TECHNICAL NOTE

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Sensitive Detection of Strychnine in Biological Samples by Gas Chromatography with Surface Ionization Detection

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ABSTRACT: Strychnine was found measurable with high sensitivity by gas chromatography (GC) with surface ionization detection (SID) and using brucine as the internal standard. The calibration curve was linear in the range of 62.5 to 1000 pg, on column. The detection limit of strychnine was about 50 pg on column (2.5 ng/mL sample); the sensitivity was 70 times higher than that reported by high-performance liquid chromatography. A detailed procedure for extraction of strychnine from human whole blood and urine was established using Sep-Pak C_{18} cartridges; the recovery of strychnine and brucine, which had been spiked in both body fluids, was about 90%.

KEYWORDS: toxicology, strychnine, brucine, gas chromatography, surface ionization detection, Sep-Pak C_{18} cartridges

Strychnine is an alkaloid obtained from seeds of nux vomica and other species of *Strychynos.* It is highly toxic and its lethal dose is 15 to 30 mg for children and 50 to 100 mg for adults [*1*]; thus highly sensitive methods are required for its analysis in biological specimens. The nux vomica plant was introduced into Europe in the 16th century for use as a rodenticide. Although its use has declined, it is still used in poisoned baits for control of vermin. Some reports show that strychnine can be also used as a therapeutic drug to alleviate symptoms of nonketotic hyperglycinemia [*2,3*]. From 1984 to 1989, nine cases of fatal strychnine poisoning were reported in Japan [*4*]. It was also reported that eight young adults sniffed strychnine in the mistaken belief that it was cocaine, and all of them developed toxic symptoms [*5*].

In 1985, Fujii and Arimoto [*6,7*] developed surface ionization detection (SID), a new technique for gas chromatography (GC). In this brief report, we propose that strychnine can be determined in biological specimens with extremely high sensitivity by GC-SID. A detailed procedure for this analysis, including rapid solid-phase extraction, is also given.

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Materials and Methods

Materials

Strychnine nitrate and brucine (10,11-dimethoxystrychnine) dihydrate (internal standard, I.S.) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sep-Pak C_{18} cartridges were obtained from Waters Associates (Milford, MA) and a DB-1 fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.32 μm) from J&W Scientific (Folsom, CA).

Extraction of Strychnine with Sep-Pak C_{18} Cartridges

Sep-Pak C_{18} cartridges were washed with 10 mL of chloroform, 10 mL of methanol and 10 mL of distilled water. This procedure was repeated at least two times. Following the pretreatment, a 1 mL aliquot of blood or urine, with or without strychnine and brucine (I.S.), was mixed with 9 mL of distilled water, and loaded onto a Sep-Pak cartridge. After washing the cartridge with 10 mL of distilled water, the compounds were eluted with 5 mL of chloroform. The chloroform layer was evaporated under a stream of nitrogen and the residue was dissolved in 100 μL of methanol. One or two μL of the residue solution was subjected to GC analysis.

GC and SID Conditions

GC analyses were carried out on a Shimadzu GC-15A instrument equipped with an SID system. A DB-1 fused silica capillary column and a split-splitless injector were used. The GC conditions were: column temperature, 220 to 300°C (2 min hold at 220°C and 20°C/min); injection and detector temperatures, 260°C and 280°C, respectively; helium flow rate, 3 mL/min.

Figure 1 shows the SID system developed by Fujii and Arimoto [*6*]. The platinum emitter is positioned between the quartz nozzle and the collector electrode. The ring electrode around the quartz nozzle is held at a positive potential of +200 V; the emitter and the ion collector are always at a negative potential of −200 V *versus* the ring electrode. Positive ion current directed to the collector is measured with an electrometer. Electronics for variable heating of the emitter filament are required. The emitter is ten-turn coiled platinum (99.9%, 0.25 mm diameter), which is capable of with-

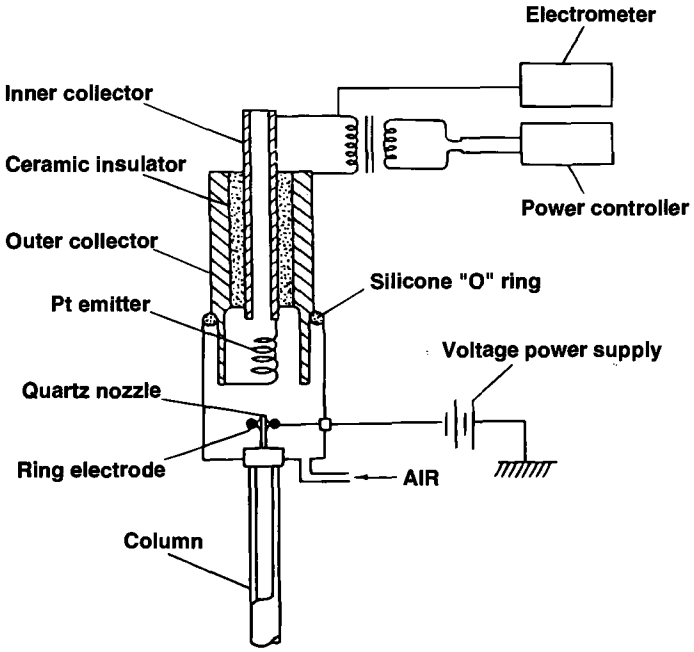

FIG. 1—*SID system with a platinum emitter. Reprinted with permission. From Ref 6 (©1985 American Chemical Society).*

standing temperatures around 1200°C for much longer than 1 month in the environment of a fast flow of a helium-air mixture. Platinum was chosen as an emitter material primarily because it has a higher work function (5.65 eV) than other typical refractory metals such as tungsten and rhenium. This property easily allows positive thermonic emission from the surface. The SID system used in this study was from Shimadzu (Kyoto, Japan).

The SID conditions were: heating current through the platinum emitter, 2.2 A; emitter temperature, about 600°C; ring electrode bias voltage, +200 V with respect to the collector electrode. The samples were injected in the splitless mode and the splitter was opened after 2 min.

MS Conditions

Mass spectra were recorded by GC-MS on a JMS-DX505H MS instrument with a Hewlett Packard Model 5890 gas chromatograph and a DB-1 capillary column. MS conditions were: accelerating voltage 3.0 kV, ionization current 300 μA, separator temperature 280°C and ion source temperature 250°C; electron energy 70 eV; reagent gas methane and chamber pressure 1 Torr. GC conditions for the GC-MS were the same as those described for GC-SID.

Results

Figure 2 shows GC profiles for 40 ng of strychnine and 250 ng of brucine, which had been added to 1 mL body fluids and extracted

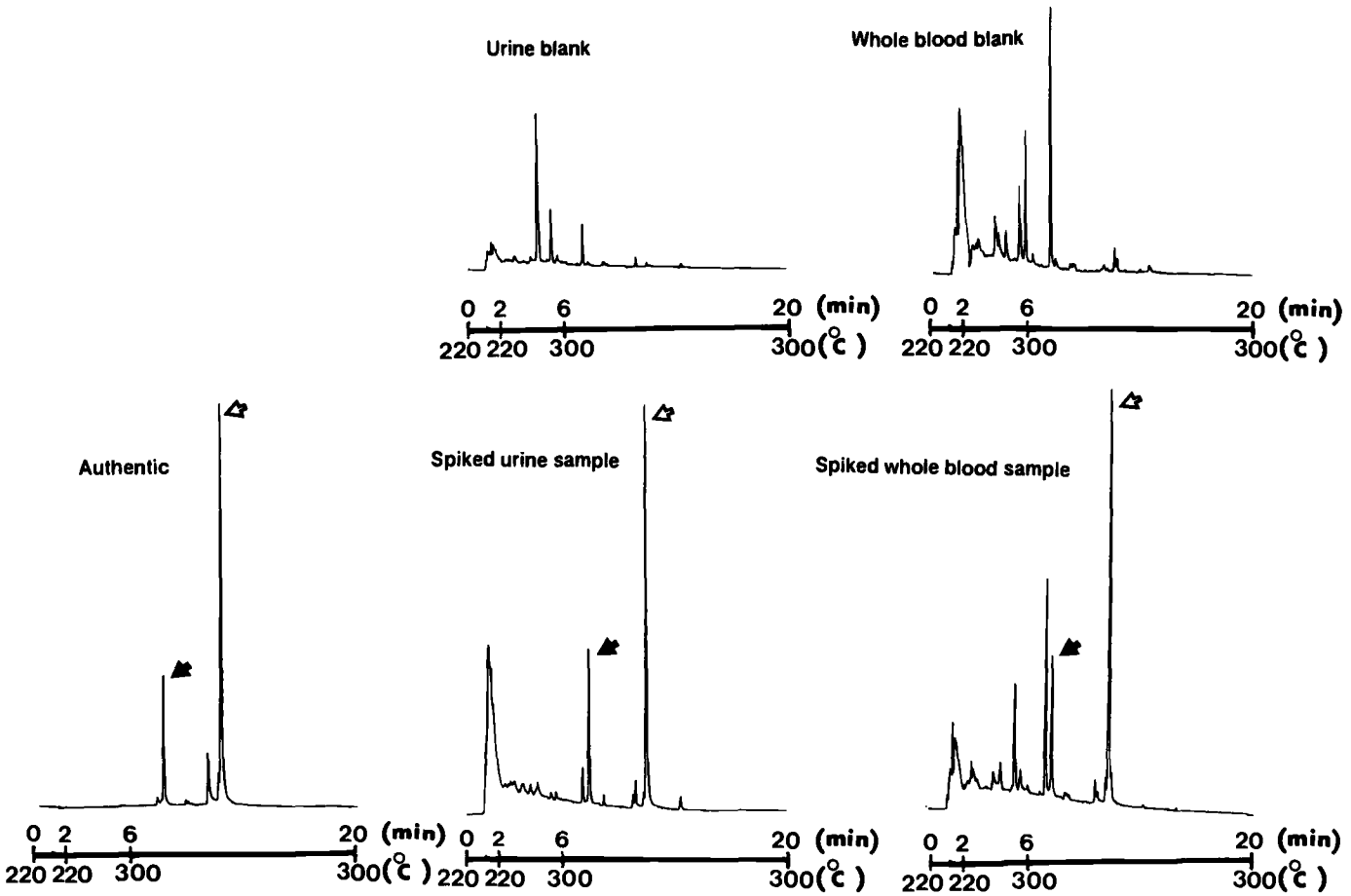

FIG. 2—*Capillary GC-SID for strychnine* (filled arrow) *and brucine* (I.S., open arrow) *extracted from whole blood and urine, and for each background with use of Sep-Pak C_{18} cartridges. The mixture of 40 ng of strychnine and 250 ng of brucine was added to 1 mL of samples. GC was carried out with a DB-1 fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm). Its condition were: column temperature 220–300°C (20°C/min); injection and detector temperature, 260 and 280°C, respectively; helium flow rate, 3 mL/min.*

with Sep-Pak C_{18} columns. There were some impurity peaks found in the both whole blood and urine blanks, but none of them interfered with the drug peaks. The retention times were about 7.6 min for strychnine and 11.7 min for brucine.

To confirm that the detected peaks are due to undecomposed strychnine and brucine, we have measured positive ion electron impact mass spectra of each peak; and have detected base peaks of molecular ions at *m/z* 334 for strychnine and *m/z* 394 for brucine.

We have examined the percent recovery of the drugs from whole blood and urine at two different concentrations of strychnine. For urine, the recoveries were 100 ± 12% (mean ± SEM, $n = 4$) at 40 ng/mL and 95.2 ± 20% ($n = 3$) at 200 ng/mL for strychnine. For brucine, the recovery was 94.0 ± 9.3% ($n = 7$) at 250 ng/mL urine. For whole blood, they were 93.4 ± 15% ($n = 5$) at 40 ng/mL and 91.2 ± 9.2% ($n = 4$) at 200 ng/mL for strychnine; also, it was 100 ± 16% ($n = 9$) at 250 ng/mL for brucine.

We made the calibration curve for strychnine using brucine as I.S. It showed good linearity in the range of 62.5 pg to 1000 pg on column. The peak areas of 1000 pg of strychnine and 2500 pg of brucine on column were 81 668 ± 5722 and 80 241 ± 6966 (arbitrary units, mean ± SEM., $n = 4$). The equation of the curve and the regression coefficient were: $y = 0.001\ 08\ x\ 0.0019$ and $r = 0.999$, respectively. The detection limit (signal-to-noise ratio = 3) was about 50 pg on column (2.5 ng/mL).

Discussion

In the original report on GC-SID by Fujii and Arimoto [*6,7*], they reported that tertiary amino compounds with straight side chain structures exhibited very high sensitivity. During our studies on GC-SID [*8*], however, it has become more obvious that tertiary amino compounds with ring structures also exhibited a relatively high response. Typical examples are dextromethorphan, dimemorphan [*9*] and meperidine [*10*]. This is also the case for strychnine, which contains two cyclic tertiary amines in its structure. As stated in the result, the sensitivity to brucine (10,11-dimethoxystrychnine) was about twice lower than that to strychnine, showing the methoxy groups affect to lower the response by SID.

Several papers have reported on chromatographic detection of strychnine. Two reports dealt with GC-flame ionization detection of the drug, but no mention was made on its detection limit [*11,12*]. The other two reports were on its analysis by high-performance liquid chromatography [*13,14*]; the detection limit in one report was 625 ng/mL [*13*] and the present GC-SID method gives the sensitivity about 70 times higher.

In this study, we have employed solid-phase extraction using Sep-Pak C_{18} cartridges for the separation of strychnine with brucine from biological specimens. No reports are available on the use of Sep-Pak C_{18} cartridges to extract strychnine and its analogs. We have used chloroform as elution solvent from the cartridge, although methanol/water or acetonitrile/water is recommended according to the manufacturer's manual. The merits of chloroform are that recovery is much better, backgrounds much clearer and evaporation time of the eluate much shorter.

Although homicidal use of strychnine is rare, accidental and suicidal poisonings still occur. The present modern method of GC-SID together with solid-phase extraction is recommended for the analysis of strychnine in biological specimen because the procedure is sensitive and simple.

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