h [*7*]. Peak concentrations of 45 to 75 ng/mL in plasma were reported for monkeys 1 h after oral doses of 1.47 mg of 6-mercaptopurine per kilogram of body weight [*6*]. The concentration of 6-mercaptopurine in blood obtained in this case was a thousand times greater than the therapeutic concentration reported. The higher concentration of 6-mercaptopurine in blood and other tissues in this case might be explained on the basis of the toxicity of 6-mercaptopurine. Liver damage and impaired renal function caused by 6-mercaptopurine might result in the slower elimination and greater accumulation of the drug in blood and other tissues. A review of the literature failed to reveal any previous report of accidental or intentional overdose with 6-mercaptopurine. This was, therefore, the first fatal case reported. The autopsy findings indicated that death was caused by bone marrow aplasia with toxic hepatitis caused by acute Purinethol toxicity.

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Address request for reprints or additional information to
Reng-Lang Lin, Ph.D.
Cook County Institute of Forensic Medicine
Office of the Medical Examiner
1828 W. Polk St.
Chicago, IL 60612