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Inhalant abuse and detection using Gas Chromatography: A review of literature

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ABSTRACT

Inhalant abuse is one of the most emerging as well as perilous problem in respect of drug abuse in adolescent children. Because of the easier availability, low cost and high psychological kick, inhalants are increasingly abused by people of all ages. However, the worst effects can be seen in children in their teens. Conventional and modern gas chromatographic methods are most frequently used for their detection in different types of samples. This review article intends to summarize all the work done in field of detection and analysis of inhalants in matrices of toxicological importance. Minimum work has been done in terms of detection of inhalants from forensic point of view. Therefore, it is necessary to develop more methods for detection of inhalants using GC and other hyphenated techniques.

Keywords: Forensic Toxicology, Drug abuse, Inhalant, Fatal Cases, Headspace Extraction, Gas chromatography – Mass spectrometry

INTRODUCTION

Drug of abuse may be defined as any chemical substance which is used for any purpose other than it is intended to. Such substances can also be defined legally as substances, the possession and supply of which are restricted by law because of its potential harmful effect on the user. A drug may

be a solvent, tobacco, alcohol, OTC (Over the Counter) drugs or may be an illicit drug. Any substances, whether natural or synthetic, which affect body's CNS (Central Nervous System) and alter mood, thinking and perception of the user, these substances may also be called as Psychoactive Substances or Psychoactive Drugs[1]. Drugs can be classified into[2] 1) Narcotics (Pain relieving drugs, analgesics), 2) Stimulants (Stimulates the central nervous system), 3) Depressants (Depresses the central nervous system) 4) Hallucinogens (Alter human perception), 5) Inhalants (Volatile solvents).

Inhalants

Inhalants are the psychoactive substances that are volatile in nature and their vapours are inhaled to get mood-altering or mind-altering effects. Inhalants can be differentiated from other classes of drugs on the basis of their route of administration i.e. inhalation, rather than their mechanism on CNS or their psychoactive effects. Inhalants are essential components in a number of household and industrial products like glue, lighter fluid, cleaning fluids, paint thinners, nail paint removers etc. Due to their domestic and industrial use, solvents are often abused by adolescent teens. Over 1400 household products are abused as inhalants [3,4].

For many people inhalants are among the one of the first substances abused and for many others, especially children, inhalants are among the most preferred substance for abuse due to its easy availability. Inhalants can either be in the form of liquids or in the form of gas, but liquids are far more abused than gases because obviously liquids are easier to carry and handle than gases [5].

History of inhalants abuse

Inhalants use can be traced as far back as 1400 B.C. by the Greeks. In ancient Greece, people used to visit an ancient Greek Oracle named Pythia, to know about their future. Pythia used to tell her predictions after entering a trance. It was believed that she used to tell the predictions by getting them from the Greek God Apollo himself. In 1990, a team of scientists finally discovered the reason behind this. Geological faults in the rocks were discovered in the Oracle's temple and a sweet smelling gas namely ethylene was found to be drifted through these cracks and due to this gas, oracle i.e. priest or priestess (here Pythia) experienced euphoria and hallucinations after entering into trance of the gas. Inhalants use is also linked to native Americans called Shamans. They used to inhale substances that caused hallucinations. Altars holding burning incense have also been found to give psychoactive effects in Ancient Egypt and Middle East[4].

In 20th century, a number of new volatile organic compounds were introduced in everyday use products. Most of these VOCs (Volatile Organic Compounds) were obtained from fractional distillation of petroleum i.e. as petroleum distillation products. Everyday household products using these VOCs included glues, cement, gasoline, paint thinner, polish and spot remover, paint and dry-cleaning products etc. along with their widespread use, these began to be abused also in 1920s, during World War II and continuing to present day. These substances were abused because upon their intoxication these gave similar effects to that of alcohol intoxication and gave mild hallucinations also. In 1940s and 1950s, a large number of reports were published about children sniffing glue (used for making model aeroplanes) because of presence of toluene in that glues. After that, toluene was removed from a large number of household products and glues. But it is still used in many other products like gasoline, paint remover, paint thinner etc. in 1960 sniffing commercial and household products like paint thinner, shoe polish, glue became very much popular among

drug abusers, especially in hippies to alter the state of mind, consciousness, because these products were in expensive to get high.

In 1993, Ralph Minnitt, a British doctor developed a machine in which nitrous oxide was mixed with air and then delivered to patients for having relief from pain, but it could not make the patient unconscious. Later, machines were more developed and air was replaced with oxygen and used by pregnant women to get relief from labour pain. Today main use of nitrous oxide is in dentistry (to make people feel less nervous), as an additive in gasoline, in cans of whipped cream and of course for abusing, much large than its use.

It was also noted that in 19th and 20th century, most drug abusers of inhalants were adults but in 21st century most drug abusers of inhalants are teenagers, because inhalants are cheaper, easy to get, easy to steal, easy to conceal and of course legal.

Categories of inhalants

Balster et al[6] have presented four criteria for the classification of abused inhalants. This include 1) Chemical structure 2) Form such as gas, vapour, aerosols or liquid 3) Product type 4) Pharmacological properties. On the basis of chemical structure, the inhalants can be classified into volatile nitrites, nitrous oxide, linear and branched alkanes, halogenated aliphatic hydrocarbons, aromatic hydrocarbons, ethers, etc. On the basis of form, the inhalants are classified into gaseous, liquid and aerosol inhalants. On the basis of product type the inhalants can be classified as adhesives, cleaning products, paint thinners, cosmetics, water repellents, room odourizers, etc. On the basis of pharmacological properties, the inhalants can be classified into CNS depressants, anaesthetics, etc. These criterions of classification of inhalants are not strict and are overlapping in nature which means that no single classification is absolute in nature. Since every classification has its own strength and weakness, often we must use more than one criteria of classification for better understanding of inhalants.

The classification of inhalants on the basis of form has been elaborated as follows[3,4]:

Volatile Solvents

Volatile solvents are those chemicals which produce vapor at room temperature. These are found in a large number of commonly available household and industrial products. It includes paint thinners and removers, dry-cleaning fluids, degreasers, gasoline, glue or model glue, correction fluid, felt tip markers, nail polish remover, permanent markers, white-board cleaner, rubber cement, shellac, shoe-polish spray etc.

Aerosols

Aerosols are liquids stored in containers under high pressure for example - the spray cans that contain propellants and solvents. The chemicals that are inhaled from aerosols include: spray paint, deodorants, hair sprays, vegetable oil spray, fabric protector spray, dusting spray, computer cleaner, air freshener, insecticide spray, spray lacquer etc.

Gases

As the name suggests, the chemicals that are in gaseous state even at room temperature are called gases. Medical anesthetics include ether chloroform, halothane and nitrous oxide. Household or commercial products include butane lighters (fluid for lighters), propane tanks (gases for cooking,

heating), refrigerants and air conditioners (freons), whipped cream dispenser (nitrous oxide), helium (used to inflate balloons).

Nitrites

Compounds of nitrites are also inhaled because of their muscle relaxing and sexual enhancing properties by dilating blood vessels. These include iso-amyl nitrite (also known as popper or snappers), iso-butyl nitrite (also known as rush, climax or locker room), cyclohexyl nitrite, used in certain diagnostic procedures.

WHY VOLATILE SOLVENTS ARE ABUSED?

The risk of volatile abuse is ever increasing and people often abuse them for various reasons. The most prevalent reason for its abuse is peer pressure. This peer pressure may be from fellow group members, friends, etc. The volatile solvents are easily available at home, shops, schools, offices, grocery stores, gas stations and are low-priced which makes them the top priority among the teenagers. The products comprising the volatile solvents are available as daily usage products which make them easily concealable which allows such products to evade suspicion[5].

A number of remarks like ‘It makes me feel good’, ‘I like the high’ and ‘You are not afraid when you are under’ have been recorded from solvent abusers. They also described the inhalants intoxication similar to that of alcohol but not identical to it. Heavy or consistent users try to cope up or treat their feelings of depression and frustration with the state of oblivion they get from inhaling solvents. Solvent abuse is regarded as superior to inebriation produced by alcohol and other drugs because upon administration, due to their volatile nature, these are easily and rapidly absorbed from the lungs to the bloodstream to the brain, giving quick results and the user experiences instant effects in their inebriation[7].

Abusing inhalants does not require any paraphernalia which is beyond the reach of some abusers especially children. All they need are plastic bags, rags or clothing, balloons etc. for huffing and these products are easily available to persons of all ages and also not recognized as inhalant paraphernalia by adults. Special blood tests can be used to detect their presence but that also be done within a few hours of solvent administration which is very difficult and quite expensive also. Even in case of paraphernalia, due to their volatile nature amount of inhalant left after abuse may be very less. Thus users do not have the fear of being punished of inhalant detection from their body[3].

Methods of abuse

Inhalants can be abused by three ways[5,7,8]:

Huffing

It is a method of taking inhalants into the body through mouth. The user first soaks the rag pieces with the chemical to be abused. Then, the rag pieces are kept into mouth and sucked or kept near the nose and the fumes are inhaled. Other variants of huffing are bagging, ballooning. In bagging the user puts the inhalant or the solvent like paint thinner or gasoline etc. into the plastic bags or polyethenes and inhale the fumes by holding the bag near to the mouth of face. In ballooning, balloons are used instead plastic bags or polyethenes and solvents or gases are put into them and the huffed by taking the balloons to the mouth.

Sniffing

It is a method of taking inhalants through nose. Inhalants can be sniffed directly from the container of the product or by emptying an aerosol by spraying it in another open container from which vapors can be inhaled easily. Some abusers sniff inhalants from their fingernails by painting them with the substance. These users then put their fingers into their nose to breathe in the fumes.

Direct spray

It is a method of taking inhalants by directly spraying the chemical substance into oral cavity or mouth or using the slim plastic straw provided with some products and spraying into the mouth. This method is used especially for aerosols. In direct spraying, sometimes the user sprays the chemicals onto his/her clothing or sleeves for breathing in vapors for the whole day.

Some abusers, especially children pour the products into the collars, cuffs, sleeves or other parts of their clothing and then raise their clothing near to face to huff or place their face or head down on arms on desk to sniff and pretend to be resting or sleeping. Some abusers soak the cotton balls or swabs into the product and then stuff up their nostrils by putting these cotton balls or swabs into them and then use to sniff them. Inhalants are also abused by decanting the product onto a scarf, bandanna, socks, cloth or baby diaper then held or tied over the face. Sniffing or huffing can also be done from a small enclosed space like cardboard shipping carton, car or closet[8].

Mode of Action

During respiration oxygen enters the body which is then absorbed inside the lungs into the blood stream. From blood the absorbed oxygen reaches the brain. When inhalants enter the body through nose or mouth, their vapours reaches to the lungs where they displace the oxygen and enters into the bloodstream and quickly reaches the brain and other body parts. The chemicals present in the solvents are lipophilic i.e. fat soluble, so after reaching the brain and other body parts, these get absorbed there quickly and effects are felt instantly.

Solvents show depressant effects on CNS and even in small amounts these can produce temporary distortions to how people sense and see things. Their effects vary from person to person and situation to situation i.e. how they are taken, how experienced is the person in using them, general and mental health of the user etc[3,8].

Effects on body

A quick high is produced within seconds of inhalants intoxication, similar to that of intoxication caused by alcohol or marijuana. The user at first becomes slightly excited but after sometimes feels drowsy, lightheaded with impaired coordination that makes him/her prone to accidents.

Immediate effects

These are the effects that last for a minute or two upon solvent intoxication. These are temporary and include hallucinations, dizziness, tremors, excitation, disorientation, emotional volatility, loss of coordination, irregular heartbeat, nausea, perception distortion, diminished sensitivity to pain etc.

Short-term effects

Inhalants excepts nitrites act like depressants but their depressing effect can vary from person to person based on user's weight, general health, amount of substance inhaled etc. User may

experience heart palpitations, dizziness, headache, problems in breathing, muscle weakness, abdominal pain, mood swings, tingling/numbness in their hands and feet, nausea, fatigue, slurred speech, slowed down reflexes, user becomes apathetic and may also experience hallucinations. Even though most of the inhalants act as depressants, some of the users feel excited with a sense of euphoria. Some users become agitated and cannot sit still. More commonly, they become relaxed and laugh a lot. Some of the users may get into fights, have unprotected sex and may injure themselves or others. Some of the users become paranoid thinking people are pursuing them and may be terrified as they try to escape from these people. Some users also develop nosebleeds after inhaling chemicals, while many of the users get sores into their mouths or nose.

Long-term effects

People using inhalants for a long time i.e. for years may suffer a wide range of physical and mental problems. Users may lose a dangerous amount of weight with long term inhalant use and it can also cause memory loss, personality disorders, nerve damage, weakness, fatigue. Severe damages to brain and other body parts occur which are described as following:

With long term use, inhalants can cause a severe and irreversible damage to several parts of brain like myelin, cerebral cortex and cerebellum. Myelin sheath is a membrane which surrounds and protects the neurons i.e. nerve cells. If inhalants are used for over years, it can damage myelin sheath because it is made of lipids due to which it is soluble in inhalants resulting in dementia or cell death. Cerebral cortex is situated towards the front of brain. It also gets affected heavily by the use of inhalants. When neurons of cerebral cortex die, user may experience hallucinations, learning disabilities, problems related to memory and personality related disorders. Cerebellum, the lower back part of brain, can also be damaged by heavy use of inhalants. A damaged cerebellum results in lack of coordination, uncontrollable shaking of arms and legs. Some inhalants cause damage to specific parts of brain like toluene is known to cause damage to ophthalmic nerve i.e. the user experiences vision related problems.

Damage to other body parts

Inhalants deprive the body of oxygen by displacing the oxygen from lungs. This condition is called hypoxia and it can damage cells throughout the body but brain cells are especially sensitive to it. Inhalants affect the kidneys adversely by destroying its ability to regulate the amount of acid in bloodstream and can cause kidney stones. Liver can also be damaged due to long term inhalant use. Inhalants get absorbed into the liver and kidneys by breaking their cells and can cause the organs to fail. Chronic inhalant use can also lead to muscle weakness, decreased strength, reduced muscle tone and damage to bone marrow. Inhalants can also cause hearing loss because these damage the cells that carry sounds to the brain. Inhalant abusers get breathing and other respiratory related problems. Some chemicals in inhalants like DCM (dichloromethane) adversely affect body's ability to carry oxygen. The lungs and heart are at particular risk. Most inhalants slow the activity of the heart to a dangerous level, taking the user to the risk of cardiac arrest and finally death. While others like toluene may damage chromosomes, injure eggs and sperm, lead to premature birth or death of foetus, cause birth defects in babies and long term problems after child birth. In some cases, if mother abuses inhalants during pregnancy, then the baby can also suffer from ill effects of inhalants[5,7,8].

Signs and symptoms of solvent abuse

Persons abusing solvents exhibit certain characteristics or signs. Abuse of solvents by someone can be recognized from these signs and symptoms. These may be in the form of physical symptoms or in the form of possession of paraphernalia with the abuser. Physical symptoms include chemical odour in breath and clothing, paint or chemical stains on face or hand, red pimply rash around the nose or mouth, excitability along with disorientation, giddiness, dazed appearance, slurred speech, nausea, loss of appetite, inattentiveness, sore tongue, irritability, depression, severe headache and lack of coordination. They may also possess paraphernalia used for getting solvents into the body like plastic bags or polyethenes with chemical stains or smell, empty product containers, rags, empty product containers, rags, clothes, cotton balls with chemical odour, empty balloons.

International and Indian Scenario of Inhalant Use

A US survey, combining data from 2002 to 2006, found that an annual average of 593,000 teens aged 12 to 17 had used inhalants for the first time in the year before they took the survey. In Nairobi, Kenya, an estimated 60,000 children live on the streets and almost all are addicted to some sort of inhalant. In the Pakistani city of Karachi there are an estimated 14,000 street kids, of whom 80% to 90% sniff glue or solvents. In the US, the 2006 National Survey on Drug Use and Health found that 1.1 million youths aged 12 to 17 had used inhalants in the past year.

According to NSDUH (National Survey on Drug Use and Health) 2014, by SAMHSA (Substance Abuse and Mental Health Services Administration), there has been a recent decline in inhalant abuse from (4.5-2.6) % since 2003-2012. Decline in inhalant use has also been recorded from (2011-2012) among males (3.1-2.1) % and females (3.5-3.1) % and among teenagers of age 13 years (3.7-2.6) %, 14 years (4.3-3.4) %, 15 years (3.4-2.6) %, 16 years (3.2-3.0) % and 17 years (2.7-2.2) %[9].

In India, inhalant use has existed since 1970. A study was conducted for ten years (2002-2011) at Drug De-addiction and Treatment Centre in North India to report the demographic and clinical profile among inhalant abusers of North India. It was found that all the inhalant abusers were males of mean age (18.9±4.12) years, mean age of onset of inhalant use was (16.3±4.22) years, mean education of (9.8±3.42) years and mean family income of (7676±7343.15) rupees per month. Majority of subjects were unmarried (89.7%), urban resident (79.3%), and from a nuclear family (78.2%) and half of the subjects were students (50.6%). The most common reason for initiating physical use of inhalants was curiosity. 85.1% of the inhalant abusers showed dependence[10]. The type of inhalants that has been commonly found among the adolescents and young adults attending de-addiction centres in India are typewriter correction fluids, solvents, gasoline and other volatile hydrocarbons.

Analysis of Volatile Solvents abused as Inhalants

Jain et al (1971) described about an extremely simple and rapid method of gas chromatography for the determination of methanol, ethanol, acetone, isopropanol and low boiling hydrocarbons simultaneously. Iso-butanol was used as internal standard. Hy-Fi 600-D gas chromatograph packed with 30% Carbowax M on acid washed 60/80 mesh Chromosorb W with FID detector was used. Nitrogen, hydrogen and oxygen at the rates of 35 ml/min, 28 ml/min and 100 ml/min respectively ware used as carrier gases. No extraction, distillation or sample preparation of the sample was performed. Quantitation was done based upon peak heights. It was observed that compounds have longer retention time at high column temperature, except for ethanol, that showed a shorter retention time at high column temperature. Varian Model 600D came out to be the ideal gas

chromatograph due to its moderate price and long injection port (that can retain residues from blood)[11].

Anthony et al (1978) described about HS-GC technique for the determination of paraldehyde, toluene and methylene chloride from three different fatal cases. In GC, column containing 0.2% Carbowax 1500 on Carbopack C was used with nitrogen as carrier gas at the rate of 15 ml/min. n-butanol was used as internal standard in toluene fatality case, n-propanol was used as internal standard in methylene chloride fatality case while n-pentanol was used as internal standard in paraldehyde fatality case. Two assays were developed for the analysis of volatile compounds using one internal standard. The first assay was run at 80°C and the second assay was run at 125°C. In both the assays, n-propanol was used as internal standard with retention time 3.0 minutes in first assay and 1.25 minutes in second assay and nitrogen was used as carrier gas at the flow rate of 15ml/min. Thus in cases of glue sniffing and industrial accidents involving solvents, second assay prove to be the most useful[12].

Balkon et al (1979) developed a screening method for the detection and measurement of trapped volatile and gaseous compounds using headspace gas chromatography-mass spectrometry (HS GC-MS). For GC analysis, HP5982 A gas chromatograph packed with Carbowax 1500 on Carbopack C column was used. Helium was used as carrier gas at a flow rate of 25ml/min. Bromochloromethane and 1,4-dichlorobutane were used as internal standards. Substantial and reproducible recoveries from HS concentration were observed. A wide range of volatile organic materials were easily isolated from biological materials. Thus it was found that up to 30m different gaseous and volatile toxic substances can be isolated and measured using given methodology at concentration of up to parts per million level[13].

Forester et al (1981) gave a review article about the analysis of volatile substances that are important from toxicological point of view, by GC. Gas chromatography with a number of different types of columns viz porapacks, carbowax, silicones, dodecyl phthalate and FFAP and with different detectors like FID, TCD, ECD was employed for the analysis of volatile substances. They described about the different methods like headspace (HS) analysis or extraction methods for the separation of analyte from the mixture. Extraction can be done either by solvent extraction or by purge and trap methods, out of which, the latter one came out to be more efficient with respect to 100% recovery of volatile substance from the sample. They observed that out of number of different columns used for separation of volatiles in gas chromatograph, Tenax 60/80 Carbopack B, found to be the optimal universal column for volatiles. Out of their study they concluded that GC, with Tenax column with HS or extraction as separation method, came out to be the most promising and comprehensive analytical technique for the analysis of volatile substances[14].

Bellanca et al (1982) analysed tissues and body fluids qualitatively and quantitatively for multiple volatile organic compounds employing GC and GC/MS. Analysis was done with HS/GC-FID (Nitrogen as carrier gas and with packed column with Carbopack B), HS/GC-FID (Helium as carrier gas and with fused silica capillary column) and with HS/GC-MS (Helium as carrier gas and 15% SP-1000 column Chromosorb W). Stock standards were prepared in water. They observed that use of 15% SP-1000 column gave better resolution and better sensitivity than Carbopack B. In spite of this, adequate separation of m and p-xylene was not obtained so they were grouped together in quantitative analysis. They concluded that liberation of compounds was maximal from solid tissues if they were first heated to 65°C for 40 minutes, cooled and then equilibrated at 37°C for 20 minutes.

Recoveries came out to be same from solid versus macerated tissues except in condition that more time was taken by the former than the latter for attaining equilibrium[15].

Ramsey et al (1982) analysed blood samples for the detection of volatile organic compounds used in solvent abuse cases by headspace gas chromatography-mass spectrometry (HS GC-MS). 1, 1, 2-trichloroethane and ethylbenzene were used as internal standards. Analysis was done on a Perkin-Elmer F17 gas chromatograph. Nitrogen was used as carrier gas at the flow rate of 30ml/min. FID, ECD and MS were used as detectors. It was observed and concluded that out of a number of different columns available, Carbopack C/ Carbowax 20 M column has proved to be the most promising for routine analysis of VOCs. Given method has proved reproducible and reliable for the analysis of volatile organic compounds including bromochlorodifluoromethane, n-butane, carbon tetrachloride, chlorobutanol, cryofluorane, dichlorodifluoromethane, ethyl acetate, halothane, iso-butane, isopropanol, isopropyl nitrate, methyl ethyl ketone, propane, tetrachloroethylene, toluene, 1,1,1-trichloroethane, 2,2,2-trichloroethanol, trichloroethylene and trichlorofluoromethane in solvent abuse cases[16].

Ramsey et al (1982) described about the role of laboratory in the investigation of solvent abuse. The easiest specimen to obtain from many patients is blood. Volatile compounds from blood can be detected, identified and measured using gas chromatographic method developed by the authors in which sample was first analysed by HS then separated by gas chromatograph using capillary column Carbowax 20M on Carbopack C and finally detected by detectors like FID and ECD. The method proved to be reproducible and reliable for routine analysis of solvents involved in solvent abuse cases. Another biological specimen, urine, also serves great purpose by providing additional useful information. For the determination of parent volatile compounds or their metabolites in urine, High Performance Liquid Chromatography (HPLC) remains the first method of choice. Methanol was used as internal standard. Column used ion the method was Spherisorb 5ODS. Sodium acetate trihydrate with glacial acetic acid was used as mobile phase. Vapour phase inferred spectrophotometry is preferred for the analysis of products abused. The spectra obtained from the vaporized sample can be regarded as the fingerprints of the product. The technique has the advantage over GC because in vapour phase IR reference compounds for comparison purpose are not necessary and some compounds that are difficult to analyse by GC, are readily identified by vapour phase infra-red spectrophotometry. Thus it is important to note that methods used for the analysis of volatile substance in solvent abuse cases should be specific, selective, sensitive and reproducible[17].

Takeichi et al (1986) described about a case reported of poisoning due to toluene during painting. A person while painting the bridge of a highway found dead at the bottom of the bridge pier. Blood sample of the descendent were analyzed by Shimadzu 8A gas chromatograph having glass capillary column packed with Chromosorb 101. Detection of separated samples was done using flame ionization detector. Nitrogen was used as the carrier gas and ethanol was used as the internal standard. It was observed that toluene concentrations were not found much enough to cause death but high enough to anesthetize the central nervous system. Investigation, autopsy findings and chemical examination of biological fluids using instrumental techniques revealed that general contusions, mainly cerebral contusion, due to a fall from a height were the main cause of death with acute sub-lethal toluene poisoning along with oxygen deficiency also[18].

Franke et al (1988) analyzed solvents and other volatile substances from biological material by GC using five different columns. GC and HS GC with five different columns and FID as detector was

used. Substances were determined by direct injection into gas chromatograph and by HS also after dissolving them in water and methanol (if not soluble in water alone). Hydroxy-n-alkanes were used as reference substances. They observed and concluded that GC system with Carbopack B column gave better results than system with Carbopack C showing good peak shapes with exception for glycols. GC with Tenax and Porapack Q served better for glycols. Combination of both the systems used in GC came out to be the best. It was also observed that screening took shorter time when temperature programming was used and use of 1-hydroxy-n-alkane as reference standard provided a good rational approach to systematic analysis of volatile substances[19].

Gill et al (1988) described storage and handling conditions of blood samples for its quantitative analysis for toluene by HS GC. HS GC was employed for the analysis using glass column packed with Carbopack C, Nitrogen as carrier gas and FID as detector. Ethyl benzene was used as internal standard. They observed that an interfering peak was often encountered in the gas chromatogram and that peak was identified as of n-hexanal. They also observed large differences in the stability of toluene concentration for blood samples stored under different types of storage containers. They concluded that blood samples should be stored in appropriate containers with minimum HS under cold conditions (4°C rather than at -20°C) to avoid the formation of n-hexanal as an interfering peak. Also, out of different containers available for the storage of blood samples, vials lined with glass, Teflon or aluminium foil from inside gave most satisfactory results. Vials with natural rubber stoppers were found unsuitable. These principles upon combination prove very useful for the analysis of toluene and other volatile substances[20].

Meulenbelt et al (1990) described about two cases involving acute intoxication from toluene. Two men found at the bottom of the pool by their colleagues while removing excess glue with the help of toluene from inside of a swimming pool. Blood samples from both the descendants were taken and analyzed by HS capillary GC with quadrupole GC-MS detector. Carb Erba 4130 gas chromatograph with CP WAX 57 CB capillary column was used. Hydrogen was used as carrier gas. From blood toluene concentration, it was clear that amount present of toluene had contributed to death of the two persons. The authors also observed that their method was able enough to detect and separate all the three isomers of cresol i.e. ortho, meta and para cresol, which were not separable and detectable by most published analytical procedures for determining cresols in urine[21].

Saker et al (1991) analyzed and provided information about toluene with respect to its losses during handling, its storage in different kinds of containers and stoppers at different temperature, volume of HS above the blood. GC with stainless steel column packed with Carbopack C and FID as detector was used with helium as carrier gas. n- propanol was used as internal standard and standard stock solution was prepared in glacial acetic acid and diluted with deionized water. Another GC system used was with HP-1 capillary column and ITD as detector. Toluene losses in three different blood standards stored at 24°, 4° and at -18°C were studied at intervals of 1, 2 and 7 weeks and 34 and 36 months. No interfering peaks as of hexane, hexanal or other common volatile solvent from normal blood samples were observed. They concluded that toluene can be analyzed in blood with initial concentration of 0.04mg/dl or greater even after 4 years after the specimen collection in a Vacutainer, if it is kept unopened and stored at 4°C or below while greater losses can be experienced if the container had been previously opened, even if stored at 4°C or below. Also, toluene should be dissolved in glacial acetic acid to reduce the losses of solvent encountered in preparation of blood standards[22].

Ashley et al (1992) analyzed 32 volatile compounds at parts per trillion level in 10 ml of human blood from large sample population using purge and trap GC-MS. Stock solutions were prepared in methanol. Analysis was done on HP 5890 gas chromatograph with DB-624 capillary column with helium as carrier gas and mass selective detector. Lower detection limits have been observed using magnetic sector instrument instead of a quadrupole spectrometer and doing purging of samples instead their HS analysis. Identities of analytes were confirmed using full scan data instead limited mass scan or selected ion recording. With these instrumental improvements and use of helium and purging distillation, it was possible to get lower detection limits. It also became possible to estimate normal baseline level of VOCs and a higher baseline level of VOCs in human blood and in cases of exposure[23].

Jones et al (1992) analyzed a case reported of death of a couple due to acetonitrile by headspace gas chromatography using two different columns. Cause of death was identified as ethanol by using two different columns. But, in actual inorganic cyanide, a toxic metabolite of acetonitrile was found as the reason behind the death and was mistakenly identified as ethanol. Two different materials used as column packing, one contained Carbopack B and other contained Carbopack C were used at different oven temperatures. Tertiary butyl alcohol was used as internal standard. Unknown volatile compounds were identified as ethanol. HS GC analysis was repeated after several days with three different stationary phases and n-propanol was used as the internal standard. Unknown volatile compounds were identified as acetonitrile using Carbowax 20M on Chromosorb was column packing material. Limited specificity of HS GC was observed when used for the analysis of volatiles despite two different column packing materials for GC[24].

Streete et al (1992) analyzed biological sample for the detection and identification of volatile substances by headspace gas chromatography split electron capture detector-flame ionization detection (HS GC ECD-FID). Samples were analyzed by HP 5890 gas chromatograph having SPB-1 fused silica capillary column and detection was done using FID-ECD. Helium was used as the carrier gas. Ethyl benzene and 1,1,2- trichloroethane were used as internal standards. With the use of column SPB-1, almost all the compounds were well separated except iso-butane-methanol and toluene-paraldehyde. No deterioration was observed in the peak shapes due to this column. Thus it was concluded that SPB-1 column was found superior than packed columns as used in previous studies for the headspace gas chromatographic analysis of volatile substances[25].

Astier et al (1993) gave a review article about chromatographic determinations of volatile solvents and their metabolites in urine samples for monitoring occupational exposure. A number of chromatographic methods have been developed and are used for the analysis of biological or non-biological matrix for the presence of volatile solvents. HS GC along with FID as detector has been used widely due to the applications of FID for the determination of aliphatic or aromatic solvents in biological matrices. ECD (Electron Capture Detector) came out to be useful for the determination of halogenated solvents or carboxylic metabolites in sub-microgram quantity. HS GC-MS has been found most satisfactory for the identification and determination of most volatile compounds. HPLC appeared to be a powerful tool for the determination of metabolites of volatile compounds in urine, accurately and easily. In planar chromatography, TLC and HPTLC are also used for the analysis of volatile substance due to their advantages in terms of simplicity, rapidity, low cost and simultaneous determination of several samples. In spite of so many chromatographic techniques available for the determination of volatile organic compounds, there exists no ideal method. But

the best methods do exists like GC-MS and HPLC-MS, for the determination of parent volatile solvents in blood and their metabolites in urine samples[26].

Jones et al (1994) described a new method for the determination of toluene in small quantities of whole blood using simple extraction procedure and GC-MS. GC with Helium as carrier gas, DB-1 capillary column using a thick film (1.0µm) of stationary phase and MS as detector was employed. Pentane was used as extraction solvent and toluene-d_8 as internal standard. They observed and concluded that the short, thick film capillary column provided rapid analysis of toluene. Precisions at lower concentrations can be improved by adding a small amount of internal standard. Use of internal standard of NMR solvent grade allowed simple, rapid and accurate determination of toluene in blood samples. Also using pentane as extraction solvent, low detection limits are obtained and sampling via extraction allows the determination of volatiles and semi-volatiles in a single analysis. They concluded that the method can be used as a tool for studies of toluene absorption and elimination[27].

Schuberth et al (1994) described a method for the analysis of volatile organic compounds using headspace gas chromatography ion trap detection (HS GC-ITD). Blood and other body fluids were taken as samples from autopsy cases. Samples were extracted using HP 19395A headspace auto sampler. Analysis was done using a HP 5890 gas chromatograph with DB-1 capillary column and MAT ITD800 ion trap detector. Identification of substances present in low quantities were confirmed using joint data from mass spectra searched in an online library and retention indices retrieved from literature. Volatile organic compounds were well separated using single column with a polar stationary phase, including methanol and acetaldehyde that were not separated by a polar phase. Thus the method was found to be sensitive and reliable for the analysis of volatile organic compounds involved in solvent abuse cases[28].

Schuberth et al (1996) developed a full evaporation headspace technique for the quantitative analysis of volatile organic compounds in blood and post-mortem tissues by using gas chromatography ion trap detection (GC-ITD). First, full evaporation of samples (of size less than 35mg) was done by keeping equilibration temperature at 130°C of HS apparatus for full evaporation in order to release all the volatile organic compounds from matrix, present in it. Then samples were analyzed using GC-ITD. HP5890 gas chromatograph with DB-1 capillary column was used. ITD was performed with MS run in full scan mode. Linear relationship between peak area and sample size was observed which indicated full evaporation conditions were met with reduced matrix effect. It was concluded that FET-GC-ITD almost met solid theory based method for quantitating volatile organic compounds in post-mortem samples. Thus FET-GC-ITD came out to be a sensitive, simple method for quantitating volatile organic compounds except methanol, in post-mortem samples, especially when the sample size is very less, with accuracy and precision[29].

Brewer et al (1997) analyzed non-routine volatiles in two traffic fatality cases by SPME and GC-MS. HS GC-FID, HS GC-MS and HS SPME GC-MS were employed. For HS GC-FID, stainless steel column packed with Carbopack B, t-butanol as internal standard and FID as detector was used. For HS SPME GC-MS, both polar (85-µm polyacrylate) and non-polar (100-µm polydilmethylsiloxane) films were used for SPME, DB-1 fused silica capillary column and Mass Selective Detector was used. It was observed that use of polar fibre improved the sensitivity for the analysis of ethanol, while non-polar fibre readily extracted straight chain alkanes. They concluded that out of all the methods described above, HS SPME GC-MS came out to be the most effective method for the confirmation of

volatiles with enhanced sensitivity offered by SPME. This procedure can prove invaluable in forensic toxicological investigations of volatile substances[30].

Barbera et al (1998) described about a case reported of poisoning due to benzene. A 41 years old man was found dead in a cargo ship that recently was unloaded of benzene. Blood and tissue samples were collected from the descendant's body. Samples were analyzed on DANI 3600 gas chromatograph with capillary column packed with GP 60/80 Carbopack B/5% Carbowax 20M and FID detector. Nitrogen was used as carrier gas at the rate of 30ml/min. High levels of benzene were found in blood, liver, kidneys and brain, which have resulted in death. The given method has proved sensitive, specific and reliable for the analysis of benzene in cases involving benzene poisoning or in cases of solvent abuse involving benzene[31].

Ojanpera et al (1998) developed a method for the analysis of Volatile Organic Compounds (VOCs) from blood samples taken from autopsies, by GC-FTIR using purge and trap as extraction method. Samples were first separated by GC the analyzed by FTIR and FID. Diethyl ketone was used as internal standard. Standard solutions were prepared in methanol. TEKMAR 3000 was used as purge and trap concentrator. GC of Perkin Elmer 8600 with capillary column Pora PLOT Q and FTIR spectrometer of PE Spectrum 2000 was used. Helium was used as carrier gas with a flow rate of 3.8ml/min. Better identification limits were observed than those in previous studies due to more sensitive spectrometer, modified extraction method and more appropriate design of GC-FTIR interface. Low molecular compounds were readily recognized using FTIR. Also, at the concentration level studied, optimal extraction conditions were not obtained for methanol and ethanol, as they showed irreproducible absorption behaviour in purge and trap concentrator. Thus it was concluded that although the method remained well suited for high throughput screening i.e. method is limited to the analysis of up to five samples per day (purge and trap as limiting factor), but it has proved an invaluable tool for the analysis of volatile substances[32].

Goulle et al (1999) described about a fatal case of dichloromethane (DCM) poisoning in which a 47 years old was found dead while measuring stock amount of DCM left in the tanks of DCM. Analysis of biological samples was done by HS GC-MS. HS GC-FID was also used for the quantitation of DCM in biological fluids and viscera. 1-propanol was used as internal standard. Standards were prepared in distilled water. VARIAN 3400 GC with DB-5MS capillary column and ITD 800 MS detector was used. Helium was used as carrier gas at the flow rate of 1ml/min. For quantitation of samples, GC-FID was employed. Column used in GC-FID was DB-WAX with helium as carrier gas at the flow rate of 2ml/min. Along with DCM, other volatile compounds viz. acetone, methanol, iso-propanol, ethanol were also observed by HS GC-FID that had contributed to death[33].

Hori et al (1999) analyzed blood of Wistar rats after exposing them to the vapours of methanol and toluene simultaneously. Analysis was done using HS GC-MS. Blood was sampled from the rats after 6 hours, 7.5 hours and 10 hours of exposure to toluene and methanol vapours. Metabolites of toluene and methanol i.e. hippuric acid and formic acid were also determined in the urine of rats after 6 hours, 24 hours and 48 hours of exposure. They observed that metabolism tends to be inhibited when organic solvents are exposed simultaneously, especially in cases of high vapour concentration. But they also observed that metabolism of toluene was enhanced by methanol. It was also observed that excretion rate become significantly lower during (6-24) hours and no significant difference was observed between (24-48) hours of exposure. Thus methanol vapour enhanced the toluene excretion. Thus results suggested that methanol enhanced the metabolic rate of toluene because biological half-life period of toluene in blood in simultaneous exposure was

shorter than that in the single exposure. On the other hand, no significant difference was there in biological half life time of methanol in blood and its metabolite's concentration formic acid in urine[34].

Dehon et al (2000) described a simple and sensitive method using HS SPME GC for the determination of tetrachloroethylene and trichloroethylene from tissue samples in a fatality case. HS SPME GC with ECD and MS detectors was used. GC systems were equipped with SPB-1 column coated with 100% polydimethylsiloxane and nitrogen was used as carrier gas. for biological fluids, chloroform was used as internal standard while for tissues, carbon tetrachloride was used as internal standard. They observed and concluded that poisoning by tetrachloroethylene found in biological fluids as well as tissues while poisoning by trichloroethylene only found in lipo-soluble tissues and HS SPME GC-MS came out to be the simple, precise and sensitive analytical technique for volatile solvents analysis[35].

El-Haj et al (2000) conducted a study for the detection of toluene and ethylbenzene by GC-MS in cases of volatile substance abuse. HS GC with HP 5MS column (cross linked 5% methyl phenyl silicone). Helium as carrier gas and MS as detector was used. Derivatization of sample was also performed using BSTFA (1% TMCS). It was observed that all the ten blood samples, 5 glue preparations, 3 dry cleaning products, 3 degreasing products, 3 paints and 3 paint strippers were all tested positive for toluene by GC screening and confirmed positive by the given method. They concluded that the given method proved highly selective for toluene and ethyl benzene and recommended for the confirmation of presence of the same in blood or glue or petroleum, or in solvent abuse cases. Also the problems with other detectors in GC like interference from other volatile substances in case of GC-FID, low sensitivity and interference of water from biological specimens in case of GC-FTIR have been overcome by the use of given method of HS-GC-MS[36].

Sharp et al (2001) analyzed biological fluids for volatile organic compounds by using headspace gas chromatography. Tertiary butyl alcohol was used as internal standard. VARIAN 3400 GC with TEKMAR 7050 HS auto sampler and FID as detector were used. Dual columns DB-1 and DB-WAX were used with helium as carrier gas. Analysis was also done using single column DB-624 in GC-FID with HS 40 headspace auto sampler. HS GC peaks were then identified by GC-MS employing HP 6890 GC with DB-1 and DB-WAX columns and HP 5973 MS detector. Comparison was also done between dual columns and DB-624 single column use. It was observed that with the use of dual columns, more than 40 compounds were identified and none of these compounds interfered with the identification of ethanol. It was concluded that for the confirmation of few compounds that did not give unique retention times on both columns, DB-624 column or HS GC-MS can be used. Thus this HS GC-MS method provided a reliable, comprehensive screening in forensic cases involving VOCs in biological and other fluids[37].

Sharp et al (2001) described about the stability of ether in blood samples in an ether assisted suicide case, in which an individual hanged himself while inhaling fumes from a mask (a cloth piece). Case samples were analyzed by two separate HS-GC systems, one with Varian 3400 GC-FID and another with Perkin Elmer Auto system XL GC-FID. No changes were observed in the concentrations of ether over the 8-week study period in stored blood vials. No loss of ether occurred from the blood samples during these 2 months of refrigerated storage. They concluded that ether remains stable in preserved, refrigerated blood for at least 2 months over a concentration range of (0.1-5.0)mg/ml, covering entire range of analgesia, anaesthesia and overdose and eliminates an important source

of uncertainty in cases of forensic samples because of their collection in preserved tubes, for interpretation of ether levels[38].

Bouche et al (2002) analyzed blood samples for the determination of n-propane, iso-butane and n-butane quantitatively by HS GC-MS in cases involving intoxication cases by inhalation of lighter fluid. Samples were analyzed using 6890 series gas chromatograph having CP-Select 624 fused silica capillary column and MS detector. Helium was used as carrier gas at a flow rate of 1ml/min. Stock solutions were prepared in t-butylmethylether and 1,1,2- trichlorotrifluoroethane was used as internal standard. They observed and concluded that environmental temperature for all practical work should be approximately 20°C. Internal standard chosen gave perfect retention time and typical mass spectrum. HS extraction came out to be very efficient with respect to its rapid sample processing capacity and suitability for direct quantitative determination of volatile substances in different matrices. The given method of GC with thick film stationary phase is sensitive, automated and fully validated in combination with MS detection whose sensitivity can be enhanced by using SIM mode for obvious identification and quantitation of n-propane, iso-butane and n-butane[39].

Collison et al (2002) analyzed a case reported of death by paint thinner. Biological samples as exhibits were analyzed for drugs, petroleum and the results were finally confirmed by GC-MS. Analysis was performed for alkaline drugs, weak acidic drugs, fire debris and for paint thinners. Chlorobutane was used as an extractant for alkaline drugs and ethyl acetate: hexane (75: 25) was used as an extractant for weak acidic drug analysis. α-methyl-α-propylsuccinimide + 7-(β-chloroethyl)theophylline) was used as internal standard for analysing weak acidic drugs. Ignitable liquids were adsorbed using ACS (Activated Charcoal Strips), and then ACS was eluted using carbon disulphide. After extraction GC-FID and GC-MS were used for the analysis of the same. For alkaline drug analysis GC with DB-5 (0.25µm) capillary column, helium as carrier gas and NPD and MS detectors were used. For weak acidic drugs, GC with DB-5 (1.2µm) capillary column, helium as carrier gas and FID and MS as detectors were used. For ignitable liquid analysis GC with DB-1 (0.25µm) capillary column, helium as carrier gas and FID and MS as detectors were used. No hydrocarbons from paint thinners were detected in vitreous samples. This may be due to insufficient sample size or due to the lower solubility of hydrocarbons in aqueous samples. But the data from liver and urine suggested that metabolites of various compounds of paint thinner were present and were a contributing factor to death[40].

Zuba et al (2002) determined acetaldehyde, acetone, methanol, ethanol, 1-propanol and 2-propanol in blood samples headspace solid-phase micro extraction gas chromatography. Analysis was performed on a Perkin Elmer Auto system gas chromatograph having DB-WAX capillary column and flame ionization detector. Hydrogen was used as the carrier gas. Salting-out agents were also applied in order to improve the extraction efficiency of analytes. Thus the given method of HS SPME GC was found rapid and simple showing good precision, wide working ranges for all analytes, good linearity, low detection and quantification and sensitive enough to detect volatile compounds that were present in blood in very low concentration. The method was thus recommended for forensic and clinical cases involving volatile substances[41].

Perbellini et al (2003) analyzed breath, blood, and urine samples for the determination of 1,3-butadiene, 2,5-dimethylfuran and benzene from environmental exposure, by using headspace gas chromatography. Samples were analyzed using HP 6890 gas chromatograph having Pora-PLOT Q capillary column and HP-5 mass selective detector. Deuterated benzene was used as the internal standard. 1,3-butadiene was identified in the blood samples of all the subjects under study while it

was never found in previous reported cases and it was found slightly higher in smokers. It was concluded that benzene and 1,3-butadiene are detectable and quantifiable in human breath, blood and urine samples due to their all-over presence in environment while 2,5-dimethylfuran was only detected in biological samples of smokers as it was not present in the environment[42].

Wasfi et al (2004) analyzed ethanol and commonly abused inhalants viz. dichloromethane, ethyl acetate, benzene, toluene, xylene qualitatively in biological fluids by static HS GC-MS. HS GC-FID along with HS GC-MS for confirmation was used. In case of HS GC-FID stainless steel column packed with Porapack Q, helium as carrier gas and FID as detector was used. While in case of HS GC-MS, DB-ALC 1 column was used along with helium as carrier gas and MS as detector. For ethanol analysis, n-propanol was used as internal standard and for inhalant analysis trichloroethane was used as internal standard. No interference was observed with the analyte by any endogenous materials present in blood. It was concluded that the given method came out to be specific, linear, sensitive, robust and reproducible for analysis of ethanol and inhalants[43].

Dixon et al (2005) analyzed rat blood and tissue samples for trichloroethylene, by using SPME-GC/MS. For trichloroethylene extraction by SPME, polydimethylsiloxane fibre was placed into the GC injection port at temperatures 50°C, 100°C and 200°C. Analysis was done on an Agilent 5890 gas chromatograph with Phenomenex ZB-5 capillary column. Helium was used as the carrier gas and Micromass Autospec Magnetic Sector was used as mass spectrometer. Several advantages were observed by using SPME-GC/MS instead traditional LLE-GC/MS, like it was able to measure trichloroethylene in blood by SPME-GC/MS, while it was not so for LLE-GC/MS. Less sample preparation and no use of organic solvent gave additional advantages of using SPME-GC/MS. It was also observed that heating of blood and liver samples prior to inserting fibre, improved the sensitivity because heating overcomes trichloroethylene binding to heme containing proteins in blood and liver. Thus it was concluded that for the analysis of trichloroethylene, SPME-GC/MS proved to be superior than LLE-GC/MS. Also SPME-GC/MS was found more sensitive than HS GC-MS due to sample pre-concentration by SPME-GC/MS[44].

Cox et al (2006) analyzed two cases reported of ether fatality by HS GC-MS from heart blood samples. GC-MS was performed with an RTX-1 capillary column with mass selective detector. Helium was used as carrier gas. MEK (Methyl ethyl ketone) was used as an internal standard in de-ionized water. All calibrator, control and sample preparations were performed in a 4°C refrigerator to preserve ether concentrations. Blood samples were tested for volatile substances (viz. methanol, ethanol, acetone, iso-propanol by HS GC), therapeutic and abused drugs (by GC-NPD) in both the cases. A peak close to acetone was observed in the blood volatile chromatogram and identified as of diethyl ether by GC-MS. They concluded that VSA (Volatile Substance Abuse) is quite common among children of age (12-17) and that cases can be analyzed using GC-MS qualitatively and quantitatively due to its specificity, sensitivity and reproducibility[45].

Hahn et al (2006) analyzed a case reported of a motor vehicle accident fatality due to 1,1-difluoroethane by GC-FID and GC-MS. In GC-FID, analysis was carried out on an HP 5890 GC with helium as carrier gas and FID as detector and in GC-MS, HP 6890/5973 gas chromatograph with helium as carrier gas and MS as detector was used. It was found that the tissues high in lipid content retained volatile compounds longer and attained comparatively higher volatile compound levels like from brain and liver. It was concluded from the study that the given method for the analysis of inhalants is efficient, provided there should be proper collection of multiple tissue samples in sealed containers within a short period of post-mortem[46].

Martinez et al (2006) analyzed a case reported of drug facilitated sexual assault using aromatic solvents. For analysis GC-NPD, GC-FID and GC-MS were used. GC-FID analysis was performed with and HP 5890 gas chromatograph, helium as carrier gas and mass selective detector while in GC-MS gas chromatograph used was HP 5971. Aromatic solvents were detected and quantified by GC-FID, while diazepam was detected and quantified by GC-NPD. All the detections were confirmed by GC-MS. It was observed from results that 3 solvents viz. toluene, benzene and xylene under study were similar in their spectrum of toxicity and well absorbed both after inhalation and dermal contact. Also this was the first reported DFSA (Drug Facilitated Sexual Assault) case until 2006, in which mixture of aromatic solvents was involved. They concluded that using these analytical techniques, good recovery, reproducibility, sensitivity and linearity can be obtained for volatile solvents and for analysis of mixture of aromatic solvents[47].

Avella et al (2008) described a method for the quantitation of DFE (1,1- Difluoroethane or Freon 152A) using HS GC-FID technique applying ideal gas law. GC was performed with Restek RTX-BAC-1 column and FID detector. Ideal gas law (PV= nRT) was applied for preparing standards in methanol as solvent. No interfering peaks of matrix were observed in DFE enriched blood, urine or brain specimens and not in blanks also. They concluded from the results that gas in equilibrium method eliminated the use of solvent based standards in favour of gaseous standards for the preparation of calibrator and hence eliminated solvent as variable and the given method is readily adaptable to a variety of volatile compounds[48].

Barua et al (2008) presented a method for the quantitative analysis of styrene and toluene in low concentrations in very small volumes of biological samples using headspace solid phase micro-extraction gas chromatography with flame ionization detector (HS SPME GC-FID). The chromatographic analysis was performed using an HP 6890 GC with fused silica capillary column and with FID as detector. Standard solutions were prepared in methanol as solvent. Five operating parameters viz. desorption time, adsorption time, adsorption temperature and sample volume were evaluated. It was observed that, with an increase in desorption time, the detection of styrene and toluene also increased, but it also caused undesirable peak broadening and tailing. It was also observed that the highest levels of toluene and styrene found in adipose tissue. No adverse effect on toluene and styrene recovery was observed up to a serum lipid content of 25% and 10% for toluene and styrene, respectively. They concluded that the given analytical technique can be applied to the estimation of styrene and toluene in small volumes as low as 10µl of biological fluids such as blood, serum an in tissue samples, which also reduced matrix effects[49].

Chakroun et al (2008) have studied exposure levels of benzene, toluene, ethyl benzene, xylenes, and n-hexane in 44 Tunisian adolescents and children suspected of volatile substance abuse. High performance liquid chromatography was used for the determination of urinary trans, trans-muconic acid (tt-MA), hippuric acid (HA, toluene metabolite), mandelic acid (MA), and methyl hippuric acids (MHA) while gas chromatography was used for the determination of urinary o-cresol (o-Cr, toluene metabolite) and 2,5-hexanedione (HD, hexane metabolite). Spot urine samples were collected from the subjects. O-Cr and HD samples were extracted using dichloromethane then analyzed by Agilent 6890 gas chromatograph having Chrompack- CP Sil 8 CB capillary column. t,t-MA samples were analyzed by HPLC having Waters Spherisorb ODS2 column and photodiode array detector. Acetic acid: methanol (90: 10) was used as the mobile phase in HPLC with a flow rate of 1ml/min. Mandelic acid (MA), hippuric acid (HA) and methyl hippuric acids (MHAs) were simultaneously analyzed by HPLC using Waters Symmetry C_{18} column with acetonitrile and

perchloric acid as mobile phase at the flow rate of 1.2ml/min. O-Cr has always been preferred to HA (both are metabolites of toluene) in solvent abuse cases because o-Cr is more specific than HA and not influenced by dietary intake. But in the study conducted, HA allowed easy detection of toluene inhalation abuse. Also, a great correlation was found between o-cresol and HA in the study. However, o-Cr still continues to be a sensitive biomarker of toluene. From results, it was also found that a large number of subjects were exposed to n-hexane and toluene than other volatile substances. Thus the method described allowed unique and concurrent sample preparation with high sensitivity and can be used in cases of solvent abuse for the detection and identification of volatile substances[50].

Liu et al (2008) analyzed seven sites of Pearl River Data region of China for the presence of VOCs. Whole air samples from seven different sites of Pearl River Data (PRD) region of China were collected using fused-silica lined stainless steel canister. (C_2-C_4) alkanes and alkenes were analyzed by GC-FID having non-polar capillary column. (C_5-C_{12}) hydrocarbons were analyzed by GC quadrupole MS. Helium was used as carrier gas in both the methods. A great difference was found in the chemical compositions of selected seven different sites, showing the heterogeneous distribution of VOCs sources in the region. Six out of seven sites were found to have presence of large percentage of alkanes. The seventh site was found to be dominated by a heavy percentage of aromatics[51].

Netto et al (2008) analyzed unaltered urine samples of workers of car repair shops for the presence of toluene using headspace solid phase micro extraction gas chromatography with flame ionization detector (HS SPME GC-FID). Stock standard solutions were prepared in methanol. In SPME, two different extraction phases of polydimethylsiloxane (PDMS) and Caboxen PDMS were used. Gas chromatograph 1000 GC with HP-Innowax capillary column and FID detector was used. Nitrogen was used as carrier gas at the flow rate of 3ml/min. It was observed that HS GC-FID alone was not sensitive enough to detect solvents in unaltered biological samples but on coupling it with SPME as extracting technique, allowed detection of solvents in unaltered biological samples. Carboxen PDMS came out to be better than PDMS with respect to chromatographic efficiency, limits of quantification, precision and repeatability[52].

Smith et al (2013) presented a method for the analysis of propane using a validated HS GC-MS method. Gas chromatographic analysis was performed with 6890 GC and with a specially designed column made up of two capillary columns set in parallel viz. a PLOT capillary column and a Poraband Q with helium as carrier gas and MS as detector. Deturated propane was used as internal standard which was produced by the reaction of propylmagnesium chloride and deuterated water. No interfering peaks were observed in the chromatogram and good linearity was obtained. So it was concluded that the method proposed is very selective and sensitive for the quantification of propane even from poor quality samples and thus the method provided reliable, accurate and repeatable results[53].

Tisicone et al (2013) analyzed volatiles like acetone, toluene, methyl ethyl ketone (MEK) etc. from human blood and urine by HS GC-FID and HS GC-MS. Analysis was performed with 7890 GC, DB-ALC1 capillary column and with FID and MS as detectors. Helium was used as carrier gas. n-propanol was used as internal standard and the standards were prepared in three matrices (deionized water, human urine and human blood). Toluene and MEK were prepared in methanol. No matrix interferences were observed in the results. So they concluded that the proposed method

is robust for the identification and quantification of a wide variety of volatiles by dual detection with FID and MS from biological samples[54].

Tong et al (2015) analyzed VOCs from the ambient air of a paper mill by gas chromatography-mass spectrometry (GC-MS). For samples of air were collected in glass tubes containing activated charcoal granules, using air sampler. Then extraction of substances (by desorption operations) in activated carbon tubes were extracted using two solvents viz. carbon disulphide and dichloromethane. Extracted samples were then analyzed by GC, using DB-5MS capillary column. Helium was used as carrier gas. Carbon disulphide was found favourable solvent for non-polar compounds while dichloromethane remained favourable solvent for polar compounds. The main VOCs that were identified in the air samples of paper mill were of alkanes, phenols, esters, ethers and benzene series[55].

Coppe et al (2016) developed and validated an analytical method of HS GC-MS for the qualitative and quantitative analysis of solvents used as inhalants of abuse, from oral fluid. Working solutions were prepared in methanol at a concentration of 2000mg/l. Samples were analyzed by a 5975 GC with HS automatic injector and 7890 A MS detector. Two columns ZB-BAC 1 Zebron and Carbowax were used with helium as carrier gas at the flow rate of 1.4ml/min. method was validated according to the recommendations of USA Food and Drug Administration (FDA) on the basis of parameters selectivity, matrix effects, residual effect, linearity, accuracy, precision, limits and detection of quantification and stability. Method was found more specific when all the solvents were analyzed by Carbowax column. Compounds with close retention times gave correct results when analyzed by GC-MS. Thus sensitivity was increased with GC-MS instead GC-FID as structural information was additionally provided by GC-MS and not by GC-FID. In conclusion, oral fluid proved to be a promising biological matrix for the analysis of inhalants in toxicological cases. The validated method proved to be fast and sensitive for monitoring inhalants of abuse in forensic and clinical cases for qualitative analysis of chloroethane, n-propanol, toluene, xylene, CFCs and for the quantitative analysis of ethanol, diethyl ether, dichloromethane, chloroform, ethyl acetate and n-butanol etc[56].

CONCLUSION

The present review was directed in hopes to summarize the work done in field of various methods used to detect and analyse various inhalants using Gas Chromatography and other related hyphenated techniques. From this review it may be concluded that GC-MS is the best instrument for detection, identification and analysis of various inhalants. Major work has been done in field of environmental and industrial toxicology and it is very important to evaluate this problem from forensic point of view. Moreover, more sensitive methods must be designed in order for the analysis of trace amounts of inhalants available from body fluids of victims and various substrates used by the abuser for inhalation of such compounds.

CONFLICT OF INTEREST

None to declare.

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What's New in This paper

Overview What is already known on the subject Inhalant abuse is a very prominent and challenging issue all over the world. Individual components abused as inhalants have been analysed and detected in various studies using gas chromatography.

What this study adds

It is very important to select an appropriate method while using Gas chromatography – mass spectrometry (GC-MS) for the analysis of inhalants. There are variety of compounds which can be abused as inhalants. In this review article, an attempt has been made to summarize the work done so far in field of detection and analysis of different compounds used as inhalants. This will help in enriching our knowledge of methods of analysis of inhalants along with their advantages and limitations.

Suggestions for further development

With new advancements in analytical instruments, it is necessary to constantly update our knowledge to select an appropriate method for the analysis of inhalants.