

A NOVEL TECHNIQUE TO DETECT CHLOROFORM AT TRACE LEVELS IN BIOLOGICAL SPECIMENS AND OTHER ITEMS

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ABSTRACT

The ability to confirm chloroform at trace levels in autopsy specimens and relevant contaminated items is a challenge to the forensic toxicologist.

This paper reveals how sensitive and selective headspace gas chromatography-mass spectrometric method was developed to detect chloroform at trace levels in blood in two cases in Sri Lanka.

Chloroform in suspected items was extracted into methanol. Separation was performed on a 60 m x 0.32 mm capillary column GC-GASPRO. The qualitative and quantitative analysis of chloroform was performed in the scan and single ion monitoring (SIM) modes respectively using mass spectrophotometric detector. The method was validated in terms of linearity ($r^2 = 0.993$) for the concentrations ranging from 5-100 mg/L, repeatability (RSD – 4.1 % at 10 mg/L, n= 6), limit of detection 1 mg/L and limit of quantification 3.3 mg/L.

In a case of sexual assault toxicological analysis of the contents of the chemical bottle revealed the presence of chloroform at a concentration of 422 mg/L and traces of chloroform on a piece of cloth.

In the case of hanging Chloroform was identified in the concentration of 72 mg/L in blood and traces were found in the empty bottle found at the scene.

INTRODUCTION

Chloroform is used in the chemical industry for synthetic purposes and laboratories as an extraction solvent. Hence, people have free access to chloroform which can be used for criminal activities. Chloroform (Trichloromethane, CHCl_3) is commonly known as “knockout drug” as it has a sedative property that causes central nervous and respiratory system depression instantaneously.¹⁻⁴ Historically, chloroform has been used as a surgical anaesthetic. However, its use has become obsolete due to adverse side effects such as hepatic and renal cancer.^{1,5} Chloroform-induced toxicity is due to production of reactive metabolites during the metabolism of chloroform in liver and kidney. Oxidative dichlorination of chloroform leading to the formation of phosgene (COCl_2) which causes the depletion of glutathione will induce cellular toxicity.⁴ Reduction of chloroform in the body results the formation of cancer triggering reactive dichloromethyl radical ($^*\text{CHCl}_2$).^{4,5} In literature, there have been several cases reported on chloroform poisoning by forced inhalation during criminal activities such as homicide, suicide and sexual assault.^{1,2,4,6-9}

The critical factor in toxicology is to identify chloroform at trace levels in biological post mortem samples and other suspect items associated with criminal activities. In early days, steam distillation or organic solvent extraction methods have been used to extract chloroform from blood and gas chromatograph with electron capture detector or colourimetric methods used for its detection.^{6,10} Later a few methods using headspace gas chromatograph coupled with mass spectrophotometer detector have been reported for the determination of chloroform in trace levels.^{1,2,6,8} Only a single method acquired more sensitive single ion monitoring mode (SIM) for the quantitative analysis was found in the literature.² This study describes a more reliable method for the detection of chloroform in post mortem specimens and related items.

OBJECTIVE

To develop a sensitive and selective headspace gas chromatography-mass spectrometric method (HS-GC/MS) for the detection of chloroform at trace levels in biological specimens and other items.

MATERIALS AND METHOD

Analytical grade Chloroform (purity 99.5%) and methanol (purity 99.8%) were used in the analysis. The instrument used was Agilent 7697A headspace auto sampler gas chromatograph (Agilent 6890) equipped with Agilent 5973N mass spectrophotometer. Capillary column 60 m x 0.32 mm GS-GASPRO, headspace vials (22 mL), vial septum and aluminium caps were used. The mass detector was linked to a data-handling system with Agilent MSD ChemStation integration software (E.02.02.1431) for data acquisition. Helium was used as carrier gas.

Chloroform stock solution was prepared by dissolving chloroform in methanol. The working

solutions were prepared by diluting the stock solution with 20% of methanol. Chloroform in suspected chloroform soaked items was extracted into methanol by addition of 5 -10 mL of methanol and mixed well, then squeezed methanol into container which can be tightly cap. Chloroform spiked blood samples were prepared by adding blank blood (0.5 mL), known concentration of chloroform working solution (0.1 mL) and deionized water (0.4 mL) into a headspace vial. Test samples (blood or suspected liquid) 0.5 mL with 0.5 mL deionized water or methanol extract (1.00 mL) was transferred into headspace vial and rapidly sealed with a septum and aluminium cap to prevent any loss of chloroform.

The sealed vials were introduced in an auto-sampling system for volatilization (90 °C for 12.5 min) and GC cycle time 30.0 min. transfer line temperature was set to 100 °C and carrier gas flow rate was 2 mL/min. Split mode injection was done with split ratio 1:1 and front inlet temperature was set to 200 °C, pressure 15.7 psi. The initial oven temperature was 50 °C for 2.0 min and was increased to 230 °C at 25 °C/min and held for 3.0 min. The total run time for gas chromatography was 12.2 min. Identification of chloroform was performed in the scan mode (m/z 25 to 250). Obtained mass spectra were compared with NIST-14 library. The method was validated according to Eurochem guidelines.¹² Quantification of chloroform was performed single ion monitoring mode (SIM); m/z 83, 47.1 and 35.1 using calibration curve (5-100 mg/L). Limit of detection (LOD) and limit of quantification (LOQ) were obtained by injecting six blank samples spiked with lowest concentration of chloroform. LOD was calculated by; mean value of above six replicates + 3 x standard deviation and LOQ was calculated by; mean value of above six replicates + 6 x standard deviation.

Case I

A woman declared that her former boyfriend smothered her with a cloth soaked in some chemical. She had fallen asleep with intermittent spells of wakefulness during which she has screamed for help. A neighbour had heard her screams and entered the house with others. The victim had been lying naked with her hands and legs tied to a bed. The police was informed immediately. Investigation revealed a piece of cloth on one of the pillows, a screw capped bottle containing a colour-less liquid and used condoms. The piece of cloth and bottle were sent to the forensic toxicology laboratory, Government Analyst's department for toxicological investigation. During this time the suspect was apprehended.

Case II

A 30 year old male was found hanging in his residence. His nose and mouth were said to have been covered with a face mask with a plastic cup inserted. Post-mortem blood and an empty bottle found at the scene were sent to the laboratory for toxicological investigations.

All the samples of case I and II were stored in cold room at +4 °C.

RESULTS AND DISCUSSION

The developed technique was applied to the above cases and found to be acceptable with respect to all validation parameters.

In blank blood sample, no interfering peak was detected at the retention time of chloroform. This confirmed the absence of interfering endogenous substances. The correlation coefficient (R^2) for the calibration curve concentration ranging 5-100 mg/L was 0.993. This revealed that the method is linear. The inter day relative standard deviation (RSD) for ten injections of 10 mg/L chloroform solution was 4.1 % which is an acceptable value (< 5%) for a validated method. Limit of detection and limit of quantification were 1 mg/L and 3.3 mg/L respectively.

Table 1: Method validation parameters for chloroform

Chloroform validation parameters						
Calibration range	RT	R^2	Calibration equation	RSD	LOD	LOQ
5-100 mg/L	9.27 min	0.993	$y = 370.73x + 1972.8$	4.1 %	1 mg/L	3.3 mg/L

RT- retention time

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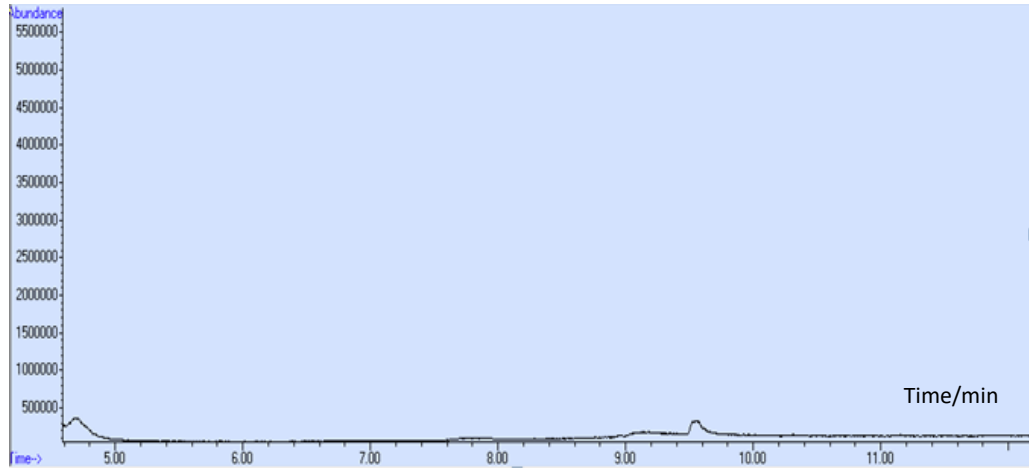


Fig.1: HS-GC/MS; Total Ion Chromatogram (TIC) for blank blood

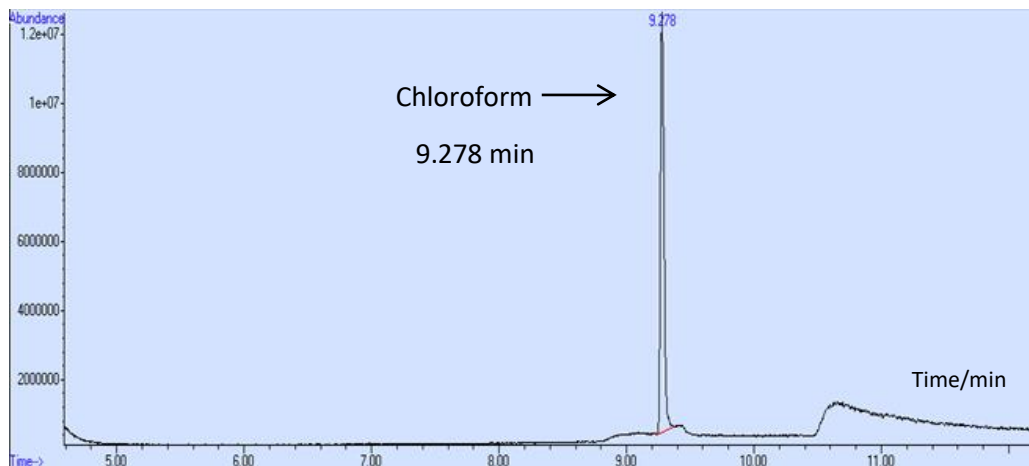


Fig.2: HS-GC/MS; Total Ion Chromatogram (TIC) for chloroform in blood

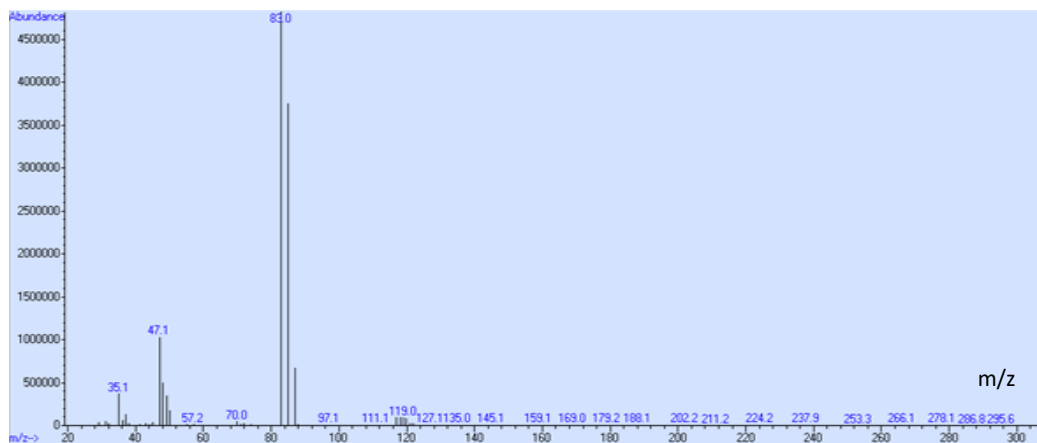


Fig.3: HS-GC/MS; Mass Spectrum of chloroform obtained from chloroform positive blood

On the toxicological analysis of samples in case I, chloroform 422 mg/L was identified in the liquid in the suspected bottle and traces of chloroform were detected on the piece of cloth. Analysis by HS-GC/MS of case II samples revealed the presence of chloroform. The post-mortem blood contained 72 mg/L of chloroform and traces in the empty bottle. Baselt and Carvey reported that concentrations of chloroform ranging from 10-194 mg/L with an average of 64 mg/L have been found in fatal cases.¹¹ Riße et al have reported chloroform levels of 73 to 115 mg/L in postmortem blood of homicides.⁸ Further Meichsner et al have reported that 47 mg/L of chloroform was found in postmortem blood of suicidal poisoning by chloroform inhalation.¹³ Chloroform exposure was lethal for the victim of suicidal hanging in case II. Riße et al reported that total loss of chloroform from blood stored at +4 °C is markedly less.⁸ Hence, there was no chloroform loss in analysed blood sample as we stored it in the said condition.

Conclusions

The reported cases were the first cases where trace levels of chloroform were identified in blood and objects in Sri Lanka using headspace gas chromatography mass spectrometry.

The study illustrates that chloroform can be detected and quantified at trace levels from blood. Further, the analytical findings provide corroborative evidence to the criminal intent.

ETHICAL ISSUES

None

CONFLICT OF INTEREST

None

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AUTHOR CONTRIBUTIONS

LSH: Design of study, Experimental work, Analysis of data and Writing of manuscript;
AW: Design of study, Experimental work, Analysis of data and Writing of manuscript;
WDVK: Design of study, Experimental work, Analysis of data and Writing of manuscript;
TRMCDM: Design of study, Experimental work, Analysis of data and Writing of manuscript

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