



Analytical method for the determination of strychnine in tissues by gas chromatography/mass spectrometry: two case reports

E.P. Marques^{a,*}, F. Gil^b, P. Proença^a, P. Monsanto^a, M.F. Oliveira^a,
A. Castanheira^a, D.N. Vieira^a

^aLaboratório de Toxicologia Forense, Instituto de Medicina Legal de Coimbra,
Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal

^bDepartamento de Medicina Legal y Psiquiatría, Facultad de Medicina, Universidad de Granada,
Granada, Spain

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Abstract

This paper describes an analytical method for strychnine determination in biological samples by gas chromatography/mass spectrometry and their application in the investigation of two cases involving strychnine ingestion: A fatal case and a clinical one. The strychnine is isolated from biological samples using a liquid–liquid extraction procedure. The clean-up procedure is performed using an acid solution. Papaverine is used as internal standard in the quantification of strychnine. In the analysed specimens, the limits of quantification were 0.1 µg/ml or 0.1 µg/g. The recovery rate ranged from 75.0% to 98.7% and the coefficients of variation ranged from 4.8% to 10.5%. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Strychnine is an alkaloid which is mainly extracted from the seeds of *Strychnos nuxvomica*, a tree native to India. This compound is highly toxic to humans. It is rapidly

*Corresponding author. Tel.: +351-239-854-230; fax: +351-239-820-549.

E-mail address: imltoxic@ci.uc.pt (E.P. Marques)

absorbed from the gastrointestinal tract and acts upon the central nervous system (CNS), causing excitation of all parts of the CNS. Strychnine poisoning was quite frequent in the last century and in the first half of this century. Strychnine has not been commercialised in Portugal since 1974, either in pharmaceutical preparations or in rodenticide formulations. Although strychnine poisoning is now a rare event, it may still occur, because even though the substance is not available in our country, small amounts of strychnine may still remain in storage, mainly in rural areas, or street drugs adulterated with strychnine may be consumed [1].

The authors describe two cases of acute poisoning by strychnine (one fatal case and one clinical case) detected by the Forensic Toxicological Laboratory (FTL) of the Institute of Legal Medicine of Coimbra (IMLC). In both cases, poisoning was due to strychnine ingestion. The analytical methods for determining this compound in tissues and body fluids by means of colour reactions [2], thin layer chromatography (TLC) [2,3], high performance liquid chromatography [4,5] and gas chromatography [2,3,6–8] have been previously described. Strychnine was detected in our Laboratory in the 1960s in experimental studies on frogs and by means of colour reaction, using Mandelin's reagent [9]. From the 1970s up to 1995 identification was performed by TLC and gas chromatography/nitrogen phosphorus detection (GC/NPD). In this work a new analytical method is described. Extraction was done according to the method described by Winek et al. [6], except that we used papaverine as internal standard and an acid solution in the purification procedure. Gas chromatography/mass spectrometry (GC/MS), SIM mode were used for identification and quantification.

2. Case histories

2.1. Fatal case

In 1995, a 47-year-old woman was found dead at home with a bottle near the body. The bottle contained a small amount of a liquid. Information provided by the family indicated that the victim suffered from depression and had a propensity for suicide. She was a housewife. Autopsy was performed approximately 12.5 h after the discovery of the body. Body length and weight were 145 cm and 50 kg, respectively. No abnormal external findings were noted during the post mortem examination. The internal pathology consisted on congestion and edema of the viscera. The cause of death could not be determined on the basis of these data, because they correspond to those that are normally found in poisoning cases.

2.2. Clinical case

In 1996, a 45-year-old man was admitted in the Intensive Care Unit of the University Hospital of Coimbra (HUC) with strong convulsions and violent contractions. According to the HUC records, the patient had previously been submitted to a gastric lavage in a Regional Hospital. The treatment in the HUC consisted on prompt control of convulsions and contractions, with adequate mechanical assistance by intubation and adminis-

tration of muscle relaxants, to prevent respiratory and heart stoppages. The HUC physicians strongly suspected strychnine poisoning, and therefore collected biological fluids to confirm this suspicion by toxicological analysis.

3. Experimental biological material

Body tissues were collected in the fatal case and fluids were collected in both cases. All the analysis were performed in triplicate. Free post-mortem strychnine biological samples were collected from traffic accident cadavers whose autopsies were performed in the IMLC, and a strychnine free urine sample was taken from a personnel laboratory.

3.1. Chemicals

Analytical solvents were of HPLC grade and reagents were of analytical grade. They were obtained from Merck, Darmstad, Germany. Strychnine and papaverine standards were purchased from the Sigma Chemical Co, St. Louis, MO, USA. Stock solutions were prepared in methanol at 1.0 mg/ml. They were diluted to 100 µg/ml to obtain the working solutions and stored at 4°C.

3.2. Equipment

The equipment and the GC/MS conditions are shown in Table 1.

3.3. Extraction procedure

After the addition of 50 µl of papaverine (100 µg/ml) to blood (5 ml) or to urine (5 ml) and 20 µl of papaverine (100 µg/ml) to each tissue sample (2 g), cut in very small pieces, strychnine was extracted using the previously mentioned method [6]. A saturated solution of sodium bicarbonate was added and the tissue samples were homogenized at 4°C using ultrasound 600 W for 10 min, with liver, lung, kidney and small intestine, and for 20 min in other tissues (heart and stomach). Strychnine extraction was performed with 8 ml of toluene–*n*-heptane–isoamyl alcohol (67:20:4) for tissue homogenates and body fluids. After vortex agitation and centrifugation, the organic layer was removed to 15 ml glass tubes and the extraction process was repeated.

3.4. Purification procedure

The organic layer was purified twice by extraction using 4 ml of 4 N hydrochloric acid solution. The acid solutions were made alkaline and extracted twice, using the same solvents as before. The organic layer was evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was reconstituted in methanol to be analysed by GC/MS.

Table 1
Experimental conditions for the determination of strychnine by gas chromatography/mass spectrometry

GC/MS	Hewlett Packard (HP) 5890 series II GC; 5970 MSD
GC	<p>Column: HP1 fused silica capillary column with a stationary phase of cross-linked methyl silicone (12.5 m×0.2 mm i.d., 0.33 µm film thickness)</p> <p>Injector: Split injection (1:10)</p> <p>Carrier gas: Helium (1 ml/min)</p> <p>Temperatures:</p> <p> Injector: 250°C</p> <p> Interface MS: 280°C</p> <p> Oven Temp.: 1=170°C</p> <p>Time: 1=1 min</p> <p>Ramp rate: 1=20°C/min</p> <p> Temp.: 2=270°C</p> <p>Time: 2=7 min</p>
MSD	<p>Ionization mode: EI (70 ev)</p> <p>Electron multiplier: 2000 V</p> <p>Select Ion Mass mode</p>

3.5. Recovery

Strychnine free biological samples were spiked with this compound at 0.1, 1.0, 10.0, 50.0 and 100.0 µg/ml or µg/g concentrations (Table 2) and were then extracted and purified according to the above described method. Papaverine was added to the residues at 1 µg/ml or µg/g concentrations just prior to GC/MS analysis. Recovery was

Table 2
Strychnine recovery and precision (CV) in biological specimens at different concentrations using GC/MS method^a

Concentration added (µg/ml or µg/g)	Recovery Mean±standard deviation (%)					
	Blood	Liver	Kidney	Heart	Lung	Urine
0.1	85.0±6.1	98.7±7.2	85.0±6.3	84.5±10.4	80.0±10.2	97.9±5.4
1.0	80.1±5.2	91.8±5.8	82.0±7.5	86.7±8.2	77.8±11.5	96.2±4.8
10.0	84.2±5.9	90.1±6.1	84.3±7.3	78.6±6.7	78.2±8.4	90.8±5.0
50.0	77.1±4.2	89.0±5.4	79.8±5.0	77.2±5.1	75.0±8.4	91.2±4.9
100.0	77.2±5.6	88.99±6.2	80.1±5.2	77.3±5.1	76.3±8.0	90.2±4.5
Concentration added (µg/ml or µg/g)	Precision CV (%)					
	Blood	Liver	Kidney	Heart	Lung	Urine
10.0	6.2	5.9	6.9	7.2	10.5	4.8

^a n=3

performed comparing the sample results with methanol standard solutions at different concentrations, with papaverine, always at the same concentration (1 $\mu\text{g}/\text{ml}$ or 1 $\mu\text{g}/\text{g}$).

3.6. Precision

Strychnine free biological samples were spiked with this compound at 10.0 $\mu\text{g}/\text{ml}$ or 10.0 $\mu\text{g}/\text{g}$ concentration and with papaverine at 1.0 $\mu\text{g}/\text{ml}$ or 1.0 $\mu\text{g}/\text{g}$ concentration (Table 2), and were analysed by the method previously described.

4. Results and discussion

In the fatal case, the presence of strychnine was confirmed in the vestigial liquid contained in the bottle by GC/MS, mode SCAN. The strychnine concentrations found in post-mortem specimens are presented in Table 3. The quantity determined in the stomach is higher than a lethal dose of strychnine through ingestion. In the tissue samples, the liver had the highest concentration, being more than twice that in blood. Similar toxicological findings were found in some of the earlier reports [2,3,6–8,10–12]. Table 4 shows the strychnine distribution in biological samples from published investigations of fatalities. In the fatal case described in this paper there was no family or clinical information concerning to the cause of death. The autopsy result was not specific, although the pathological findings agree with those in published fatalities from acute poisonings [2,3,5–7,9,10] which do not mention a specific pathology. Strychnine poisoning was only diagnosed from the results of the toxicological analysis performed in the FTL. In the clinical case, the patient showed typical symptoms of strychnine poisoning. The clinical picture of strychnine poisoning can be confused with epilepsy, tetanus and hysteria, but the clinicians strongly suspected strychnine poisoning. The strychnine concentrations are shown in Table 3.

The strychnine level was low in the gastric contents, because the patient had previously had a gastric lavage. The blood concentration found (0.35 $\mu\text{g}/\text{ml}$) was lower than the blood concentration (0.8 $\mu\text{g}/\text{ml}$) detected in a clinical case of strychnine poisoning caused by a pharmaceutical preparation reported by Creusot et al. [13]. It was also lower than the concentration limit (0.8 $\mu\text{g}/\text{ml}$) reported by Dumont et al. [9] for patients that do not show any tonic contractions or respiratory arrest. The concentration

Table 3
Strychnine concentrations in human tissues and body fluids taken from a clinical and a fatal case ($\mu\text{g}/\text{ml}$ or $\mu\text{g}/\text{g}$)^a

	Blood	Liver	Kidney	Heart	Lung	Urine	Small intestine	Gastric contents
One clinical case	0.35	–	–	–	–	15.3	–	0.1
One fatal case	21.2	49.8	15.6	16.1	31.9	–	3.3	281.5

^a $n=3$

Table 4
Published data on strychnine tissue distribution from fatal cases ($\mu\text{g/ml}$ or $\mu\text{g/g}$)

	Blood		Liver		Kidney		Lung		Urine		Small intestine		Gastric contents	
	\bar{X} or range	<i>n</i>	\bar{X} or range	<i>n</i>	\bar{X} or range	<i>n</i>	\bar{X} or range	<i>n</i>	\bar{X} or range	<i>n</i>	\bar{X} or range	<i>n</i>	\bar{X} or range	<i>n</i>
1979 [2]	0–61	5	0–209	8	0.07–90	6	–	–	1.0–7.7	3	–	–	1–480 ^a	6
1982 [10]	0.5–61	–	5–257	–	0.07–106	–	–	–	1–3	–	–	–	7.5–100 ^a	–
1985 [3]	2.3–9.7	2	18.5–80.4	2	12.9–32.0	2	–	–	2.0	1	39.0–109.0	2	160.0–1030.0	2
1985 [11]	3.3	1	6.2	1	3.2	1	–	–	1.4	1	4.1	1	213.5 ^a 175.0	1
1986 [6]	3.30	1	6.20	1	3.20	1	–	–	1.40	1	4.10	–	175.00	1
1989 [12]	1.0–20.3	68.7	1	–	–	–	6.9	1	1.0–3.2	–	–	–	–	–
1994 [7]	–	–	–	–	6016	1	–	–	–	–	–	–	–	–
1995 [8]	7.7	1	515	1	46	1	–	–	3.2	1	–	–	3000.0	1

^a Total contents (mg).

found could be explained once that the sample was collected for analysis some time after the ingestion of the compound. The urine concentration ($15.0 \mu\text{g/ml}$) further illustrates this fact. Clinical treatment in this case was aimed at stopping the convulsions. Respiratory support was efficient. Table 5 gives the retention times and the selected ion masses for strychnine determination. Fig. 1 shows a total ion chromatogram (TIC) of a blood sample spiked with strychnine and internal standard at $1.0 \mu\text{g/ml}$, the selected ions and their abundance in SIM mode.

The strychnine presents two isomers, the major one was used in for the quantification. The interfering peaks on the chromatograms of the different specimens were non-existent. The limit of quantification by this method was $0.1 \mu\text{g/g}$ or $0.1 \mu\text{g/ml}$ for the analysed specimens. The calculated recoveries vary from 75.0% to 98.7% (Table 2). The recovery values were the lowest for lung samples and highest for the liver. The coefficients of variation (CV) ranged from 4.8% to 10.5% (Table 2), with the lung sample being the one which showed a greater variation.

5. Conclusion

This study emphasizes the importance of toxicological analysis in detecting acute strychnine poisonings. In the fatal case the cause of death was only determined from the

Table 5
Retention time and selected ion mass for determination of strychnine

Compounds	Retention time (min)	Ions (<i>m/z</i>)	
		Identification	Quantification
Strychnine isomers	10.03; 10.88	334; 162	334
Papaverine	7.84	338; 324	338

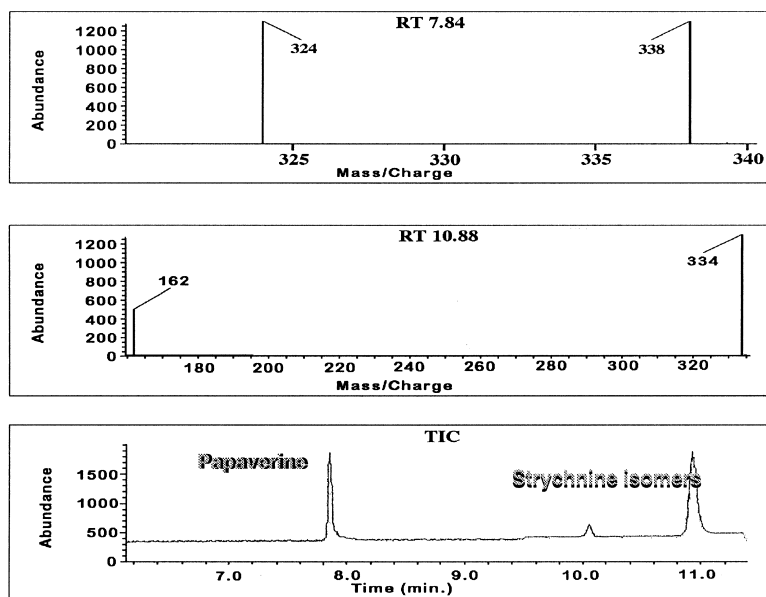


Fig. 1. TIC of a blood sample spiked with strychnine and papaverine at 1.0 $\mu\text{g}/\text{ml}$ and its selected ions.

results of the toxicological findings, and in the clinical case the diagnosis was confirmed by the analytical data. Pathological data and post-mortem concentrations of strychnine are consistent with the published data. The analytical method described is simple, specific and sensible, and provides recovery and precision for the identification and quantification of strychnine in forensic and clinical investigations.

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