Asian Journal of Chemistry Vol. 16, Nos. 3–4 (2004), 1685–1690

Gas Chromatography-Mass Spectroscopy (GC-MS) Study of Endosulfan in Biological Samples

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Mass spectrometry method has been used with capillary gas chromatography in electron ionization mode to identify the presence of α, β and endosulfan sulfate in human serum samples. The fragmentation ions were obtained due to ring cleavages, rearrangement and remote charge process. Solvent extraction procedure was used for isolation of compounds from serum sample. A detection limit as low as 100 ppb for α, β and sulfate could be easily achieved for confirmation. For endosulfan and sulfate the m/z values were obtained at intervals of M^{2+}. The m/z values of α and β-endosulfan are identical but they differ in retention time. Therefore the spectra reported might serve as reference spectra for identification of different types of organo-chlorine pesticide (OCP) in human serum sample.

Key Words: GC-MS, Endosulfan, Insecticide, Serum sample, Human.

INTRODUCTION

Endosulfan is a chlorinated hydrocarbon insecticide and acaricide of the cyclodiene subgroup and used primarily on a wide variety of agricultural crops including cotton, tea, coffee, fruits, vegetables and grains. Several methods were reported for the characterization and determination of endosulfan and sulfate in vegetables[1–7], animal tissue[8, 9], water[10, 11] and soil samples[12]. The GC-MS has been employed to quantitatively detect at low ppb levels of α and β-endosulfan in human serum, urine and liver but this failed to separate α, β-isomers. GC equipped with a microcoulometric detector (GC-MC)[8] was used to measure the levels of endosulfan in human blood at ppb (μg/L) level. In this paper we used GC-MS technique for the identification of α, β and endosulfan sulfate in human serum samples.

EXPERIMENTAL

α, β and endosulfan sulfate were procured from CCSRI, Excel Estate, Mumbai, India. The purity of the above standards was determined to be 99% by EI GC-MS analysis. Hexane (HPLC grade) was purchased from Qualigen Pvt Ltd., India.

Human Subject: Civil Hospital, Ahmedabad (Gujarat), India referred a case of acute poisoning to the Institute. The blood sample from the patient was collected and analyzed for identification of endosulfan.

Isolation of samples: 0.5 mL serum was mixed with 6.0 mL of hexane. The extraction procedure was conducted for 2 h on a low speed rotorac shaker. After the settlement of the contents, 5.0 mL upper layer of hexane extract was taken in a separate tube and concentrated to dryness under a stream of N_2. The residue was made up to appropriate volume in hexane and 1 μL sample was analyzed.

Instrumentation: Mass spectra and GC-MS measurements were acquired using CP-3800 GC equipped with Saturn 2000 (Varian India Pvt. Ltd.) mass detector with data system. For GC, 30 m DB-5 column (0.25 μm id; 0.25 μm thickness) was used in a splitless injection mode. The initial GC oven temperature (80°C) was then increased to 250°C at the rate of 5°C /min for 6 min. The total running time was 40.0 min. The injector, transfer line, trap temperature and manifold were set at 250, 270, 170 and 40°C respectively. Mass spectrometric analysis was performed in the auto EI, MS at the flow rate of 1 mL/min ultra pure helium.

RESULTS AND DISCUSSION

The GC-MS analysis of standard mixture of α-endosulfan, β-endosulfan and endosulfan sulfate clearly defines that the molecular ion peaks were obtained at m/z 339, 337, 422 with retention time at 28.8, 30.9, 32.4 min respectively as confirmed by NIST and Saturn library search technique. Endosulfan and endosulfan sulfate are organochlorine pesticides; therefore, due to the presence of isotopic abundance ($Cl^{35, 37}$) the m/z were obtained at intervals of M^{2+}. Tables 1 and 2 show the m/z value, % relative abundance of study samples, standard samples and reported spectra[13] for α-endosulfan and endosulfan sulfate. The spectrochromatogram of β-endosulfan has been shown in Fig. 3.

α-Endosulfan: The EI mass spectrum of α-endosulfan (Fig. 1) exhibits an abundant M^+ ion at m/z 339 due to loss of 68 μ mass from molecular ion. Consecutive losses of Cl_2 from M^+ yield m/z 337 (M^+-$C1_2$) and m/z 341 (M^+-SO_2,

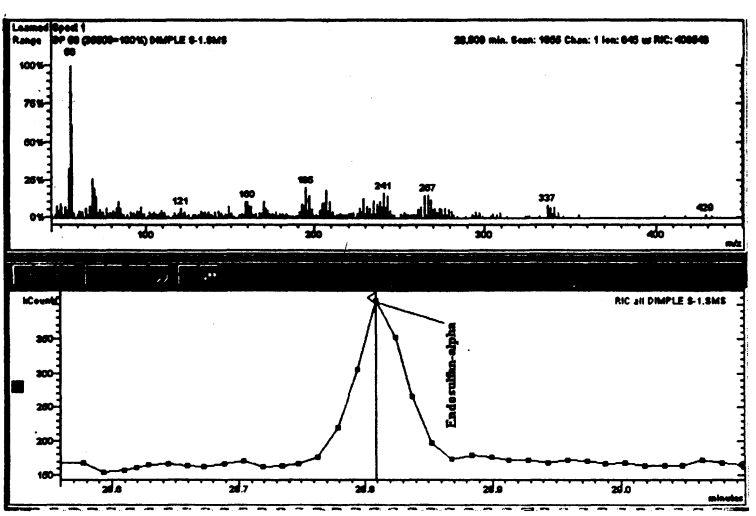

Fig. 1. GC-MS Spectrochromatogram of α-endosulfan study sample

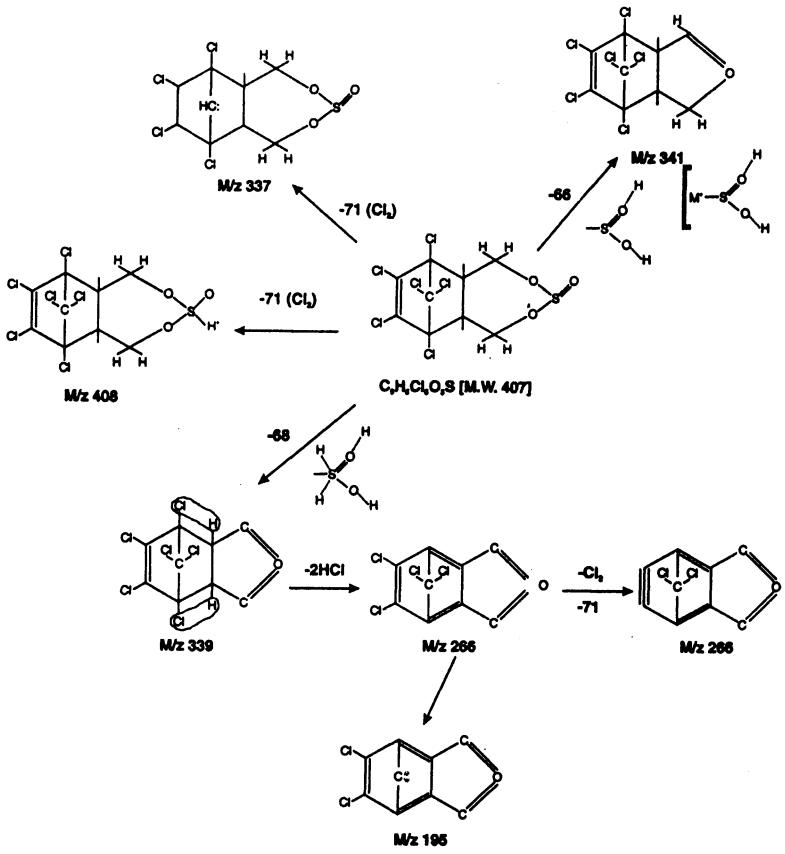

Fig. 2. Fragmentation schemes of α-endosulfan

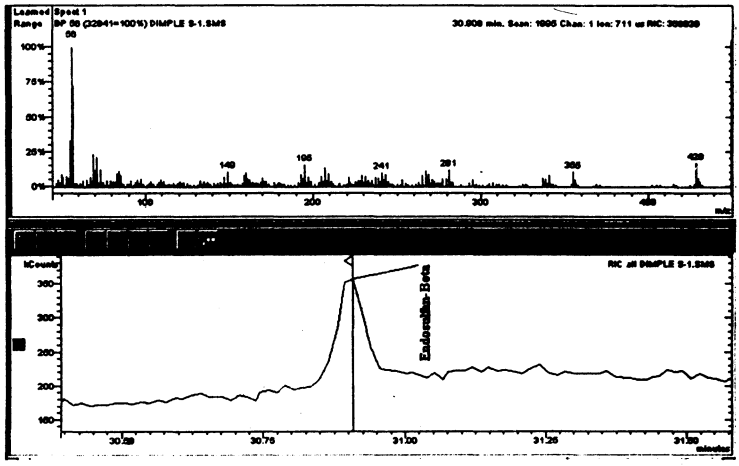

Fig. 3. GC-MS spectrochromatogram of β-endosulfan of study sample

H_2). The m/z 266 ions may represent (M^+-2HCl). Corresponding ion was observed at m/z 195 in EI spectrum of α-endosulfan due to loss of Cl_2 from m/z 339 peaks. The fragmentation schemes are given in Fig. 2. Table-1 clearly suggests that almost all m/z values (339, 341, 337, 265, 237, 195 and 159) were matched with the standard and reported spectra[13] in case of α-endosulfan.

TABLE-1
m/z (% BASE) OF STANDARD, STUDY SAMPLE AND REFERENCE SPECTRUM OF ENDOSULFAN

Endosulfan std. (% base)	Serum sample (% base)	Reported[13] spectra
339 (62)	339 (61), 337 (41)	339 (17)
265 (45)	265 (41)	265 (51)
237 (70)	237 (65)	237 (84)
195 (100)	195 (100)	195 (100)
159 (70)	159 (66)	159 (77)

Table-2
m/z (% BASE) OF STANDARD, STUDY SAMPLE AND REFERENCE SPECTRUM OF ENDOSULFAN SULFATE

Endosulfan sulfate std. (% base)	Serum sample (% base)	Reported[13] spectra
420 (8)	420 (18), 422 (36)	420 (3)
387 (49)	387 (100)	387 (33)
272 (41)	272 (89)	272 (100)
227 (23)	227 (40)	227 (76)
85 (11)	85 (9)	85 (48)

Endosulfan sulfate: The peak at m/z 422 corresponds to the protonated molecular ion (Fig. 4). The endosulfan sulfate showed M^{2+} at m/z 422 ions due to isotopic elements of $Cl^{35,37}$. The fragmentation schemes are shown in Fig 5. The intense peak at m/z 387 was due to loss of O_2 and H group from m/z 420, which confirmed the presence of oxygen atom in the molecule. The m/z at 387 also may be due to the removal of chlorine or HCl from M^+. m/z 385, 389 showed isotopic abundance of Cl atom. The m/z 227 confirmed the removal of $C_5H_2O_4SCl$ group (193 Dalton mass) from m/z 420. Intense peak at m/z 272 corresponds to loss of $C_4O_4SH_6$ group from M^+.

Conclusion

The results revealed that the GC-MS is very useful to confirm the α, β and endosulfan sulfate in human serum sample using NIST and Saturn library search. The fragmentation scheme of these insecticides was for the first time rationalized.

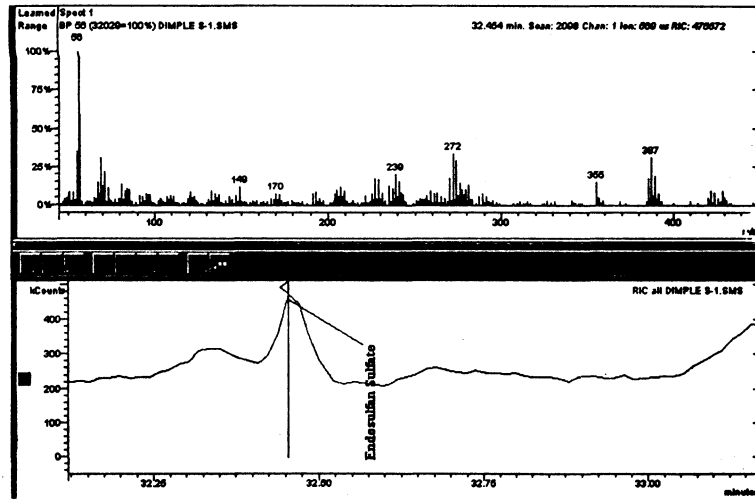

Fig. 4. GC-MS spectrochromatogram of endosulfan sulfate of study sample

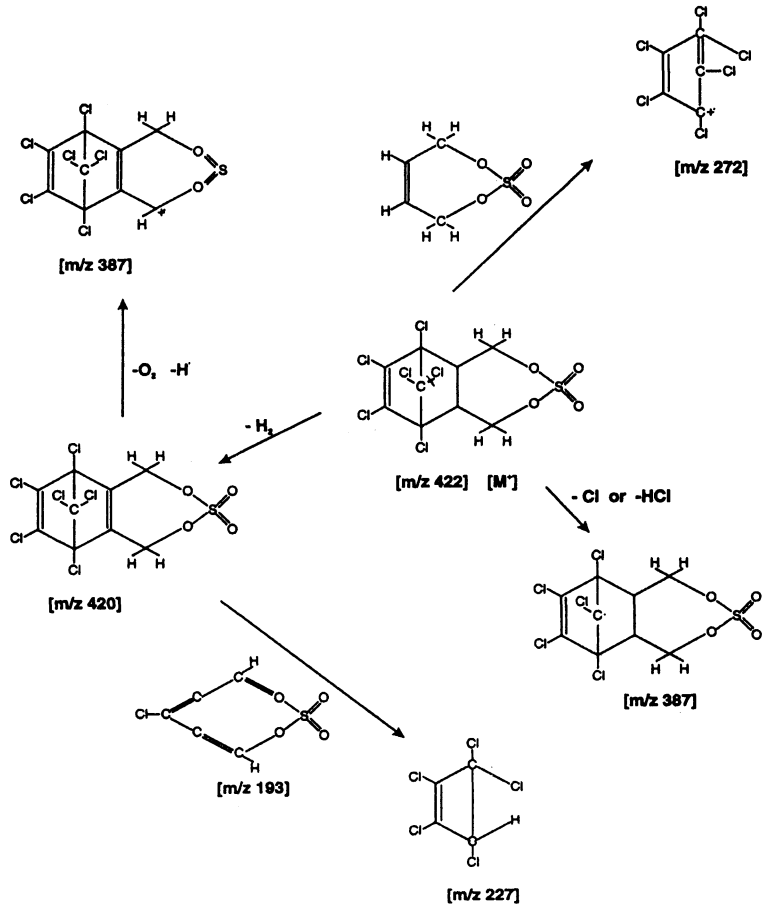

Fig. 5. Fragmentation schemes of endosulfan sulfate

The m/z peak for the study sample is completely matched with the standard spectra and reference spectra[13] for α-endosulfan, β-endosulfan and endosulfan sulfate. The fragmentation schemes of an α-endosulfan and β-endosulfan are similar because they are isomers but the retention time differs. These fragmentation schemes will be very useful in the identification of different types of OCP in biological samples.

ACKNOWLEDGMENTS

The authors are thankful to Dr. D.J. Parikh (Deputy Director Sr. Grade) for the help rendered during the study. We are also grateful to Dr. V.K. Bhatnagar and Rekha Kashyap (Senior Research Officer) for procurement of study sample.

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(*Received*: 12 April 2004; *Accepted*: 12 May 2004) AJC-3425