CASE REPORT

Takeshi Saito,[1] *B.S.; Masanao Kojimahara,*[1] *M.D.;*
Kimiko Aoki,[2] *Ph.D.; and Sanae Takeichi,*[1] *M.D., Ph.D.*

Detection of Hydroquinone in a Poisoning Case

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ABSTRACT: A fatality due to the ingestion of photographic developer solution containing hydroquinone is described. Hydroquinone was extracted from autopsy materials and identified using gas chromatography-mass spectrometry (GC-MS). The concentration of hydroquinone in the urine, liver, and kidney were 3.4 μg/mL, 0.5 and 0.2 μg/g, respectively.

KEYWORDS: toxicology, hydroquinone, photographic developer, gas chromatography-mass spectrometry, chemical analysis

In this case, a photographic developer solution was used for purpose of suicide. Photographic developer solution contains hydroquinone and metol for reducing agent. Therefore, the purpose of this study was to analyze the distribution of hydroquinone in autopsy materials using a GC-MS. Death was due to shock due to the rupture of transverse colon.

Hydroquinone has been investigated in detail for a long time. Zeidman and Deult reported two autopsy cases and their clinical histories of hydroquinone poisoning [*1*], but hydroquinone concentration in autopsy materials were not described. A few papers have reported hydroquinone and its metabolites concentrations in humans or experimental animals [*2–5*]. These studies show that hydroquinone is eliminated in the urine as the glucuronide conjugate or sulfuric conjugate. There is no reported analysis of the distribution of hydroquinone in man. Many methods have been described for the analysis of hydroquinone, using high-performance liquid chromatography (HPLC) [*5–7*], gas chromatography (GC) [*8*] and GC-MS [*9,10*].

This paper describes the GC-MS analysis of hydroquinone in autopsy materials.

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[1]Forensic Chemist, Assistant and Professor, respectively, Department of Forensic Medicine, Tokai University School of Medicine, Kanagawa, Japan.

[2]Lecturer, Department of Biochemical Toxicology, Pharmaceutical Sciences, Showa University, Tokyo, Japan.

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Case History

The decedent was an alcoholic 46-year-old printer who had also been neurotic. One morning, his wife found him in his workroom, falling and vomiting but able to walk. He said he had committed suicide by ingesting a photographic developer solution; and he was admitted to hospital. On admission, he was semiconscious and did not respond to questions.

Extensive gastric lavage and forced diuresis were performed immediately on admission. Blood and urine sample collected on admission showed hemolysis and myohemoglobinuria. Subsequent urine samples were myohemoglobinuria until 9 h after admission. Subsequently the volume of urine decreased, at last he had become anuretic. He died after 7 h, and underwent an autopsy 22 h after the death.

Materials and Method

Reagents and Chemicals

Each sample was stored in the freezer (at −20°C) until chemical analysis was performed. At the time of analysis each sample was thawed to room temperature.

Limpet acetone powder (Sigma Chemical Co. Ltd., Type I), *l*-ascorbic acid, catechol, hydroquinone, trichloroacetic acid, *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and other chemicals (Wako Co., special reagent grade) were used.

GC-MS Conditions

A Hewlett Packard 5971 series mass selective detector was used in combination with a Hewlett Packard 5890 series II gas chromatograph for analysis, equipped with a J & W dimethyl polysiloxane fused silica capillary column (DB-1, 30 m × 0.25 mm I.D., 0.25 μm film thickness) and using helium as carrier gas at 10 psi. Splitless injection with manual and a valve off time of 0.75 min was used for injection. The GC-MS was run in the selected ion monitoring (SIM) mode. The temperature was programmed from 100° to 200°C at 10°C/min. The injector and interface temperatures were 200°C and 280°C, respectively.

Extraction and Derivatization for GC-MS Analysis

Each 1.0 g sample of tissue was homogenized at 4°C with 4 mL of 0.1% ascorbic acid and 5 μg of catechol (internal standard), then centrifuged at 12 000 rpm for 20 min, deproteinized with 1.5 mL of 20% trichloroacetic acid, and transferred. pH values of the transferred supernatant were adjusted to 3.0 with 0.5 *N* sodium hydroxide. The supernatants were extracted with 4 mL of ethyl ether and the mixture were centrifuged at 3000 rpm for 5 min. The ethyl ether phases were added to the same quantity of saturated sodium chloride solution and the mixture was shook for 20 min. The ethyl ether phases were then evaporated to dryness with a stream of nitrogen. Then the residue was trimethylsilylated with 100 μL of BSTFA at 65°C for 30 min and 0.1 μL of the sample was injected to GC-MS.

A 1.0 mL sample of urine was incubated with 3.0 mL pH 4.5 acetate buffer, 4 drops of chloroform, 40 mg of limpet acetone powder (glucuronidase and sulfatase) and 5.0 μg of catechol at 37°C for 24 h in glass tube. The extract was then processed as previously described.

The concentrations of hydroquinone in all the samples were calculated from the stan-

dard curve for hydroquinone, which was prepared with known concentrations of hydroquinone following the same method.

The mass ions (m/z) used for quantitation were 239 and 254 for catechol and hydroquinone.

Results

Qualitative and quantitative analyses of hydroquinone in autopsy materials were performed by GC-MS. The GC-MS system described above separated hydroquinone and catechol completely. A chromatogram of trimethylsilylated derivative of ethyl ether extract from postmortem liver tissue sample is shown in Fig. 1. Hydroquinone has a retention time of 9.4 min and catechol of 8.2 min. The mass spectrums for a hydroquinone standard and the liver extract are shown in Fig. 2. The concentrations of hydroquinone in autopsy materials are shown in Table 1. The detection limit for hydroquinone (signal-to-noise ratio >2) was 16 ng/g.

Discussion

All of the tissues extracted and analyzed in exactly the same way. Any metabolites were not detected. Gad-ElKarim [*6*] and Anwar [*7*] were used of the ethyl ether as an extracting solvent at pH 3.0. O'Grodnick [*8*] was used of ethyl acetate as extracting solvent. In our analysis, ethyl ether extraction was done earlier than ethyl acetate. At pH 3.0, the extraction efficiencies of hydroquinone were maximum.

Although hydroquinone concentrations were determined, these value were considerably low. Chakraborty [*11*] reported that male black and albino rats ingesting hydroquinone for 7 days (25 mg/day) accumulated 0.597 mg/g (black rat) and 0.627 mg/g (albino rat) in the liver. Our results were lower than these values. The therapeutic transfusion might be reflected in the results.

There were higher concentrations of hydroquinone in the liver and kidney than in other tissues. The results showed approximately similar to the examination of rats by Divincenzo [*5*]. It has been suggested that liver or kidney is a better sample than other tissues for hydroquinone determinations.

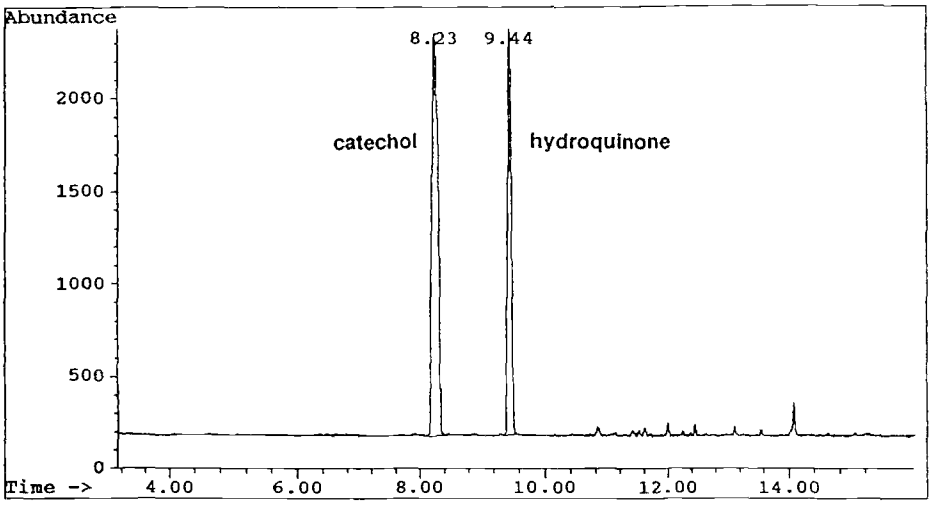

FIG. 1—*GC-MS chromatogram of trimethylsilylated derivative of ethyl ether extract from liver tissue sample.*

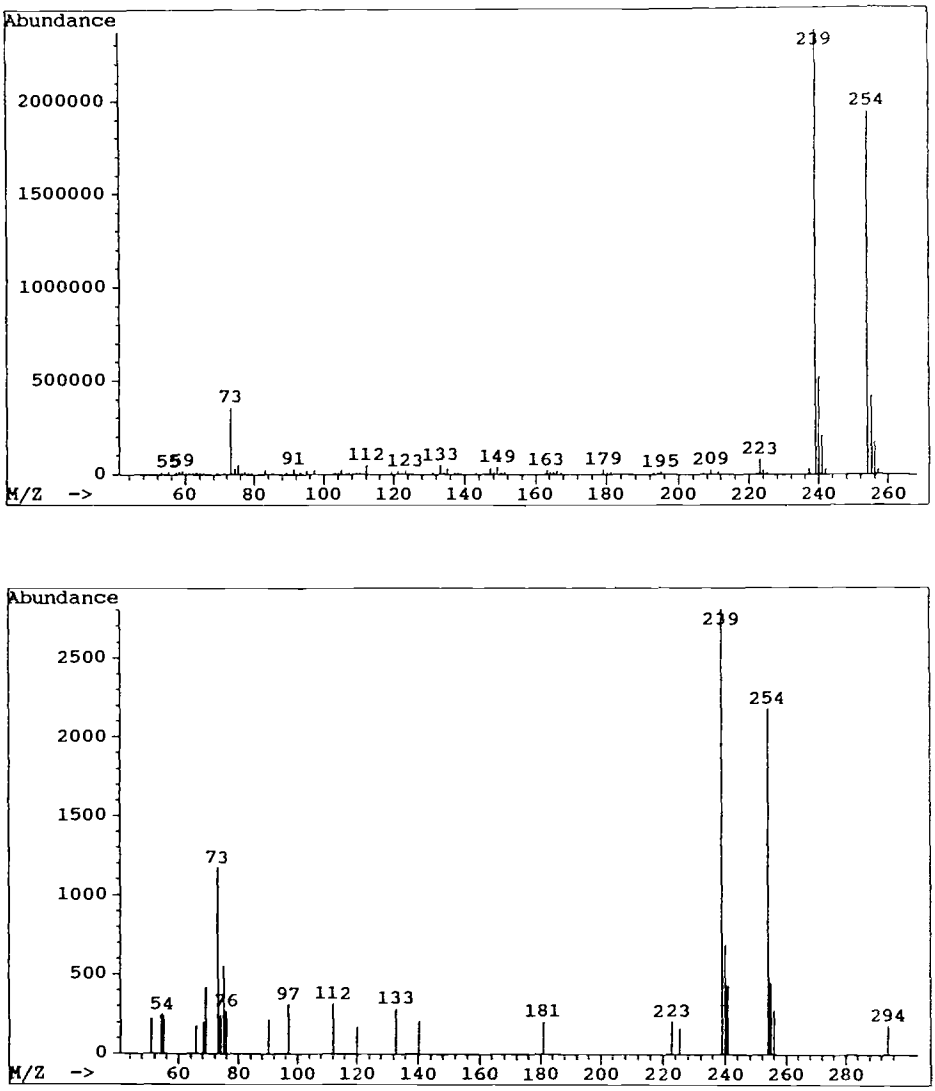

FIG. 2—*Mass spectra of the BSTFA derivatives of hydroquinone standard* (top) *and liver extract* (bottom).

TABLE 1—*Concentrations of hydroquinone.*

Sample	Hydroquinone ng/g (mL)
Urine	3390.0
Brain	62.5
Liver	457.0
Kidney	212.0
Lung	0.0
Spleen	124.5
Pleural effusion	0.0

In another study, conjugated metabolites were treated with β-glucuronidase to hydrolyze glucuronic acid conjugate and/or hydrolyze sulfuric acid esters prior to analysis, respectively [5,7]. In our case urine hydrolysis performed with using limpet acetone powder. The limpet acetone powder contains β-glucuronidase and sulfatase. Hydroquinone concentration in the urine were 1080 ng/mL before enzyme hydrolysis. David and Robert [2] reported that male volunteers ingesting up to 0.5 g of hydroquinone per day excreted 8 to 15% of dose unchanged and 38 to 42% as conjugates in urine. In this patient excreted 31.9% of the hydroquinone unchanged and 68.1% as conjugates in urine. High concentration of the hydroquinone were obtained in urine, but not detected from lung and pleural effusion. It may be the natural consequence of forced diuresis. Although the patient have taken the treatment a general method of emergency treatment, it was not special treatment for hydroquinone poisoning. Hydroquinone concentration could not be analyzed in heart blood due to lack of blood. The exact cause has not been completely explained.

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Address requests for reprints or additional information to
Takeshi Saito
Dept. of Forensic Medicine
Tokai University School of Medicine
143, Shimokasuya, Isehara-shi
Kanagawa 259-11
Japan