Case Report

Detection of Endosulfan Isomers and Metabolites by GC-MS in the Exhumed Body of an Infant

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ABSTRACT

Endosulfan is a broad spectrum, non-systemic contact, and alimentary insecticide. It is a mixture of two isomers. The analysis of this insecticide, its isomers a and b endosulfan and other metabolic fragments, especially endolactone and a chlorinated dicarboxylic product in the autopsy material of an infant was performed by GC-MS in an alleged case of infanticide related to a dowry demand case. It confirmed that the child had been poisoned with endosulfan prior to death. No autopsy material, except ash and bone were left of the mother, as her parents-in-law had cremated her body.

A new method was developed on GC-MS for the detection of this insecticide, which can be highly useful for routine analysis of insecticides in forensic laboratories. A study of various metabolites of endosulfan was also performed which may be informative for metabolic pathways studies.

Key Words: GC-MS, Gas chromatography–mass spectrometry, Autopsy, Postmortem

Introduction

An individual registered a case on 14 Feb 2006 against the parents-in-law of his daughter under sections 498-A, 304-B, and 201 of the Indian Penal Code at the Raini Police Station, in Alwar District of Rajasthan. The case was registered for torturing and murdering his daughter in the course of demands for dowry. It was alleged that when the accused realized that the girl was not in a position to meet their demands, they killed her as well as her 9 month-old son. They then allegedly burnt the body of the mother without prior information to her father, and buried the body of the infant. The body of the child was exhumed one month later, and after postmortem examination on 21 march 2006, some autopsy material was sent for chemical analysis to the Forensic Science Laboratory, Jaipur, Rajasthan.

Postmortem findings indicated insecticide poisoning, and so, thin layer chromatography (TLC) and gas chromatography–mass spectrometry (GC-MS) analysis were performed. The detection of endosulfan (a chlorinated hydrocarbon pesticide) confirmed that the autopsy suspicions were well founded. The study of isomers and metabolites can add more significance, not only to the analysis work,[1] but also to solve the murder mystery. This will help pave the way for opening a murder investigation of the child's mother.

Materials and Methods

a) **Material**

The materials sent for examination comprised ash and burnt bones of the mother, and autopsy samples of the child - stomach, small intestine, lungs, liver, spleen, kidneys, and brain.

b) **Chemicals**

Acetone, Hexane, Sodium sulphate, Diphenylamine,

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ethanol, TLC precoated silica gel-G plates (all from Merck Company).

c) **Instruments**

Microwave Solvent Extraction Lab Station (Ethos Sel) for digestion from Milestone, HPTLC from (Camag), Trace GC 2000 and Trace DSQ from Thermofinnigan.

d) **Procedure of Extraction**

About 50 gm of viscera sample was minced to a fine slurry, and 50 gm of sodium sulphate and 50 ml of acetone were added to it. Microwave solvent extraction device was used for digestion and extraction. The material was kept in ethos sel, and temperature programming was done. The material was heated from room temperature to 80ºC in 5 minutes, and retained at that temperature for 20 minutes. The system was then cooled down to room temperature. The material was filtered and taken in a separating funnel, where 100ml of hexane was added in three aliquots to extract thrice the digested liquid. Each time, the hexane layer was separated and mixed together, and passed through a bed of anhydrous sodium sulphate. The liquid was collected in an evaporating porcelain bowl. The dried material was dissolved in acetone, and made up to 2ml.[2,3]

e) **Separation by TLC & Identification by Chromogenic Reagents**

The material was spotted on precoated silica gel glass plates using HPTLC (high performance thin layer chromatography) applicator. The plate was developed in hexane:acetone (8:2). The developed plate was sprayed with 1% biphenylamine in ethanol (this gives well-separated greenish-gray spots).[4,5] The spots that were obtained tallied with the control sample of endosulfan. The other developed plate was sprayed with 20% solution of sodium hydroxide, followed by nickel amine reagent. Greyish-black spots were obtained.

f) **GC-MS Method**

Those spots which were unsprayed, were scratched and dissolved in HPLC grade acetone. One µl of it was applied to GC injector at 200ºC.The material was separated by capillary column: EC™ –5 Alltech of length 30 metres, ID –0.25millimetre, film thickness – 0.25 µm.[6]

Properties of the Column –

5% phenyl and 95% methylpolysiloxane supplied by Alltech as EC™-5. It is similar to DB-5, SPB-5, Rtx-5, HP-5, ULTRA-2, CPSil, - 8CB, OV-5, BP-5, RSL-200 with temperature limits from 60ºC-350ºC. Capillary column has immobilized stationary phase with long life and stability.

Mass- Spectrometer –

Mass spectrometer detector was used to fragment gaseous molecules into ions by electron ionization, using high-energy electron. Positive ions were scanned on the basis of their mass to charge ratio. A mass spectrum was obtained by monitoring the ions passing through the quadrupole filter, where only certain specified mass to charge ratios pass through, and all other ions are thrown out of their original path.[7]

Results & Discussion

The extract of visceral material from the body of the child when applied to GC-MS as stated above, using EC TM –5-Alltech capillary column gave rise to the following observations (**Table 1**):

The chromatogram (**Fig 1**) exhibited distinctive peaks at Rt-8.16 and 8.70. These correspond to endosulfan. The other peaks in the chromatogram correspond to octadecanoic acid (Rt - 8.09) and erucic acid (Rt-9.87). The peak at Rt-8.16 shows mass spectra for endosulfan (**Fig 2**). The major peaks obtained at Rt-8.16 were 196.90, 158.91, 206.89, 192.89, 240.84, 238.84, 264.83, 68.99, 80.99, 268.86, 119.98, 276.85, 95.01, 278.82, 338.75, 132.92, 336.86, 340.87, 306.86, 342.87, and 405.94.

The spectrum was matched with Wiley and Mainlib libraries, and tallied with endosulfan. Its isomers a-endosulfan & ß-endosulfan were identified in 18.98 and 28.39 percentage ratio. Chromatographic peaks at Rt-8.70 also give similar results but the ratio of a-endosulfan and ß-endosulfan was identified in 10.81 and 40.48 percentage ratio. The corresponding mass spectrum is shown in **Fig 3**. The major peaks obtained at Rt-8.70 were 194.91, 196.87, 207, 208.90, 84.93, 80.97, 242.86, 158.91, 238.80, 268.84, 276.83, 118.94, 134.98, 278.84, 336.83, 306.88, 342.92, and 405.78. These peak patterns matched with endosulfan. Besides these mass fragments, the following were also observed: 3,6-Dimethanonaphth [2,3-b], oxiren 8-ol 3,4,5,7,6,9,9, hexachloro-1, 1a, 2,2a, 3,6,6a, 7,7a octahydro stereoisomer, and for 4,7-

methanoisobenzofuran-1 (3H)-one 4,5,6,7,8,8, -hexachloro -3a, 4,7,7a-tetrahydro i.e., endolactone and bicyclo (2,2,1) hept-5-ene-2, 3-dicarboxylic acid, 1,4,5,6,7,7, hexachloro molecules. Generally, in technical formulations, the isomeric concentration for a & ß is about 64% and 26%, which was reversed in this case. This corresponds to earlier studies, which indicate that a-isomer is more toxic but less stable then ß-isomer. During metabolic conversion processes these changes occur, where a-endosulfan undergoes more pronounced conversion change than ß-endosulfan.[8,9]

These results confirm that the child had been by endosulfan. It is probable that the mother may also heve been administered the same poison and the body cremated to conceal evidence of the crime.

Table 1 Temperature Programming Standard Operating Procedure

GC OVEN		MASS DETECTOR PARAMETER	
Initial temperature °C	80°C	Source Temp.	200°C
Initial hold time (min)	1.00	Scan Rate	500.00
Number of ramps	1.00	Scan mode	EI
Rate deg/min	20ºC	First mass	40.0
Final temp°C	270°C	Last mass	550.0
GC run time (min)	11.33	Vacuum compensation Electron Energy Mass Transfer Line Temperature	78 psi 70 eV 275°C

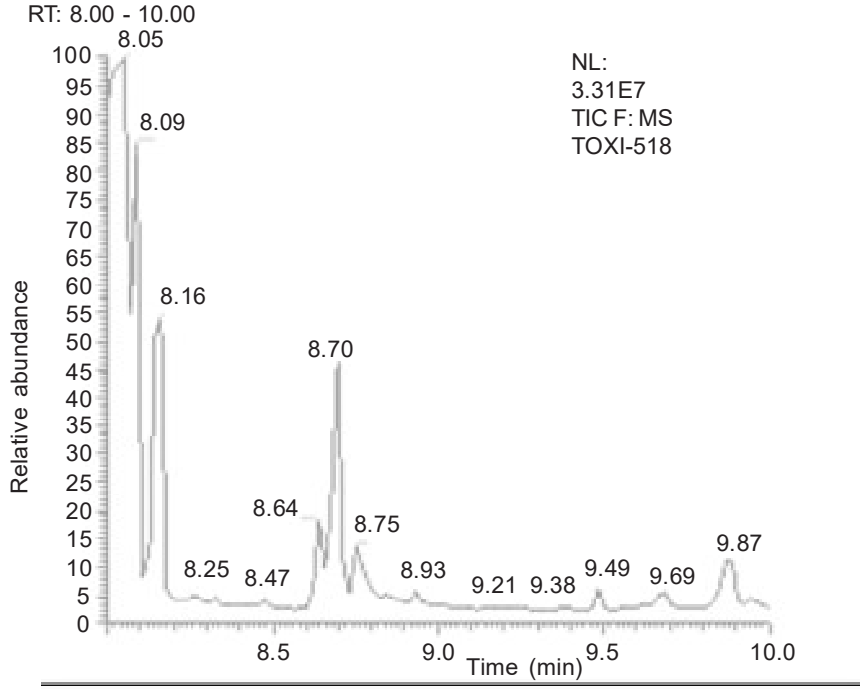

Fig. 1

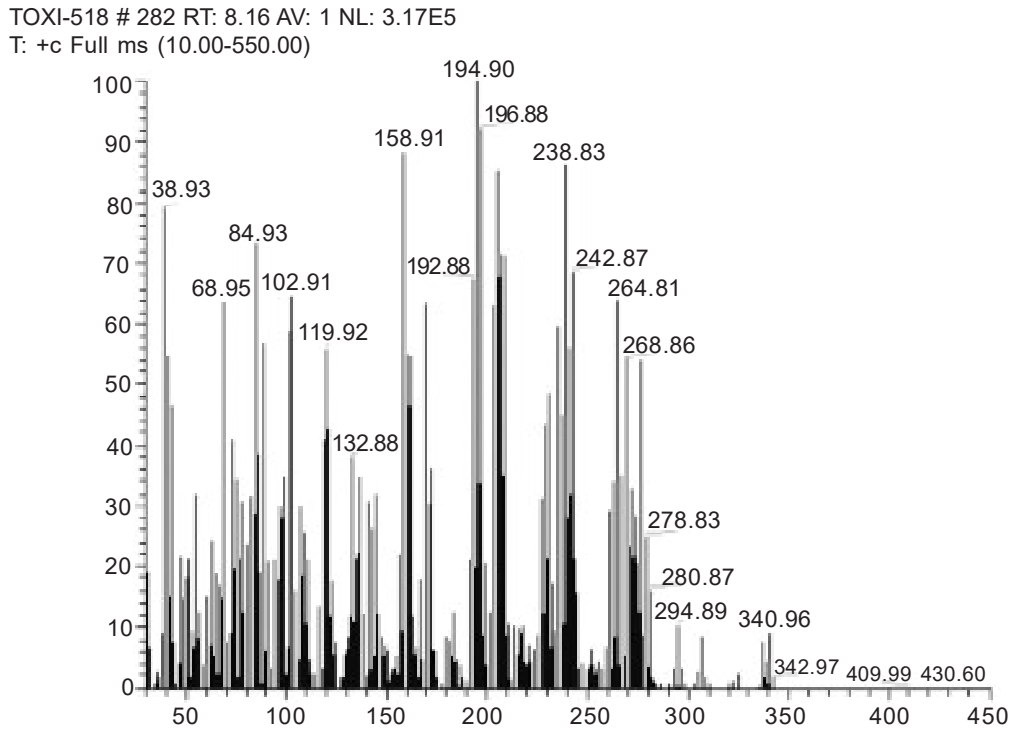

Fig. 2

Conclusion

The finding of a & ß isomers of endosulfan, endolactone and dicarboxylic chlorinated products in the autopsy material of the child confirmed that he had been poisoned with endosulfan. The standard operating procedure shown in Table 1 can be applied to other such applications where there is suspicion of insecticide poisoning.

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