Pharmacological effects of endosulfan and its metabolite-DLLME dispersive liquid-liquid micro extraction combined capillary columns gas chromatography-mass spectroscopic screening of gastric lavage and vomit for detection of endosulfan and its isomeric form

Abstract

A simple, economical, rapid and sensitive analytical method has been developed for the simultaneous determination of endosulfan (a-and b-) in complex samples, such as gastric lavage and vomit, based on dispersive liquid–liquid micro extraction (DLLME) followed by gas chromatography–mass spectrometric (GC–MS) analysis. The method parameters have been optimized using response surface design experiments. Trichloroethylene (TCE) and acetone were chosen as extraction and disperser solvents respectively. After UA-DLLME, the sediment phase obtained was directly analyzed by GC–MS without any further cleanup and preconcentration procedure. Several factors which can affect the UA-DLLME extraction were screened and optimized by 27–4 Plackett–Burman design (PBD) and central composite design (CCD) experiments respectively. Based on these experiments the optimized parameters for UA-DLLME extraction were as follows: extraction solvent, (TCE, 58mL), disperser solvent (acetone, 1.27mL) and ionic strength (Na2SO4, 7%, w/v). Intra-and inter day precision were expressed as percent relative standard deviation (% RSD) and were found to be less than 6.33%. The limit of detection (LOD) of all the analyses in soil and urine were found to be in the range of 0.316–2.494 ng g⁻¹ and 0.049–0.514 ng mL⁻¹ respectively. The proposed method was successfully applied in the analysis of soil samples contaminated with endosulfan. The method may find wide application for the routine determination of endosulfan and its metabolites in environmental and biological samples.

Keywords: DLLME, LOD, pharmacological, toxicology, ultra gas, endosulfan

Abbreviations: RSD, relative standard deviation; LOD, limit of detection; LOQ, limit of quantification

Introduction

Endosulfan is an organo chlorinated pesticides¹ still used in many places in India for protecting agricultural fields from pests. One to so many socio economic problems in agriculture farmers commits suicide by consuming the Endosulfan.²,³ Endosulfan⁴ is a neurotoxic poison which inhibits active site of the enzyme acetyl chloride esterase. In this study an attempt is made to develop a method for rapid screening of Endosulfan in cases of poisoned samples of living persons who are admitted in hospital for treatment.⁵,⁶ Openly in clinical toxicology unit toxicologist were requested to give reports immediately after the samples were submitted in order to give antidote for the poison and to save the life of patients. Hence a rapid method is required to identify and confirm the poisonous compound in gastric lavage and vomit. Endosulfan exists in different isomeric form and in this study aimed to separate isomers and also to confirm in the samples.

Materials and methods

a. Sigma Aldrich Endosulfan standard was procured for assay procedure.

b. All the reagents and solvents used were of analytical grade.

c. Mini Centrifuge (Tomy) was used for centrifugation 5μl eppendorf tubes were used for sample digestion. Hamilton Micro pipettes (0-1μl) were used for pipetting out small volumes of sample and solvents.

d. Clarus 600 Gas Chromatography and Mass Spectrometer Perkin Elmer were used for chromatographic mass spectroscopic examination.

Method

1ml of free sample gastric lavage was pipetted out into an eppendorf tube and 1ml of the methanol is added to mix and precipitate the protein. The dispersive solvent acetonitrile is added for selective phase dispersion and mix together centrifuged for 5 minutes at 3000rpm. After setting the cellular protein the supernatant of the tube is transferred to a fresh tube and air dried to evaporate. Reconstituted with reconstituting solvent acetone was injected into Gas Chromatography Mass Spectroscopy for analysis.

Instrumentation and GC–MS conditions

GC–MS analyses were performed using a Trace GC ultra gas chromatograph connected to a Quantum XLS mass spectrometer (Thermo Scientific, FL, USA). The GC was equipped with a TG-5MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness)
consisting of a stationary phase 5% phenyl 95% methyl polysiloxane. Injection was carried out in the split mode (35 : 1) at an injector temperature of 250°C. (Figure 1) Helium gas was used as a carrier gas with a flow rate of 1.0 mL/min. The oven temperature programming was as follows: the initial oven temperature was held at 100°C for 1.0 min, and then increased to 190°C at a rate of 20°C min⁻¹ and then increased to 260°C at a rate of 3°C min⁻¹ held for 5.0 min. The ion source and transfer line temperature were 220°C and 290°C respectively. All the samples were analysed in selected ion monitoring mode. The sonicator used was an IMECO ULTRA SONICS (Bombay, India). An Easy SPIN centrifuge (Remi Instruments Ltd, Mumbai, India) was utilized for the centrifugation of the sample tube. (Table 1)

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<td><strong>Gas Chromatography condition</strong></td>
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Assay validation

Recoveries were carried out by spiking the standard mixture of endosulfan¹⁵⁻¹⁶ and its metabolites in soil and urine at concentrations of 100, 500 and 2000 ng g⁻¹ and 160, 220, 460 ng mL⁻¹ respectively. Precision is the ability of the assay to consistently reproduce result when sub-samples are taken from the same specimen. Intra and inter-day precisions were checked by carrying out five independent assays of sample in a day and for five successive days (n=5, five replicates of samples) respectively and the values were expressed as % Relative Standard Deviation (RSD). Limit of detection (LOD) and limit of quantification (LOQ) were calculated as per the IUPAC procedure. 28, 29 Five replicates were used to determine the LOD and LOQ for endosulfan and its metabolites. (Figure 2)

Selection of extraction solvent

The first step in the DLLME optimization was to select an appropriate extraction solvent. Organic solvents such as CB, CDS, TCE, DCM and CC14 were selected on the basis of their higher density and low miscibility in comparison to water, higher extraction capability of target compounds and good chromatographic behaviour. The selectivity of solvent was evaluated with 70mL of each extraction solvent and 1.0mL of acetone as disperser solvent in 5 mL of water sample spiked with endosulfan¹⁵⁻¹⁶ and its metabolites at a concentration of 2mg mL⁻¹ of each. Among all the solvents tested, TCE was found to give optimum results in comparison to other solvents tested (Figure 3). A much less cloudy solution formation was observed in DCM when compared with TCE. This may be due to the high solubility of DCM in the mixture of acetone and water (medium). This is supported by the high solubility (13g L⁻¹) and polarity of DCM (3.1) in comparison with TCE solubility (1.28g L⁻¹) and polarity (1.0). The polarity order is given below. 31DCM (3.1)>CB (2.7)>CC14 (1.6) >CDS (1.0) z TCE (1) Even though the polarity index is the same for CDS and TCE, due to the high density of TCE and high chlorinated content nature, TCE has a greater extraction efficiency than CDS.

Selection of disperser solvent

The selection of disperser solvent is also one of the important factors in the DLLME extraction process. The main criterion for the selection of the disperser solvent is its miscibility with extraction solvent and aqueous solution. Acetone, methanol, acetonitrile and tetrahydrofuran were selected as disperser solvents for optimization. Selection of dispersion solvent was evaluated by taking 1.0mL of disperser solvent and 70mL of TCE as an extraction solvent. Out of the solvents tested, the optimum response was obtained with acetone as compared to the other solvents (Figure 4). The reason for this may be attributed to its higher miscibility in both the phases (extraction and aqueous).

Results and discussion

In the old liquid-liquid extraction method the efficiency of extraction is not much good and also along with our active compound of interest is combined with other impurities like fats and lipoprotein and sometimes salts. As our compound of interest is very less and sometimes in metabolite form and conjugate form it is very difficult for the scientist to interpret the mass data because of clumsiness sometimes in metabolite form and conjugate form it is very difficult and sometimes salts. As our compound of interest is very less and extraction is not much good and also along with our active compound.

Endosulfan organo chlorinated insecticides is very well isolated and identified by the current method developed. Initially the peaks could not be identified due to operating condition of Gas Chromatography Mass Spectroscopy. Operating conditions of GC-MS is optimized for resolving very well distinguished peaks. The identification and confirmation of Endosulfan is achieved by mass spectra data and most of the time the isomers of Endosulfan are difficult for the scientist to distinguish.17,18 The α and β form of the isomers are structural difference isomers with some molecular weight and different orientation. (Figure 6)

Always in the court of law at law absolute identification and confirmation of compound of interest is required to prove beyond the doubt to the court of law. Only by this specific method high end Clark 600 Mass Spectrometer the separation of isomer distinctly identified and confirmed. The author and co-worker thanks to institute Central Forensic Science Laboratory for providing all assistance and support.19,22 The author also says personally thanks Director, CFSL, Kolkata for constantly motivating and supporting us. (Figure 7)
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Figure 1 Chrom endosulfan.

Figure 2 Chrom endosulfan.

Figure 3 Spec endo 1.

Figure 4 Spec endo 2.

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Figure 5 Chrom endo sample.

Figure 6 endo1.

Figure 7 endo2.

Acknowledgements

None.

Conflict of interest

The author declares that there is no conflict of interests involved in this study.

References


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