Original Paper

Simultaneous Detection of Strychnine and Brucine in Biological Matrix by Gas Chromatography-Mass Spectroscopy[#]

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ABSTRACT

A new gas chromatography-mass spectroscopy (GC-MS) method is being presented for the separation and detection of strychnine and brucine, alkaloids of *Strychnos nux vomica* in a single run. The analysis was carried out using 5% phenyl methyl silicone capillary column, electron impact ionization mode and quadrupole mass analyzer. The extracts of the exhibits were analyzed using the new method.

The peaks of the two alkaloids were found to be well resolved, and there was clear separation between the two. The retention time and mass fragmentation pattern, base peaks, molecular peaks of strychnine and brucine standard/NIST library and crime case exhibits matched, establishing the presence of the two active principles of *Strychnos nux vomica*.

The new method has the advantage of better separation of the two alkaloid peaks over the conventional GC-MS methods, and is useful for the identification and confirmation of *Strychnos nux vomica* constituents in biological matrices of poisoning cases that have ended in death.

Key Words: Strychnine; Brucine; *Strychnos nux vomica*; Gas chromatography-mass spectroscopy; GC-MS

Introduction

The study being presented is in relation to the death of a young woman who died immediately after marriage, after manifesting symptoms and signs of strychnine poisoning. The forensic pathologist who conducted the autopsy also suspected the cause of death to be due to some organic toxin of plant origin. The police handling the case forwarded the viscera/blood preserved by the pathologist, together with some other samples collected from the scene of crime. Extracts from *Strychnos nux vomica* are used in various systems of medicine (unani, siddha, homoeopathy) for the treatment of a variety of ailments. This plant contains the alkaloids strychnine and brucine as active principles.[1] Though such extracts in small quantities may not do much harm, when taken in excess, can cause lethality.

In the present case, the usual procedure for extraction, isolation, and detection of strychnine and brucine from biological matrix was followed.[2-4] But since the gas chromatography-mass spectroscopy (GC-MS) method available in the existing literature for confirmatory detection of the alkaloids does not show satisfactory results as the peaks of the two separated alkaloids are not properly resolved and often overlap each other, a modified technique was utilized in order to enable simultaneous detection of both the alkaloids in a single GC-MS run.

Materials and Methods

Chemicals: All the reagents and solvents used were of analytical grade. Methanol of chromatographic grade was used for the GC-MS analysis. Precoated 20 x 20 cm Silica Gel G F254 Merck TLC plates were used for thin layer chromatography. Strychnine and brucine standards of NIST traceability were procured from E-Merck India

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Ltd. Visceral exhibits of stomach, intestine, liver, spleen, kidney, blood, and spinal cord sent by the forensic pathologist constituted the biological matrices, while other crime scene exhibits included a steel vessel, a sieve for filtering, and a polythene milk packet.

Apparatus: The following equipment was used for the study -

Standard TLC apparatus from E-Merck
Gas chromatograph-Mass spectrometer, Perkin Elmer
GC Model: Perkin Elmer
MS Model: PE-Turbo Matrix.
Software: Turbo Mass.

Preparation of Standard Solution: Standard solution of strychnine and brucine was prepared by dissolving 10mg each in 10 ml pure methanol to obtain a concentration of 1mg/ml.

Extraction from Biological Matrices: The method used for the extraction was basically from the working manual of the Directorate of Forensic Science (DFS), New Delhi, with minor modifications to suit the nature and quantity of the exhibit.[5]

50 gm of macerated tissue of stomach and intestine was mixed with 50 ml of rectified spirit in a conical flask and acidified with tartaric acid. The conical flask was heated on the water bath for about 1 hour. The resultant mixture was then filtered through a filter paper. The filtrate was evaporated and the residue added to 50 ml of warm distilled water and filtered through a Whatman filter paper. The filtrate was transferred to a separating funnel and rendered alkaline with ammonia solution, and extracted with a diethyl ether solvent, in portions of about 25 ml at each extraction for three times. The extract was purified by dissolving it in about 20 ml of water acidulated with dilute sulphuric acid and filtering through a Whatman filter paper. The filtrate was extracted with diethyl ether solvent in portions of 25ml at each extraction for three times. These extracts were evaporated to dryness for analysis.

From the above extract, after the extraction procedure, the following colour test was performed as per the DFS working procedure manual. The results are mentioned in **Table 1**.

TLC Conditions:
Solvent System: Cyclohexane: Toluene: Diethyl amine (75:15:10)
Plate: Silica gel G (0.2 mm thickness)
Development: Ascending technique.
Spray reagent: Dragendorff's Reagent
Colour of spot: Orange

Gas Chromatographic Conditions:
Column: Capillary column packed with 5% methyl phenyl silicone.
Length: 30 mts, internal diameter 0.25mm.
Carrier gas: Helium.
Flow rate: 1ml/mt.

Temperature programming -
Oven temperature: 150C for 1 min, Ramp1-9C degree/min to 280C hold for 8 min.
Injector temperature: 150C.
Temperature of interface: 300C
Injection volume: 1microlitre.

Mass Spectroscopy Conditions:
Reagent Gas for Tuning and Calibration: Heptacosa tributyl amine.
Mode: Full scan mode.
Ionization: Electron Impact ionization.
Source Temperature: 250C.
Interface Temperature: 250C.

Results and Discussion

For the detection of the two nux vomica alkaloids (strychnine and brucine), TLC and colour test were performed as per standard procedure. The observations are given in **Table 1**. The observations of Rf values pertaining to TLC, and the results of the colour test indicate the positive presence of the alkaloids in all the exhibits.

Fig 1 shows the total ion chromatogram of the strychnine and brucine standards, while **Fig 2** shows the total ion chromatogram of the exhibit. TLC shows well resolved chromatographic peaks, both in the standards as well as exhibits, which are not generally achieved with the conventional method. Experimental condition retention time of strychnine standards were found to be 12.08, 15.04 respectively. However, the retention time of the two corresponding peaks in the exhibits were found to be matching (**Table 2**).

Fig 3 shows the mass spectra of standard strychnine and brucine peaks, while **Fig 4** shows the mass spectra of the exhibits. **Table 2** shows the base peaks, molecular peaks, and major fragments of both the standards and exhibits. All the parameters with reference to standards and exhibits for both the peaks were found to be matching, which is sufficient confirmation of the presence of strychnine and brucine in all the exhibits.

Conclusion

A reproducible and sensitive single-run GC-MS method for simultaneous determination and confirmation of strychnine and brucine in exhibits of forensic interest has been developed. The method developed is useful in the toxicological analysis of biological matrices for the detection of strychnine and brucine which are active principles of *Strychnos nux vomica*, and has some advantages over the conventional method.

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Table 1 Retention Time and Colour Test Results of Standard Strychnine & Brucine & Exhibits

Serial No.	Name of the Exhibit	Colour Test 1	Colour Test 2	Retention Time
1.	Exhibit 1 (stomach and intestine)	+ve	+ve	0.484,0.40.
2.	Exhibit 2 (liver, kidney and spleen)	+ve	+ve	0.484,0.40.
3.	Exhibit 3 (spinal cord)	+ve	+ve	0.484,0.40.
4.	Exhibit 4 (blood)	+ve	+ve	0.484,0.40.
5.	Exhibit 5 (leaves and stalk of plant)	+ve	+ve	0.484,0.40.
6.	Exhibit 6 (steel bowl)	+ve	+ve	0.484,0.40.
7.	Exhibit 7 (metal sieve)	+ve	+ve	0.484,0.40.
	Strychnine	+ve	+ve	0.484
	Brucine	+ve	+ve	0.40.

Table 2 Retention Indices (Gas Chromatography) of Strychnine & Brucine & Exhibits

Serial No.	Name of the Exhibit	Retention Indices
1.	Exhibit 1 (stomach and intestine)	12.29,14.97
2.	Exhibit 2 (liver, kidney and spleen)	12.29,14.97
3.	Exhibit 3 (spinal cord)	12.29,14.97
4.	Exhibit 4 (blood)	12.29,14.97
5.	Exhibit 5 (leaves and stalk of plant)	12.29,14.97
6.	Exhibit 6 (steel bowl)	12.29,14.97
7.	Exhibit 7 (metal sieve)	12.29,14.97
	Strychnine	12.09
	Brucine	15.41

Table 3 Molecular Weight, Base Peak, Major Fragments of Strychnine, Brucine and Exhibits

Sample	Retention time	Molecular weight or M/Z	Base peak/ Molecular peak	Major fragments
Strychnine	12.09	334	334	130,120,107
Brucine	15.41	394	379	379,203,197
Exhibit 1	12.29,15.04	334 & 394	334,394	130,120,203,197
Exhibit 2	12.29,15.04	334 & 394	334,394	130,120,203,197
Exhibit 3	12.29,15.04	334 & 394	334,394	130,120,203,197
Exhibit 4	12.29,15.04	334 & 394	334,394	130,120,203,197
Exhibit 5	12.29,15.04	334 & 394	334,394	130,120,203,197
Exhibit 6	12.29,15.04	334 & 394	334,394	130,120,203,197
Exhibit 7	12.29,15.04	334 & 394	334,394	130,120,203,197

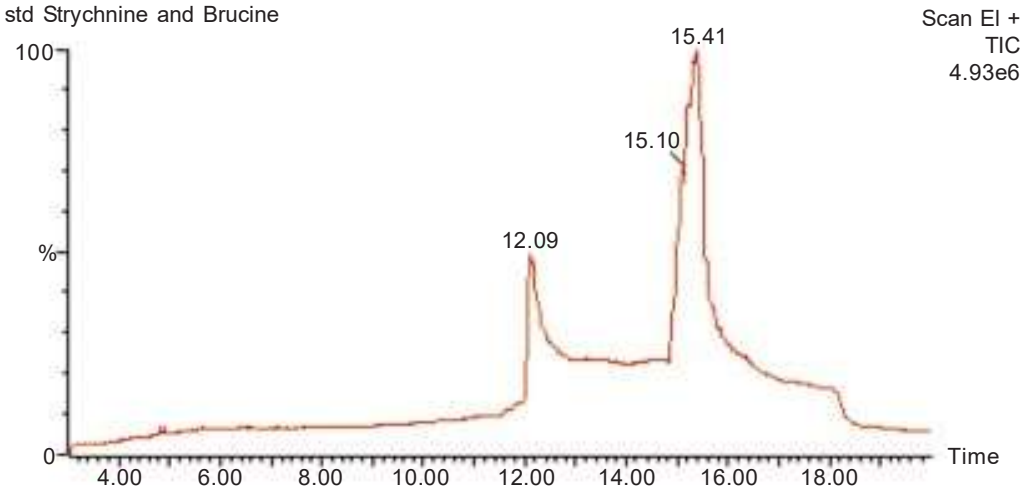

Fig. 1

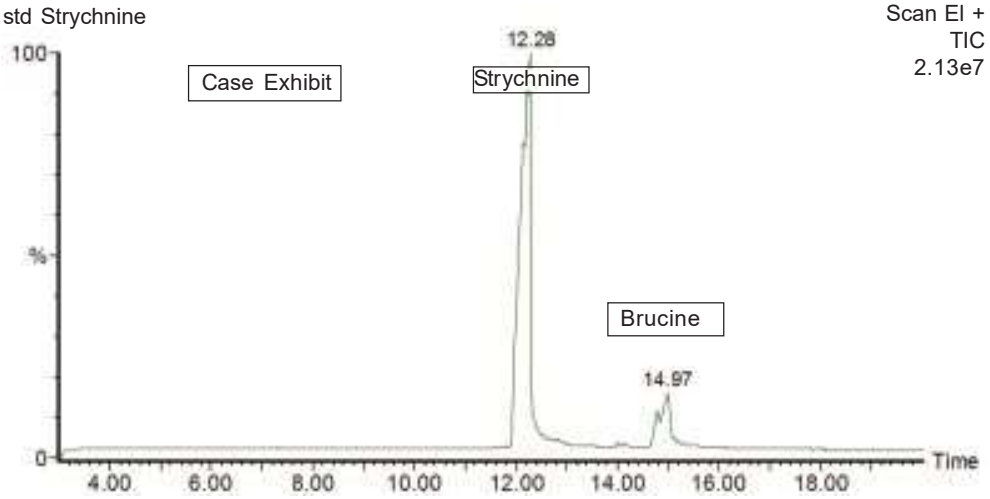

Fig. 2

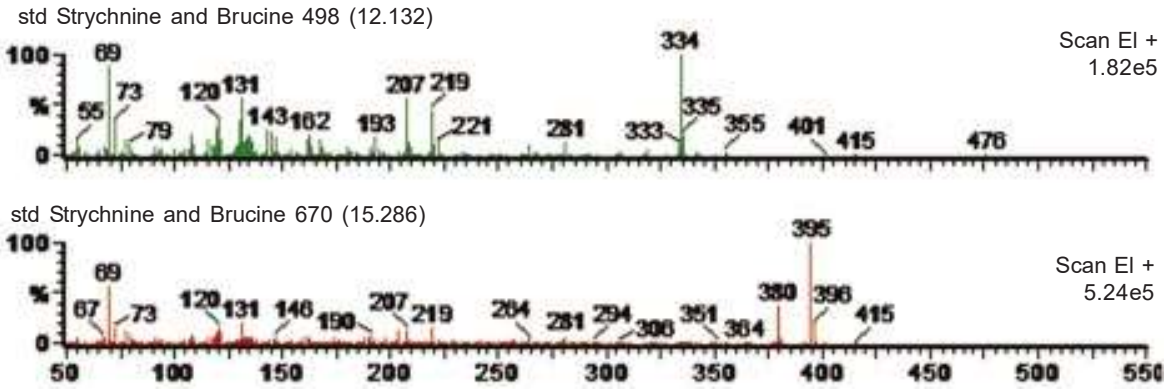

Fig. 3

Hit	Rev Library	For	Compound Name	MW	Formula	CAS
1	688 Nist	420	STRYCHNINE	334	$C_{21}H_{22}O_2N_2$	57-24-9

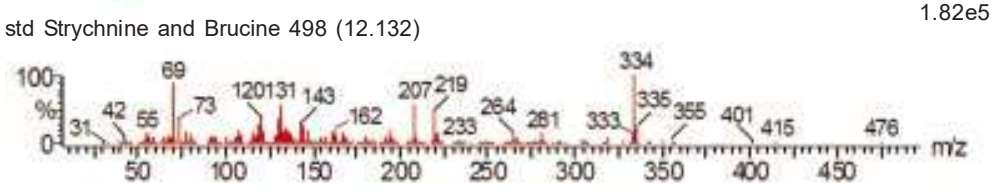

Fig. 4

Hit	REV	For	Compound Name	MW	Formula
1	761	314	BENZENEHEXACARBOXYLIC ACID, HEXAMETHLY ESTER	426	$C_{18}H_{18}O_{12}$
2	404	312	BRUCINE	394	$C_{23}H_{26}O_4N_2$

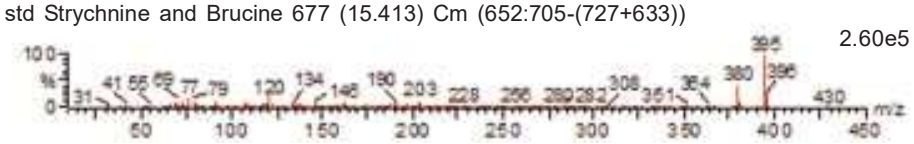

Fig. 5

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