Review

Determination of volatile substances in biological samples by headspace gas chromatography

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Abstract

This review surveys headspace gas chromatographic (HS-GC) methods as used in the determination of volatile substances in body fluids and tissues from the viewpoints of the design of HS-GC instrumentation, partition coefficients and matrix effects of biological samples, additives to the liquid phase and stabilities of volatile substances in biological samples. It includes extensive tables that detail published static HS-GC methods that have been applied in forensic, clinical and environmental analyses.

Contents

1. Introduction

Volatile substances are defined as those which vaporize easily when warmed. The detection and determination of volatile toxic substances is very important in forensic science [1], e.g., in deducing causes of death and in the investigation of driving offences involving alcohol. Within clinical practice, they include the routine monitoring of industrial workers for exposure to hazardous compounds and the detection of organic endogenous metabolites in certain clinical diagnoses. In anaesthesiology, the solubility of inhaled anaesthetics in blood and tissues has been determined. In the environmental sciences, certain volatile compounds in biological samples have been measured as a means of monitoring environmental pollution. The headspace gas chromatographic (HS-GC) method was originally developed for use in anaesthesiology [2], toxicology [3,4] and food science [5] research. This method is now a powerful tool for analyses for volatile substances in a wide range of research and practical applications; including forensic science, clinical chemistry, anaesthesiology, environmental science, food science, polymer science and basic physico-chemistry (i.e., in the determination of physical constants).

There are several monographs [6–8] and reviews [9–14] covering the HS-GC method. However, there has never been an extensive review of the HS-GC method as applied to biological analyses. This paper, therefore, centres on the static HS-GC method and its use in the determination of volatile substances in body fluids and tissues in relation to forensic science, and in addition briefly discusses its applications in clinical chemistry and environmental science. Obviously, many types of body fluids and tissues from humans and animals are the objects of analysis and the complexity of these samples alone presents a unique challenge to the researcher. Representative liquid samples include blood, urine, vitreous humor and milk. Solid biological samples include tissues such as the liver, lung, brain, kidney, muscle and various types of fatty tissue. In addition, clinical samples requiring analysis may originate from patients with a wide range of conditions including healthy, diseased, burned or deceased (i.e., forensic examination). The analysis of breath is excluded from this review as it is considered a gaseous sample. In addition, the analysis of other biological samples, along with foods and beverages, is also excluded as these subjects have been reviewed elsewhere [15].

The aim of this paper is to provide a critical review of the HS-GC method as applied to the determination of volatile substances in body fluids and tissues. In addition, it provides reference methods and special precautions necessary for toxicologists engaged in the establishment of a practical HS-GC method. An extensive review of the literature concerning the fundamentals of HS-GC methodology is presented in terms of application to biological sampled. This literature review is presented in tables that are organized according to the chemical class of the analyte.

2. Gas chromatographic method for the determination of volatile substances

The determination of the level of volatile substances in samples has been accomplished through the use of titrimetric, spectrophotometric or chromatographic methods. The titrimetric and spectrophotometric techniques lack specificity and usually do not provide sufficient sensitivity. In contrast, the detection of volatile substances using gas chromatography (GC) is both qualitative (with respect to retention time) and quantitative (with respect to peak signal intensity). As a result, GC analysis is generally used for these types of determinations [16]. The three methods used for the preparation of samples for

GC analysis are as follows: (1) solvent extraction (SE), (2) direct aqueous injection (DAI) and (3) direct injection of the headspace volume (HS). The SE method involves the extraction of the volatile analyte(s) of interest from the sample using an organic solvent. An aliquot of this extract is then introduced into the GC column. This method is used extensively for the pretreatment of non-volatile analytes, allows for the concentration of analytes, thereby increasing sensitivity, and is also effective for volatile substances with high boiling points. However, the SE method has several disadvantages, including a time-consuming and laborious extraction procedure. In addition, precise determination of the analyte(s) cannot be expected, especially for substances with low boiling points. The solvent peak itself can interfere with the elution of analytes and many non-volatile components can be co-extracted, thus interfering with peak detection. This method requires ultra-high-purity organic solvents because the sensitivity is limited by background signals which may occur due to any trace contaminants.

The second technique, DAI, is a GC injection method in which minute amounts of the sample are injected directly into the GC column. The advantages of this procedure include a rapid and simple preparation of the sample. However, contamination of the GC injector and GC column with sample matrix is a serious disadvantage and necessitates frequent maintenance of the injector port. Also, attaining a stable baseline is often difficult and this method does not provide high sensitivity owing to the limited sample volume. In addition, water vapour can also disturb the chromatographic separation. For these reasons, the DAI method is now used almost exclusively for the analysis of aqueous samples only.

The HS technique was developed as a viable alternative to the above two methods. This method involves the equilibration of volatile analytes between a lower liquid (or solid) phase and an upper gaseous phase, with only the gaseous phase sampled. This method has significant advantages over the previous two. For example, gaseous sampling avoids any contamination of non-volatile components which may be found in the sample matrix or in an organic solvent.

The HS-GC technique can be divided into the two following categories: static (equilibrium) HS and dynamic (non-equilibrium) HS, also referred to as the "purge and trap" method. The static HS method involves the equilibration of volatile analyte within the sample with the vapour phase at a defined temperature. The vapour phase containing the analyte is then injected into the GC column in a closed system. This method is simple, minimizes the number of artifacts during analysis, can provide precise quantification and can effectively measure volatile substances with relatively low water solubility. A drawback, however, is that it is considerably less sensitive when analysing volatile substances with high water solubility. The second technique, dynamic HS, involves passing a carrier gas over the sample for a specified period of time and trapping the analyte in a cryogenic or adsorbent trap. The concentrated analyte is then introduced using pulsed heating. Several variations of this technique have been developed apart from the simple dynamic HS method (the inert gas is purged over a liquid sample), including through-solution gas stripping (the inert gas is bubbled into the liquid phase) and thermal desorption (the sample is retarded and heated on a solid support while the gas is purged). In general, the dynamic HS method is effective for the measurement of volatile substances of moderate to high water solubility. In addition, this method offers increased sensitivity when compared with static HS, DAI and SE methods owing to the concentration after trapping of the volatile analyte. The dynamic HS technique, however, is difficult and costly owing to the complex instrumentation necessary. Precise quantification of analytes suffers from incomplete recovery after the purging, trapping and desorption steps. The dynamic HS method may also result in artificats due to impurities present in the purging gas. There are many commercially available automated dynamic HS machines.

3. General

3.1. Theory concerning static HS-GC method

According to Raoult's law, in a closed system where a volatile component is in thermodynamic equilibrium between the liquid phase and the gaseous phase, the following equation holds:

$$P_i = P_i^0 x_i \gamma_i \tag{1}$$

where P_i = partial vapour pressure of the volatile component i, P_i^0 = vapour pressure of the pure component i, x_i = mole fraction of component i in solution and γ_i = activity coefficient of component i in solution. If the components do not interact, $\gamma_i = 1$. In contrast, non-ideal solutions can show positive or negative deviations from Raoult's law. The extent of deviation depends on the types of volatile substances analysed and on the solvent [17]. The value of γ_i is constant when the concentration of volatile substance in the liquid phase is below 1% [18], and Raoult's law can be simplified to Henry's law. The concentration of analyte in the gaseous phase is then proportional to that in the liquid phase:

$$C_L / C_G = k \tag{2}$$

Considering the mass balance of volatile analyte between two phases, the following equation is valid, as illustrated in Fig. 1:

$$C_L^0 V_L = C_L V_L + C_G V_G \tag{3}$$

where C_L^0 represents the analyte concentration in the liquid phase prior to HS equilibrium, C_L and C_G represent the concentrations in the liquid and gaseous phases, respectively, after equilibrium and V_L and V_G represent the volumes of the

liquid and gaseous phases. The partition coefficient (k) and phase ratio (β) are defined as C_L / C_G and V_G / V_L, respectively. Eq. 3 can be transformed as follows:

$$C_G = C_L^0 / (k + \beta) \tag{4}$$

Eq. 4 demonstrates that the GC sensitivity is related to C_G and is dependent on k, β and C_L^0. The k value is constant with a dilute analyte [18], constant temperature and identical liquid matrix. The k value decreases with increase in temperature and changes according to the character of the matrix. The principles and applications of HS-GC methods have been reviewed elsewhere [10,18].

3.2. System and instrumentation

Originally the HS-GC technique was applied to the measurement of the solubility of anaesthetics [2,19–21] and to the analysis of gases [3,4,22–24], alcohols [25,26] and solvents [27,28] in biological samples. The instrumentation and procedures were simple, but there were many drawbacks, a major one being the lack of precise quantification. Through improved instrumentation and further theoretical developments, the HS-GC method has been refined to overcome previous drawbacks and can now provide accurate quantitative analyses.

The HS-GC system consists of an HS element (pretreatment) and a GC element (measurement). The HS element can either be manual or automated and consists of a vaporization container where equilibrium is obtained, a heating device which keeps the HS container at a constant temperature and an injection device which transfers the vapour phase from the HS container into the GC column. Initially, the HS element consisted of a glass vial sealed with a rubber septum with transfer through a gas-tight syringe [2,4,19,20,25–27,29]. In general, a hard glass vial is recommended as the container. The container is sealed by either a screw-cap or a crimped cap. A septum is necessary for sealing the container, maintaining the physical integrity of the sample vial with increase in internal pressure, and allowing for sample withdrawal via
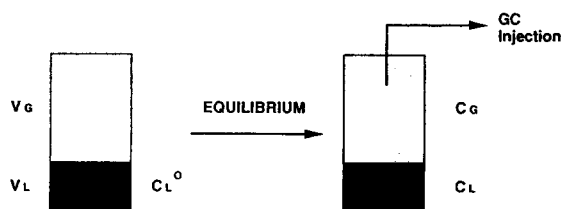

Fig. 1. Schematic diagram of static headspace gas chromatography. For symbols, see text.

a syringe. Originally butyl rubber or silicone rubber septa were used but were found to introduce serious errors due to adsorption of the analyte on these materials, resulting in a time-dependent decrease in vapour concentration [30]. Currently, septa are coated with either polytetrafluoroethylene (PTFE; Teflon) or aluminium foil to prevent adsorption. All components of the HS container and injection equipment which contact the sample must be composed of chemically inert materials [31].

Even stir bars made of PTFE have been found to adsorb benzene and toluene [32]. Because of the limitations on sample volume and vapour volumes which can be injected into the GC system, HS containers with capacities between 5 and 25 ml have frequently been used. Manual injection with gas-tight syringes is the transfer method of choice. Unless special pressure corrections are employed [33,34], the use of pressure-lock-type syringes is recommended to prevent the loss of sample vapour. The syringe should be prewarmed to a temperature higher than the HS temperature in order to prevent analyte or water condensation. Contamination of the syringes is a major concern as it can lead to non-quantitative results [35]. It is possible to minimize contamination by cleaning the syringe with solvent and drying under vacuum at high temperature between analyses. Septumless injection equipment has also been devised to prevent cross-contamination [36–40]. A further disadvantage of the manual injection method is its low reproducibility due to sampling inaccuracies, and therefore automated injection instruments have been developed and are now commercially available. At present, there are two types of automated HS samplers: the sampling loop type [41] and the pneumatic control type [42–44]. The sampling loop type is a system where the HS vial is pressurized by a GC carrier gas for a constant time. At a given point, the vapour phase is released to atmospheric pressure, fills the sample loop and is subsequently introduced into the GC column. The pneumatic control type is a system whereby the HS container is pressurized by a GC carrier gas for a constant time and the vapour phase is injected

into the GC column under pneumatic control at a given time. A stainless-steel transfer line of the HS sampler has been found to introduce ghosting problems, and it has been overcome by lining the transfer tube with deactivated fused silica [45].

Other HS containers that have been used are glass syringes, facilitating easy introduction of the HS vapour phase [2,3,21,28,46–50]. In addition, specially designed devices have also been used where carrier gas is employed either to sweep vapour over the HS phase or to by-pass it by a switching valve [40,51–54].

The ratio of the gaseous phase to liquid phase (β) and the injection volume will both vary depending on the HS-GC analyses. In general, phase ratios for most common analytes are 5–20. Injection volumes that offer appropriate sensitivity and peak resolutions should be low enough that they do not disturb the HS equilibrium. Usually, approximately 10% of the gaseous phase (0.05–2 ml) is introduced into the GC system.

Because the GC aspect is beyond the scope of this review, only the most important GC advances developed for increasing the sensitivity and resolution and shortening the analysis time of HS-GC are considered, namely the use of capillary columns [55–60] and cryofocusing systems [44,56,57,61–64].

3.3. Partition coefficients

Static HS-GC determination of volatile analyte requires the determination of a partition coefficient. Because matrix effects are unpredictable, special consideration must be given to the effects that the matrix may have on the determination of partition coefficients.

3.3.1. Data on partition coefficients

Published data on partition coefficients are widely available. In the physico-chemical meaning, the partition coefficient is equal to the Ostwald solubility coefficient, and so may be calculated from available physical solubility data (as in the case of priority pollutants) [65]. In anaesthesiology, the partition coefficients of

anaesthetics in various liquid phases such as water, oil and biological fluids are needed in order to obtain pharmacokinetic information such as the anaesthetic uptake and body distribution. These values are published in comprehensive tables [66–69]. Partition coefficients are also necessary to develop physiologically based pharmacokinetic models used to access uptake, distribution, metabolism and elimination of volatile pollutants [70–75]. Harger et al. [76] reported partition coefficients for ethanol in biological fluids by using spectrophotometric measurement of the vapor phase. Aqueous phase partition coefficients are available for components of food [77], organophosphorus compounds [78] and solvents [79] as determined by static HS-GC methods.

Partition coefficients are based on many parameters, e.g., polarity, volatility and molecular mass. In general, partition coefficients of volatile substances in water increase with increasing water solubility and therefore the following order among chemical classes is observed: aromatics > cycloalkanes > alkenes > alkanes. Within each chemical class a decrease in partition coefficient occurs as the molecular mass increases [77].

Partition coefficients also differ according to the properties of the liquid phase [80,81]. Partition coefficients in biological samples are significantly influenced by the matrix components. Anaesthesiological and toxicological studies have indicated that partition coefficients of untested volatile substances within complex biological samples can be predicted based on a knowledge of the oil and saline partition coefficients and a knowledge of the relative lipophilicity and hydrophilicity of various biological fluids and tissues [72]. The relative ranking of increasing lipid character and decreasing aqueous character based on these coefficients is water < urine (body fluids) < blood < muscle < liver < fat tissue < oil. The partition coefficients for aromatic hydrocarbons in blood are closely correlated with the product of the partition coefficients for these compounds in both water and oil, but coefficients for ketones are more dependent on the coefficients determined in water [72]. For

congeners of alkylbenzenes and chlorinated aliphatic hydrocarbons, those with a short alkyl chain are more soluble in water but less soluble in blood and olive oil than those with a long chain [72,73]. Although the partition coefficient can be regarded mainly as a simple function of lipid content, it can also be affected by other components within biological samples, such as water, salts, proteins and carbohydrates. Diethyl ether represents the only known exception to the rule that compounds show greater solubility in protein solutions than in water [67]. Partition coefficients are not identical for all types of protein or lipids within a liquid matrix and any variation in the composition of the matrix can alter the partition coefficient [67].

3.3.2. Effect of salt addition

The solubility of many non-electrolytes decreases in the presence of salts. This phenomenon, known as the "salting-out effect", produces an increased activity coefficient (γ) with increasing salt concentration. In contrast, a decrease in salt concentration produces a "salting-in effect". The degree of change in k values is correlated with the concentration and type of salt and volatile substance. For example, the chemical properties of both the salt ion and the volatile analyte, presumably charge density, size and resultant effects on bulk water structure, affect this behaviour. Theoretical predictions of the magnitude of this effect may be made and are based mainly on electrostatic theory [82]. The addition of salt causes a decrease in hydrogen bonding between analyte and water as free water is sequestered by the tight hydration shell surrounding the salt ion. For example, partition coefficients of ketones, aldehydes, alcohols [83] and ethyl acetate [84] are all decreased by the addition of sodium sulphate. A salting-out effect is also observed on addition of sugar compounds [85].

3.3.3. Matrix effects of biological samples

Biological samples consist of complicated components such as water, proteins, lipids, saccharides and salts, all in varying concentrations. Biological samples also differ in chemical compo-

sition, specimen type, specimen origin (species and individual) and clinical conditions. For example, blood samples can differ in haematocrit value, haemoglobin concentration and lipid content. Frequently encountered complications include alterations in the condition of the sample through post-mortem changes such as putrefaction, or the contamination of blood with other body fluids. These considerations make the preparation of a matrix identical with that in the unknown samples, for use in quantification and calibration, very difficult. Forensic and clinical toxicologists should therefore be aware of the limitations of quantitative HS-GC in the analysis of biological samples. Dilution of the biological samples with water can reduce the influence of complex matrix effects.

An association phenomenon between volatile analyte and matrix components may contribute to decreased partitioning [34]. For example, the slopes of the calibration graphs for tetrachloromethane [86] and ketones [87] were shown to differ among different specimens. In addition, lipid components of blood were found to retain halothane to varying extents and thereby resulted in poor correlation across samples, differences in partition coefficients and difficulty in obtaining accurate calibration graphs [88]. Fatty components in biological samples were also found to affect the vaporization of organic solvents [89] and ethanol [90]. The variable fat content of liver, as a consequence of the state of alcohol consumption, might affect the relative partition coefficient of ethanol, other alcohols or an internal standard [91]. Blood samples containing higher than normal cholesterol and triglyceride levels resulted in larger k values for halothane [88]. No clear correlation can be drawn between partition coefficients of ethanol and the following blood constituents; haemoglobin, albumin, total protein, urea, electrolyte and glucose levels [91]. Different matrix components within biological samples had differing effects on determinations of partition coefficients for analyte and internal standard pairs in the determination of ethanol [92] and cyanide [33], again demonstrating difficulties inherent in the internal standard method.

Despite unpredictable matrix effects in unknown samples, the partition coefficients of many volatile substances in urine and the other body fluids are similar to those in water. Differences in urine density resulted in negligible matrix effects on the partition coefficients for trichloroethanol (TCE) and the methyl ester of trichloroacetic acid (TCA) [93,94]. In addition, matrix effects have been examined for methanethiol in urine [95].

The distribution of ethanol between plasma and whole blood should be roughly the same as that of water [96]. Ethanol, a highly soluble volatile substance, equilibrates freely between the water component of biological samples and the overlying gaseous phase. Ethanol does not significantly bind to any endogeneous constituents of plasma and whole blood. The partition coefficient of ethanol was found to be correlated to the water content of the liquid phase in biological samples, and water content value (moisture ratio) is useful in the prediction of partition coefficients [97] or in the correction of results [98] related to ethanol content. The concentration of acetone in plasma was higher than that in an equal volume of whole blood, which probably reflects the greater proportion of water in plasma as compared with whole blood [91].

3.3.4. Effect of temperature

To facilitate the determination of trace amounts of a volatile substance, it is possible to alter the ratio of volatile substances in an HS container by altering the equilibration temperature. In general, raising the HS temperature decreases the partition coefficients [39,80], with a linear relationship between ln k and the reciprocal of temperature [68,78,99]. The slope of this plot differs with the type of volatile substance. The more soluble the volatile substance, the greater is the change in solubility with a given temperature change [67,68]. Raising the HS temperature is a popular technique for increasing vapour pressure and facilitating the sensitive detection of volatile analytes. However, raising the temperature can increase artifacts due to the enhanced chemical reaction in the bio-

logical samples. High temperatures are necessary for the HS-GC determination of certain volatile substances that have slow HS equilibrium values due to slow diffusion within certain biological materials.

3.4. Effect of various additives

To facilitate the HS-GC determination of volatile analytes, specific compounds can be added to the samples prior to analysis. Some additives are introduced immediately after sample retrieval, e.g., EDTA to prevent blood coagulation and sodium fluoride to prevent the growth of microorganisms. As discussed above, salts can also be added to produce a salting-out effect. The addition of salt has historically been used in the determination of ethanol [5,100], and extensive improvements have been made in this area. The particular salts to be added should be selected based on their specific salting-out effect and the accuracy and precision desired. For example, ammonium sulphate has been used for both salting-out and to prevent the oxidation of ethanol [101]. Many types of salts have been investigated for the optimized salting-out of ethyl acetate [102]. In addition, acid has been used to convert cyanide into protonated, volatile hydrogen cyanide. Alkaline salts have been added to convert amines into their volatile free bases [103–106]. Sulphuric acid has been employed to prevent the degradation of ethyl acetate by hydrolytic enzymes in tissue [107]. Anhydrous copper(II) sulphate has been used for the deproteination and dehydration (salting-out) of samples in the determination of ethanol [53]. Sodium fluoride was also found to be effective for the storage and salting-out of ethanol containing samples [108]. Formic acid has been used to overcome ghosting problems in the determination of short-chain fatty acids [45]. Simultaneous addition of sodium nitrite and sodium fluoride prevented the oxidation of ethanol and bacterial growth and resulted in a salting-out effect [109]. Sodium chloride has been used to denature hydrolytic enzymes and to produce a salting-out effect in the determination of methyl methacrylate levels in blood [110]. A combined

treatment with potassium hydroxide, olive oil and Triton X-100 has been used to solubilize tissue samples in the determination of toluene [89]. In general, the addition of olive oil has been found to reduce the matrix effect of tissue samples owing to its ability to minimize differences between the fat content of standard and unknown samples [89]. After conversion to volatile ester derivatives, non-volatile oxidized metabolites of alcohols have been detected by HS-GC [111–117]. Sodium nitrite and sulphuric acid were used for the conversion of ethanol into nitrite ester [118]. Likewise, non-volatile cationic acetylcholine has been detected by bacterial conversion to volatile trimethylamine [103]. Trichloroethanol and phenol conjugates have been converted into their respective free forms by β-glucuronidase [119,120] and sulphatase [121] treatment. Hydrolytic enzymes and derivatizing reagents were also used to convert conjugates of TCE and TCA into the volatile TCE and ester derivatives, respectively [120]. Bromide ion levels have been measured following conversion of bromoform by citric acid, potassium permanganate, manganese dioxide and sulphuric acid [122]. Sulphuric acid and ammonium sulphate have been used to convert TCA into its volatile ester and to produce a salting-out effect in the determinations of TCA and trichloroethanol [93,94,123,124]. For esters such as ethyl and methyl acetates, the addition of sodium fluoride has been recommended in order to minimize esterase activity [59].

A further reason for adding a concentrated salt solution is its ability to minimize the matrix effects of unknown samples [1]. This consequence was first reported in the determination of ethanol levels in various biological fluids [100]. Previous differences found in the partition coefficients between specimens can be alleviated by the addition of a salting-out reagent [125]. Recently, however, detailed studies that directly investigated the ability of salt saturation to minimize matrix effects have been published [92,126]. These studies demonstrated that discrepancies in the partition coefficients for ethanol in water and blood were magnified in the presence of saturating salt [92]. The matrix effect

was abolished only after substantial dilution of blood specimens [97]. Even with the introduction of a salting-out agent, the type of biological specimen still influences the partitioning of alcohols between liquid and HS vapour phases [126]. For example, the use of saturated sodium chloride did not result in equal activity coefficients across specimens, nor was the magnitude of the effect the same for ethanol and the common internal standard used in its determination [126].

3.5. Mode of quantification

One can determine the initial liquid phase concentration of a volatile analyte through the measurement of the equilibrated vapour phase concentration given a knowledge of the partition coefficients, or by using standard calibration methods. Because of its ease and simplicity, absolute calibration and internal standard methods have been the most commonly used for HS-GC analysis. Unless an automated sampling device is used, the accuracy and reproducibility of absolute calibration method may be low owing to sampling inaccuracy. The internal standard (I.S.) method does not suffer from inaccuracies due to sampling error. Experimental errors due to evaporation during storage and tissue homogenization will be minimized if the I.S. is added to the sample immediately after removal from the body. These calibration methods have additional limitations if the properties of the sample matrix are unknown, thereby prohibiting precise quantification. These limitations are based on differing lipid concentrations and other components within biological samples which influence the vapour phase concentration and can result in unreliable calibration graphs [88–90]. Watts and McDonald [92] have reported suitable internal standards for ethanol determination that take into account matrix differences. Drozd *et al.* [127] have shown theoretically that the I.S. method will produce systematic errors. Ideal internal standards are stable isotope-labelled compounds when mass spectrometric detection is possible [125,128–130].

Recently, to cancel the matrix effect of un-

known samples, three different methods have been introduced into HS-GC analyses: standard addition method, multiple HS extraction method and full evaporation technique [131]. The standard addition method involves the preparation of standard addition (SA) samples spiked with known amounts of the analyte substance of interest followed by HS-GC analysis of the unknown and SA samples. The original concentration of analyte in the sample is calculated by comparison of the GC peak intensities with and without the standard addition. The added amount of standard analyte does not change the values of V_G and V_L and also does not change the thermodynamic properties of the solution. Therefore, the partition coefficient of the analyte does not change even in a complex matrix. The main constraint of this method is that the SA samples and unknown samples must be analysed under identical conditions. Theoretical investigations of this method in aqueous solutions have been reported [61,132–134]. Koupil *et al.* [88] reported the usefulness of this method in the determination of halothane in biological samples and did not observe matrix effects, such as from differing cholesterol concentrations. This method was also found to be valid for the determination of carbon monoxide in tissues [135]. However, in heterogeneous biological samples, there may be differences in evaporation properties between the volatile analyte in the original sample and the added substance due to differing amounts of diffusion of the volatile substance through the biological matrix.

The multiple headspace extraction (MHE) method is based on a stepwise extraction of the volatile substances into a gas, with subsequent HS-GC analysis of the extract. Following each sampling step, equilibrium is rapidly regained and a volume of inert gas equal to that removed is introduced into the HS container. The vapour concentration in each step of the analysis is reduced exponentially, and it is possible to obtain the original concentration and partition coefficient by extrapolation to zero time using a plot of the logarithm of vapour phase concentration *versus* the extraction step number, irrespective of the sample conditions. The total

amount of each volatile substance can be calculated after only a few extraction steps. This method is useful for samples that are insoluble, such as certain polymers or residual solvents in printed foils, as these cannot be analysed using standard calibration methods. However, the MHE method requires special instrumentation and is a complex procedure. Suzuki *et al.* [136] first described this method as applied to the determination of organic solvents within adhesive tape. McAullife [137] described the theoretical basis for this method. Practical theory, instrumentation and many applications have also been reviewed [138–145]. Several examples of this procedure as applied to the analysis of solid samples have been published [146–148], and it has proved to be more quantitative than other methods [140].

3.6. Tissue homogenization

Owing to their liquid nature, body fluids, such as blood, urine and cavity fluids, are reliable HS samples as they attain vapour equilibrium easily and completely. Tissues such as liver, kidney and brain are also suitable for the determination of volatile substance levels when blood or urine samples cannot be obtained owing to delays in post-mortem sampling. The analysis of these tissues involves homogenization of the tissue to facilitate the vaporization of any volatile substances. The homogenization is usually done with a blender at low temperatures. Often homogenization is done in an open vessel, which results in a significant loss of volatile analytes prior to HS-GC analysis [89]. Improved recovery from tissue has been obtained by homogenization of the sample in the presence of organic solvents [149]. The recovery of ethanol levels was also optimized by homogenization in the presence of thiourea and perchloric acid [150].

Obviously, the homogenization of tissue samples should be done in a closed HS container to prevent loss of the volatile analyte. Miyaura and Isono [89] described a treatment involving the presence of KOH, olive oil and Triton X-100 in order to solubilize tissues within closed HS containers. Homogenization has also been done

through the use of ultrasonic irradiation. This method of disruption has been useful in the determination of ethanol [151] and carbon monoxide levels in coagulated blood [152] and tissues [153,154]. In addition, enzymatic homogenization of tissues using collagenase [155] and subtilisin A [59,156] has also been described. A combination of physical (ultrasonic irradiation), chemical (detergent) and biochemical (collagenase) treatments which liquify samples has been effective in the analysis of chloroform levels in various kinds of tissues [155].

3.7. Stability of volatile substances within biological samples

One purpose of toxicological analyses is to obtain a quantitative measure of volatile analyte levels within the body. Realistically, the measured results often do not reflect the true physiological levels. According to Chace *et al.* [157], the following two post-mortem alterations in volatile substances should be considered: phase I, a change in volatile substance which occurs between the time of death and the time of sampling; and phase II, the change which occurs between the time of sampling and the time of HS-GC analysis, *i.e.*, the time for storage and transfer. In addition, this author would like to add consideration of a further change, phase III: the change that can occur during analysis. These changes in analyte emphasize that HS-GC results in forensic and toxicological studies of absolute levels of volatile analytes within biological samples need to be evaluated critically.

Phase I changes have been investigated for a variety of substances. The post-mortem phase I change of inhaled ethyl acetate and toluene has been reported [158–162]. The change and distribution of toluene in eggs (used as a biological sample) after burning were investigated, in relation to an actual case of a fire [128]. Liquified petroleum gas has been found to be metabolized to more polar volatile substances [163]. The changes in the carboxyhaemoglobin percentage in blood during heating and putrefaction have been investigated [164–167]. Post-mortem formation of carbon monoxide in tissue has also

been reported [168,169]. Post-mortem ethanol formation has been considered in relation to the formation of *n*-propanol [98].

To limit the amount of phase II change there are several important storage characteristics that should be considered: type of container, temperature, time and β value (the ratio of air volume to sample volume). The stability of carbon monoxide during storage has been examined [52,157,164,165,170,171]. The stability of ethanol in blood and tissues has been extensively investigated and stabilizing procedures have been found [29,90,172–174]. Three processes could result in the loss of ethanol from stored blood samples: loss due to the growth of contaminating microorganisms (prevented by the addition of sodium fluoride), loss by evaporation due to improper capping of the storage container and decomposition that occurs in samples stored over long periods (accelerated at elevated temperatures). Storage conditions have also been investigated for toluene, mainly with respect to the use of appropriate containers [158,175–178]. The stability of carbon tetrachloride in blood and urine samples has also been examined [179], in addition to changes in cyanide concentration in blood [33,180]. An additional phase II change could involve the contamination of samples during storage. For example, chlorinated solvents commonly used in the laboratory should be considered, given that capped vials are rarely airtight. The integrity of the septum decreases with storage at low temperature and could allow gaseous contamination to reach the samples [181]. Most volatile substances are relatively stable in blood if certain simple precautions are taken [59]. It is generally recommended that samples be stored at low temperatures in the presence of preservatives, and in glass containers sealed tightly with an inert septum. Further, the containers should have minimal air space above the sample and be opened at low temperature and only when required for analysis [59].

There are several examples of phase III changes in the literature. Raising the temperature can introduce a change in analyte level and therefore requires special precautions. Ethanol concentration has been found to decline pro-

gressively during HS equilibrium [182]. Ethanol can be oxidized to acetaldehyde at elevated temperature. This reaction is catalysed by oxy-haemoglobin and is limited only by the amount of oxygen in the sealed vial [182,183]. Acetaldehyde shows rapid disappearance during HS incubation [29]. Acetaldehyde can also be formed from ethanol during deproteinization procedures prior to HS-GC analysis [184–189]. Other phase III changes include the conversion of thiocyanate into cyanide in the presence of haemoglobin and acid in the analysis of blood for cyanide [33] and the degradation of ethyl acetate to acetic acid and ethanol during the analysis of liver samples [107].

4. Detailed conditions of HS-GC methodology

Tables 1–10 present detailed HS-GC conditions for the determination of several groups of volatile substances in a variety of biological matrices. For each chemical group the following variations of HS-GC methodology are considered: HS conditions (equilibration times and temperatures, the use and types of additives, the use and types of IS, HS containers, HS sampling system), GC conditions (columns, temperatures, carrier gas, detectors) and overall HS-GC sensitivity (detection and quantification limits). Within each table references are cited in chronological order.

4.1. Definition of terms used in tables

In the "Substances" and "Additive" columns, the following abbreviations are used: AcH = acetaldehyde; ACA = acetoacetic acid; AcOEt = ethyl acetate; AcMe = acetone; MeCN = acetonitrile; AmSO$_4$ = ammonium sulphate; Bz = benzene; nBuOH = *n*-butanol; tBuOH = *tert.*-butanol; sBuOH = *sec.*-butanol; CH = chloral hydrate; ClBz = chlorobenzene; AcOH = acetic acid; BCE = 1-bromo-2-chloroethane; IBA = isobutyraldehyde; Bu$_2$NH = di-butylamine; 1,2-DCE = 1,2-dichloroethane; 1,1-DCE = 1,1-dichloroethane; cisDCE = *cis*-1,2-dichloroethylene; DCPOH = 1,3-dichloro-2-pro-

panol; Et_2S = diethyl sulphide; Me_2SO_4 = dimethyl sulphate; Me_2S = dimethyl sulphide; EtOH = ethanol; EtBz = ethylbenzene; EDTA = ethylenediaminetetraacetate; EtSH = ethanethiol; EtHgCl = ethylmercury(II) chloride; EtONO = ethyl nitrite; FBz = fluorobenzene; βG = β-galactosidase; BHB = β-hydroxybutyric acid; IAA = iodoacetic acid; BKB = β-ketobutyric acid; MeOH = methanol; AcEt = methyl ethyl ketone (2-butanone); AciBu = methyl isobutyl ketone; MeHgCl = methylmercury(II) chloride; MeHgI = methylmercury(II) iodide; AcPr = 2-pentanone; PCA = perchloric acid; PheHgCl = phenylmercury(II) chloride; nPrOH = 1-propanol; iPrOH = 2-propanol; SDS = sodium dodecyl sulphate; SSA = sulphosalicylic acid; THF = tetrahydrofuran; THP = tetrahydropyran; MeBz = toluene; TSA = toluenesulphonic acid; TCA = trichloroacetic acid; $C_2H_3Cl_3$ = 1,1,1-trichloroethane; $iC_2H_3Cl_3$ = 1,1,2-trichloroethane; TCE = trichloroethanol; C_2HCl_3 = trichloroethylene; TMB = 1,2,4-trimethylbenzene; TSA = toluenesulphonic acid; TX = Triton X-100; Xyl = xylene; oXyl = o-xylene; mXyl = m-xylene; pXyl = p-xylene.

In the "Specimen" column the following abbreviations are used: AP = adipose tissue; AQ = water; BL = blood; BR = brain; CF = cavity fluid; FT = fat tissue; FB = fermentation broth; GC = gastric content; HE = heart; KD = kidney; LV = liver; LN = lung; ML = milk; PL = plasma; MS = muscle; SR = serum; SL = saliva; SP = spleen; ST = stomach content; UR = urine; VH = vitreous humor.

In the "HS temperature" column the ambient temperature (room temperature) is abbreviated as RT.

In the "HS container" column the following abbreviations are used: VS = Van Slyke volumetric apparatus; WN = Warburg nanometer.

In the "HS injection" column, abbreviations of method are as follows: LP = automated injection method by which a constant volume of vapour phase sample in loop is introduced; MA = manual injection using syringe; PN = automated injection method by which pressurization and injection are controlled pneumatically; SW = injection method by which carrier gas is

used to sweep vapour phase; VE = injection method by which vapour phase is vacuum extracted using Van Slyke apparatus.

In the "Column" column, liquid phase (percentage concentrations and types), support particle size (mesh/mesh, mesh number × particle diameter (mm) = 14.9) and column dimensions (length × I.D.) are cited for packed column analysis. For capillary column analysis, column (liquid phase type and film thickness), dimensions (length × I.D.) and splitting ratio are cited. The following abbreviations are used: for liquid-phase material, AG = Apiezon grease; CW = Carbowax; DIP = diisodecyl phthalate; HC = Hallcomid; PEG = polyethylene glycol; for column support material, AW = acid washed; CP = Carbopack; CS = Chromosorb; DMCS = dimethyldichlorosilane; HP = Haloport; HMDS = deactivated with hexamethyldisilazane; MS = molecular sieve; NAW = non-acid-washed; PP = Porapak; SL = Shimalite; SCOT = support-coated open-tubular capillary column, UP = Uniport; UB = Unibeads.

In the "Oven temperature" column, the temperature values are cited for isothermal analysis. For temperature-programmed analysis, the initial temperature (°C) and its hold time (min) in parentheses, temperature ramp rate (°C/min) (number/m) and the final temperature (°C) are cited.

In the "Carrier gas" column, type of gas and flow-rate are cited except for special cases which describe controlled-pressure values.

In the "Detection" column, the following abbreviations are used: βID = β-ionization detection; ECD = electron-capture detection; FID = flame ionization detection; FTD = flame thermionic detection; HECD = Hall electrolytic conductivity detection; MS = mass spectrometry; MIPT = microwave-induced plasma emission detection; NPD = nitrogen–phosphorus detection; PID = photoionization detection; FPD = flame photometric detection; TCD = thermal conductivity detection.

4.2. Carbon monoxide

The determination of carbon monoxide (CO) levels in blood is important in cases such as CO

inhalation due to fires and in industrial hygiene [190]. CO binds strongly to the reduced form of haemoglobin (Hb), and therefore the carboxyhaemoglobin percentage in blood (% CO-Hb) is a critical parameter that predicts CO levels. A spectrophotometric method has been used to measure the % CO-Hb, but this method is not reliable in certain instances, *e.g.*, in forensic analysis such as fire accident victims or putrefied bodies. Denatured haemoglobin molecules give false results in this method owing to the altered absorbance spectrum. Alternatively, the HS-GC method has been used for blood CO determinations. The HS-GC procedure involves the initial liberation of CO gas from blood Hb by the addition of hexacyanoferrate(III) [to oxidize Hb to methaemoglobin (met-Hb)] or strong acid (to denature Hb). The released CO gas is then measured by GC using TCD or FID (after CO has been converted into methane by postcolumn reduction with hydrogen on a nickel catalyst). Molecular sieves (5 Å) are used as the column matrix, and provide a good separation without interference. The following two injection methods are used in this analysis; static HS injection and a method in which CO gas is liberated in a special reaction vessel and introduced into the GC system by a carrier gas swept over the HS phase.

The partition coefficient of CO is very low (0.014–0.036), and therefore all liberated CO in blood samples is available for GC analysis. In order to facilitate the liberation of CO gas from samples, saponin or detergent is added as a haemolysation reagent. The HS temperature does not need to exceed room temperature owing to its small *k* value. The HS-GC method has been shown to be reliable as compared with other methods [54,191–197].

To obtain a % CO-Hb value in blood, a reference of 100% CO-Hb needs to be measured using one of the following two methods. The first is measurement of the CO binding capacity, obtained by CO saturation of the blood sample and subsequent analysis. The second is to measure the total Hb concentration by the cyanomethaemoglobin spectrophotometric method or by analysing for total iron content by atomic absorption spectrometry. The first meth-

od is easily performed using the specially designed sweeping apparatus, but is unreliable owing to the inconsistent CO binding capacity found for special samples [51,198,199]. For instance, Met-Hb (oxidized Hb molecule having no CO binding capacity) is found in subjects who have died due to fire, does not have a complete CO binding capacity and can therefore result in incorrect % CO-Hb measurements. Detailed HS-GC conditions are presented in Table 1.

4.3. Alcohols and their metabolites

Blood ethanol levels are tested in many countries as a means of addressing and deterring citizens from driving under the influence of alcohol. Compared with other methods, such as spectrophotometry, the GC technique is advantageous because it provides both qualitative (retention time) and quantitative (peak signal) results. The direct injection technique has been used in the past, but contamination of samples in the GC injector port and column is a serious disadvantage. Pyrolysis GC has recently been developed for blood ethanol determination [206]. In European countries, HS-GC has been made a legal method for ethanol determination. Ethanol determination in post-mortem samples is important in order to determine blood alcohol levels after traffic accidents.

Numerous reviews have been published concerning ethanol determination [207–211]. Extensive improvements in HS-GC have been made such that it is now possible to determine results for numerous samples quickly and accurately [212]. The selection of the column, internal standard, additives for sample preservation and induction of salting-out effect, automated systems and data systems have been extensively discussed [212]. The critical determinates in the selection of an I.S. for ethanol determinations are that it is not an endogenous compound and that it has similar chemical properties and GC retention time, and for these reasons *n*-propanol and *tert.*-butanol are used extensively. Sodium chloride is the most commonly used salting-out reagent. Various HS temperatures have been used that either optimize sensitivity (high temperature) or accuracy (room temperature)

Table 1
Carbon monoxide

Specimen	Haemolysis reagent	CO release reagent	HS container	HS injection[a]	Column	Oven temperature (°C)	Carrier gas (ml/min)	Detection	Limit	Reference[b]	Ref.
BL tissue	Saponin	K₃Fe(CN)₆ AcOH	Syringe	MA RT	MS 5A (160) 2 m	75	He 135	TCD	20 ppm (v/v)	CO Sat	3
BL		K₃Fe(CN)₆	Vessel	SW 5 min	MS 5A 3 m	100	He 30	TCD		CO Sat	51
BL	Saponin	Lactic acid	VS	SW 3 min	MS 5A 2.4 m	70	He 15 p.s.i.	TCD	10 ppm (v/v)		200
BL	Saponin	K₃Fe(CN)₆ Lactic acid	VS	SW 2 min	MS 5A (40/60) 2.7 m	100	He 80	TCD	50 nl/ml		201
BL	TX	K₃Fe(CN)₆	Syringe	SW 10 min	Silica 2 m × 6.4 mm	30	He 60	TCD		CO Sat	52
BL		HCl, lactic acid	VS	SW 2 min	MS 5A (50) 1.8 m × 6.4 mm	105	He 135	TCD		Hi-CN	202
BL	Sterox	K₃Fe(CN)₆	Vessel	SW 5 min	MS 5A (30/80) 1.8 m × 6.4 mm	100	He 50	FID	20 nl/ml	Hb	54
BL	Saponin TX	K₃Fe(CN)₆	Syringe	MA 10 min RT	MS 5A (60/80) 1.5–1.8 m × 6.4 mm	100~ 120	He 45	TCD		Fe (AA)	90
BL	Saponin	K₃Fe(CN)₆	VS	SW 0.5 min	MS 5A (30/60) 2 m × 3 mm	50	He 50	TCD		CO Sat	170
BL	Saponin	K₃Fe(CN)₆ Lactic acid	Vial	SW 3 min	PP Q (80/100) 0.91 m × 6 mm MS 13X (40/60) 6 mm × 3.6 mm	70	He 100	TCD	50 nl/ml		191
BL	TX	K₃Fe(CN)₆	Vessel	SW 5 min	MS 5A (40/60) 3 m × 3 mm	100	He 30	TCD		CO Sat	203
BL	TX	K₃Fe(CN)₆	Vessel	SW 5 min	MS 5A (40/60) 3 m × 3 mm	100	He 30	TCD		Hi-CN	198
BL	Saponin	K₃Fe(CN)₆	Vessel	SW 5 min	MS 5A (30/60) 2 m × 3 mm	80	He 35	TCD		CO Sat	199
BL		H₂SO₄	Vial	MA 10 min RT	MS 5A (60/80) 1.65 m × 3.2 mm	110	H₂ 20	FID		CO-Oxi Hi-CN	192
BL		K₃Fe(CN)₆	Vessel	MA 5 min RT	MS 5A 2 m × 3 mm	80	He 35	TCD		CO Sat	204
BL		K₃Fe(CN)₆ Lactic acid	Tube	MA 30 min RT	MS 5A (100/120) 1.8 m × 3.2 mm	140	He 15	FID			205
BL		K₃Fe(CN)₆	Chamber	LP 75 s	MS 5A 2 m × 3 mm	80	He 35	TCD		CO Sat	152
BL	Saponin	K₃Fe(CN)₆	Syringe	MA 4 min RT	MS 5A (80/100) 1 m × 3 mm	90	He 40	TCD		CO Sat	49
BL		H₂SO₄	Syringe	MA 10 min	MS 5A 1.8 m × 6.4 mm	70	He 30 p.s.i.	TCD		Hi-CN	48
BL		K₃Fe(CN)₆	Vial	PN 1 h 90°C	MS 3A (60/80) 1 m × 3.2 mm	135	He 36 p.s.i.	FID	0.1% CO-Hb	Hi-CN	193
BL,CF	TX	K₃Fe(CN)₆	Syringe	MA 5 min RT	MS 5A (60/80) 2.1 m × 3 mm	100	He 24	TCD		Hi-CN	50 169
BL	Saponin TX	K₃Fe(CN)₆	Bottle	MA 10 min RT	MS 5A (60/80) 1.8 m × 3.2 mm	70	He 20	TCD		CO Sat Hi-CN Fe(AA)	194
BL	Saponin	K₃Fe(CN)₆	Vial	LP 10 min	MS 5A (30/60) 2 m × 3 mm	70	N₂ 0.6 kg/cm²	TCD FID		CO Sat	153
LV		K₃Fe(CN)₆	Vial	MA 5 min RT	MS 5A	80	N₂ 35	FID		Fe (AA)	154
BL		HC, lactic acid	Chamber	SW 5 min	MS 5A (60/80) 1.8 m × 3.2 mm	50~70	He	FID		Hi-CN	196
BL		K₃Fe(CN)₆	Vessel	SW 2.5 min	MS 5A (30/60) 1.8 m × 3.2 mm	90	N₂ 50	TCD		CO Sat	195
Tissue		H₂SO₄	Vial	MA 30 min 65°C	Carbosieve S-II 1.8 m × 2 mm	35 (5) 25/min 190	He 22	MS	0.5 µg/ml	Fe (AA)	135
BL		H₃PO₄	Vial	PN 70°C	PP Q (80/100) 3 m × 0.9 mm	80	N₂ 30	FID		CO gas	197

a For manual HS injection method (MA), HS equilibrium time (min) and temperature (°C) is cited. For sweeping method (SW), time (min) for CO liberation before sweeping vapour is cited.
b Method for obtaining 100% CO-Hb value as reference is cited as follows: CO Sat, sample blood is saturated with CO gas and used as reference; Hi-CN, total Hb concentration is measured by spectrophotometric cyanomethaemoglobin method; Fe (AA), total iron content is measured by atomic absorption spectrometry; CO-Ox, total Hb concentration is measured using commercial CO-Oximeter.

[108,41]. Polar stationary phases are used as column packings material, e.g., polyethylene glycol, Carbowax and porous polymer adsorbents. Anthony et al. [213] evaluated the selectivity of the column supports Carbopack B and Carbopack C for the determination of a wide range of low-molecular-mass volatiles [213]. Two- and three-column systems have been used to verify ethanol determination [212,214,215]. FID is the most popular mode of detection. Importantly, ethanol is oxidized to acetaldehyde by oxyhaemoglobin during storage or HS equilibrium [173,183] unless special precautions are taken. Although ethanol has a high partition coefficient (ca. 2000), the HS-GC method offers suitable sensitivity for most forensic determinations; the legal blood ethanol concentration for motorists is ca. 0.5 mg/ml and the background level in normal individuals is 0.001–0.0025 mg/ml [216]. The reliability of the HS-GC method has been verified in comparison with other methods [35,217–219]. Detailed HS-GC conditions for the determination of ethanol are presented in Table 2.

The determination of the level of oxidative metabolites of alcohols is important in forensic science, because aldehydes and organic acids are thought to produce much of the toxicity caused by alcohol consumption. Non-volatile organic acids can be determined after conversion into their ester derivatives in the presence of a strong acid and methanol [111–117]. Formaldehyde can also be converted into an ethoxy derivative [229].

Aldehyde levels can be detected by employing the HS-GC method used for alcohol and the assay is more sensitive because the aldehyde partition coefficient is considerably smaller. However, determination of the acetaldehyde level in blood has serious methodological difficulties. First, because of its high chemical reactivity, acetaldehyde shows rapid disappearance [29] during HS incubation at elevated temperature, owing to the chemical reaction with biological components in samples and/or further oxidation. In order to prevent this disappearance, deproteinization procedures have been conducted [29]. Supernatants from deproteinized

blood samples have been used as HS samples. Second, especially in the presence of ethanol, significant artifactual acetaldehyde formation has been observed during the deproteinization procedure or HS incubation [29,184–186,188,189], owing to non-enzymatic oxidation of ethanol by either ascorbic acid [230] and/or erythrocytes [231,232]. Although oxidation by ascorbic acid can be completely prevented by addition of thiourea [233], oxidation by erythrocytes cannot be prevented by thiourea [231,232]. Effective acetaldehyde determinations have been conducted with a rapid deproteinization procedure at low temperature using elaborate additives: perchloric acid and saline [188,232], nitric acid and sulphosalicylic acid [234] and perchloric acid, thiourea, saline and sodium azide [224]. Detailed HS-GC conditions for the determination of alcohols and their metabolites are presented in Table 3.

4.4. Non-halogenated organic solvents

The analysis of biological samples for levels of non-halogenated organic solvents has been performed for the diagnosis of solvent abuse and the monitoring of industrial exposure [239]. Organic solvents are easily metabolized in the body, and in many instances the non-metabolized parent compound is only a minor fraction of total body burden. Sensitive HS-GC methods make it possible to detect trace amounts of non-metabolized compounds. Various kinds of stationary phases have been used, depending on the polarity of the analyte. Polar materials such as polyethylene glycol, Carbowax and porous polymers are common stationary phases, although non-polar and intermediate-polarity phases have also been used, especially in capillary analysis. FID and MS are the major detection methods. Detailed HS-GC conditions are presented in Table 4.

4.5. Halogenated aliphatic hydrocarbons

Halogenated aliphatic hydrocarbons are frequently used as industrial solvents and have been used as anaesthetics in the past. The determination of these substances in biological samples

Table 2
Ethanol

Specimen[a]	Additive	I.S.	HS temperature (°C)	HS time (min)	HS container	HS injection	Column	Oven temperature (°C)	Carrier gas (ml/min)	Detection	Limit	Ref.
BL (AcH)	ZnSO4 Ba(OH)2		55	15	Bottle	MA	5% CW 1500 HP 60-F 1.2 m × 6.4 mm	100	He 50	FID		29
BL, SR, UR		AcMe AcEt	60		Bottle		15% PEG 1500 (60/80) Celite 545 15% PEG 1540 (80/100) CS W	100	N2 88	FID		25
BL, UR	NaCl		30	5	Flask	MA	1.5% Flexol 8N8, 1.5% DIP, 1.5% PEG 600 (100/120) CS P 1.8 m × 3.2 mm	75	N2 19	FID		100
BL		nPrOH	55	15	Vial	MA	25% PEG 1000 (6000) (60/80) Shimalite 0.75 m × 4 mm	90	N2 90	FID		217
BL			60	10	Vial	MA	20% CW 2M (60/80) firebrick AW (CS P HMDS) 3 m × 3.2 mm	100	N2 17 12.3	FID		35
SR	CuSO4		80	2	Tube	SW 2 min	15% Flexol 8N8, 10% DIP, 3% PEG 600 (42/60) C-22 firebrick 3 m × 4.8 mm	100	He 75	FID	1 µg/ml	53
BL, UR, SL	nPrOH		55	15	Vial	MA	25% PEG 1000 (60/80) SL	90	N2 70	FID		26
BL	NaF		27	15	Bottle	MA	15% Flexol 8N8, 10% DIP, 3% PEG 600 (42/60) C-22 firebrick 1.8 m × 6.4 mm	100	He 50	FID		108
BL, UR			27	15	Bottle	LP	PP S (80/100) 1.2 m × 3.2 mm	132	He 50	TCD		41
BL	NaCl	1,4-Dioxane	85	18	Bottle	MA	15% Flexol 8N8, 10% DIP, 3% CW 600 (40/60) firebrick 2.4 m × 6.4 mm	100	He 40	TCD	0.01% (w/v)	218
BL, UR	NaF Filter-paper		35	15	Bottle	MA	5% CW 1500 (30/60) Haloport 60F 1.2–1.8 m × 3 mm	75~100	N2 30	FID		220
BL, tissue		nPrOH	55		Bottle	MA	25% PEG 1000 (60/80) 1 m × 4 mm	90	N2 30	FID		151
BL		nPrOH	27		Flask	MA	PP S (100/120) 1.8 m × 3.2 mm	160	He 50	FID		221
BL		tBuOH	60		Vial	PN	15% CW 1500 Celite 2 m	70	N2 25	FID		42
BL		nPrOH	62		Vial	PN	PP Q 1.5 m	140	N2	FID		173
BL		tBuOH					0.4% PEG (60/80) graphite	105		FID		209
BL	NaF	nPrOH	60	3	Vial	MA	PP Q (80/100) 1.8 m × 3.5 mm	150	N2 30	FID	3 µg/ml	109
BL	PCA[b]	tBuOH	60	20	Vial	PN	15% CW 1500 (60/80) Celite 2 m × 2.7 mm SP-1000 25 m × 0.35 mm split	80 52	N2 1.5 N2 0.6 kg/cm²	FID		55
BL (AcH)	Thiourea PCA[b]	nPrOH	60	30	Vial	MA	5% CW 20M (30/60) HP F 1.8 m × 4 mm	90	He 30	FID	5 µg/ml (EtOH) 0.25 µg/ml (AcH)	222
LV		nPrOH	60	30		PN	5% CW 20M (60/80) CP B 1.83 m × 3.2 mm	65 110	N2 30	FID	3.125 mg/dl	213
BL, LV (AcH)	Thiourea PCA[b]	iPrOH	65	30	Flask	MA	PP Q (100/120) 1.8 m × 2 mm	130	He 37	FID	2.7 µmol (EtOH) 23 nmol (AcH)	150

Sample	Additive	Solvent	Temp.	Time	Container	Method	Column	Oven	Carrier gas	Detector	Conc.	Ref.
BL, BR, LV, ML		tBuOH	60	15	Bottle	PN	15% CW 1500 (60/100) Celite 2 m × 2.7 mm	160	N$_2$ 35	FID		90
BL		nPrOH	40–60	10–120	Vial	LP	PP Q 2 m × 4 mm		N$_2$ 30	FID		182
BL, UR, VH		nPrOH	60	30	Vial	PN	5% CW 20M CP B	120	N$_2$ 20	FID		223
BL	NaCl (K$_2$CO$_3$) NaNO$_2$ H$_2$SO$_4$				Vial	MA	PP Q (80/100) 2 m × 2 mm			FID		118
(EtONO)^c		EtOH- d$_3$(d$_5$)	5	10			Tenax GC (30/60) 2 m × 2 mm	60		FID MS		
BL, SR			25	30	Flask	MA	PP Q 0.305 m × 3 mm	100	H$_2$–N$_2$ 6:4	FID		97
BL	AmSO$_4$ Dithionite	nPrOH	60	30	Vial	PN	5% CW 20M CP B 1.8 m × 2 mm	65	N$_2$ 30	FID	0.001% (w/v)	101
BL (AcH)	PCA, NaN$_3$ Saline Thiourea^b	nPrOH	60	15	Vial	MA	5% CW 20M (30/60) HP F 1.8 m × 4 mm	90	N$_2$ 30	FID	6 µg/ml (EtOH) 0.1 µg/ml (AcH)	224
BL	NaCl	nPrOH	RT	30	Vial	MA	0.2% CW 1500 (80/100) CP C 2 m × 2 mm	125	N$_2$ 20	FID	2 µg/ml	219
Heart & femoral	NaF	nPrOH		30	Vial	PN	0.2% CW 1500 (80/100) CP B 1.8 m × 3.2 mm	130		FID		225
BL		nPrOH	55	12	Vial	PN	DB-1 (3 µm) 30 m × 0.53 mm DB-WAX (1 µm)	45	He 7.5	FID		214
BL		nPrOH		18		PN	0.2% CW 1500 (80/100) CP C 5% CW 20M CP B 15% CW 20M CP W	100 100 120	N$_2$ 20	FID		226
BL	NaF EDTA	nPrOH	40		Vial	PN	0.2% CW 1500 (80/100) CP C 5% CW 20M (60/80) CP B 15% CW 20M CS W 2 m × 3 mm	100 100 120		FID		212
BL, UR		nPrOH	60	10		PN	0.2% CW 1500 (80/100) CP C 2 m × 3.2 mm 10% CW 400 (80/100) CS WHMDS	90	30			227
BL, VH		nPrOH	60	35	Vial	PN	0.2% CW 1500 (80/100) CP C 3 m × 3.2 mm	120	N$_2$ 30	FID		228
BL, SR, PL		nPrOH			Vial	PN	CW 1540 CS W 2 m × 3 mm	100	N$_2$ 20	FID		96

^{a} Substances in parentheses were also analysed.
^{b} HS sample is a supernatant fraction of acidified sample.
^{c} The substance is converted by derivatization from non-volatile analytes.

Table 3
Alcohols and their metabolites

Substance[a]	Specimen	Additive[b]	HS tempera-ture (°C)	HS time (min)	HS container	HS injec-tion	Column	Oven tempera-ture (°C)	Carrier gas (ml/min)	Detec-tion	Limit	Ref.
EtOH, AcMe, MeOH, iPrOH	BL	CuSO$_4$	50	3	Vessel	SW	5% HC M-18 (5% CW 1540) (35) Teflon 6 4.5 m × 2.16 mm	56	He 20	TCD		40
EtOH, AcH, nPrOH	BL		55	15	Vial	MA	25% PEG 1000 (60/80) SL 0.75 m × 4 mm	90	N$_2$ 40	FID		172
AcH	BL	ZnSO$_4$[c], Ba(OH)$_2$, saline	55	60	Vial	MA	PP Q (50/80) 1.8 m × 3.2 mm	85	He 30	FID MS		184
AcH, EtOH	LV	PCA, thiourea, (tBuOH)	65	15	Vial	PN	15% PEG (60/100) Celite			FID		233
AcH	BL, LV	PCA, (tBuOH)	65	15	Vial	PN	15% PEG (60/100) Celite	75	N$_2$ 35	FID		230
AcH	BL	(nPrOH)[a]	55	20	Vial	MA	15% PEG 1500 (60/80) CS W 3 m × 4 mm 2 m × 4 mm	100	N$_2$ 50	FID		185
EtOH EtOH, AcMe, MeOH, nPrOH	AQ	Saline			Vial	MA	PP P (80/100) 1.8 m × 3 mm	120 130	N$_2$ 30	FID		132
AcH	BL, LV, BR	HClO$_4$[c]	65	15	Vial	PN	15% PEG (60/80) Celite	75	N$_2$ 35	FID	0.5 μM	231
AcH	BL		50	20	Vial	MA	PP S (80/100) 0.9 m × 2 mm	110		FID		235
AcH, MeOH, EtOH	LV	(nPrOH)	60	30		PN	5% CW 20 M (60/80) CP B 1.83 m × 3.2 mm	65 110	N$_2$ 30	FID		213
HCOOH (ester)	BL, PL, UR	H$_2$SO$_4$, MeOH (MeCN)	RT	20	Syringe	MA	PP Q 1.8 m × 3 mm	124	N$_2$ 24	FID	1 mM	111
AcH	BL	(iPrOH)[c] PCA[c]	60	30	Vial	MA	CS 101 (60/80) 1.5 m × 4 mm	120	N$_2$ 50	FID		187
EtOH		PCA[c]	55	20			15% PEO 1500 CS WAW (60/80) 2 m × 4 mm	110				
AcH	BL	NaNO$_2$, SSA[c] (IBA)	60	10	Vial	MA	PP Q (80/100) 1 m × 2 mm	110	He 40	FID	0.4 μM	234
HCOOH (ester)	BL	H$_2$SO$_4$, MeOH (MeCN)	45	20	Vial	MA	PP Q (80/100) 2 m × 3 mm	135	N$_2$ 48	FID	5 mg/l	113
nPrOH, EtOH	BL, MS	TSA, MeOH	55	15	Vial	MA	25% PEG 1000 (60/80) SL	90	N$_2$ 70	FID		98
HCOOH (ester)	FB	(iBuOH)[c]	80	30	Beaker	MA	PP Q (QS) 1.5 m × 4 mm	100	He 30	FID	20 μg/ml	112
AcH	BL	PCA, saline	65	30	Vial	PN	CS 101 (60/80) 1 m × 2.4 mm	130	N$_2$ 40	FID		188
EtOH		PCA, saline (nPrOH)	55	30	Vial	MA	15% PEG 600 (60/80) CS W AW 2 m × 3 mm	130	N$_2$ 55	FID		
AcH	BL	PCA, saline Thiourea[c]	65	15	Vial	PN	15% PEG (60/80) Celite	75	N$_2$ 35	FID	0.1 μM	232

Analyte	Sample	Reagent	Temp.	Time	Container	Mode	Column	Col. temp.	Carrier	Detector	Detection limit	Ref.
HCHO, MeOH	BL	(nPrOH)	60	15	Vial	MA	25% PEG 1000, 2% KOH (60/80) CS WAW-DMCS	80	N_2 60	FID	0.2 mg/ml	236
AcOH (ester)	BL	H_2SO_4, MeOH (MeCN)	55	15	Vial	MA	PP Q (80/100) 2 m × 3 mm	140	N_2 40	FID	0.2 µg/ml	114
MeOH	SR	(MeOH-d_4)	65	30	Vial	MA	CPWAX 57CB 26 m × 0.22 mm split 1:20	50	He 4 p.s.i.	MS	12 µg/ml	129
AcOH (ester), EtOH	PL	H_2SO_4 MeOH	55	30	Vial	PN	PP QS (80/100) 1.35 m × 4 mm	140	N_2 95 kPa	FID	50 µM (AcOH), 1.61 mM (EtOH)	115
EtOH, AcH, AcMe, MeOH, iPrOH, nPrOH, tBuOH	BL	NaCL	RT	30	Vial	MA	DB-WAX (1 µm), DB-1 (3 µm), DB-1 (5 µm) 15 m × 0.53 mm, DB-1 (1.5 µm) 30 m × 0.53 mm	30~40	H_2 25	FID	10 µM	58
EtOH, AcMe, tBuOH, nPrOH, MeCN, iPrOH, MeOH	BL, PL, SR	NaCl	25, 37	45	Tube	MA	PP S (80/100) 1.8 m × 3.2 mm	165	N_2 45	FID		92, 126
MeOH	BL	K_2CO_3	60	30	Vial	PN	0.2% CW 1500 CP C 2 m × 3 mm	80		FID		226
HCHO	BL	HCl, EtOH (nPrOH) (tBuOH)	56	60	Vial	MA	25% PEG 1000 (60/80) SL; 25% PEG 1000 (60/80) SL	90, 90	N_2 40; H_2 30	FID; MS	10 µM	229
HCOOH (ester), MeOH	BL, SR	H_2SO_4, MeOH (propionic acid)	35	30	Vial	MA	5% IIC M-18 (0.5% CW 600) (40/60) Teflon 6	75		FID	25 µg/ml	116
EtOH, MeOH, AcMe, iPrOH, tBuOH, AcEt	BL	K_2CO_3 (PrOH)					0.2% CW 1500 CP C (80/100)	80		FID		237
Organic acid (ester)	FB	$NaHSO_4$ MeOH	120	20	Vial	PN	Restek Stabilwax DA (0.25 µm) 30 m × 0.32 mm	70 (3) 6/min 120	N_2 30	FID		117
MeOH	KD, LV, UR, BL, HE, VH, CF, SC	(nPrOH)	60	30	Vial	PN	15% CW 15000 (80/100) CS WAW 2 m × 1.6 mm	100	N_2 30	FID		238

[a] Substances in parentheses are those which are converted by derivatization from non-volatile analytes.
[b] Substances in parentheses are internal standards.
[c] HS sample is a supernatant fraction of acidified sample.

Table 4
Non-halogenated organic solvents

Substance	Specimen	Additive	I.S.	HS temperature (°C)	HS time (min)	HS container	HS injection	Column	Oven temperature (°C)	Carrier gas (ml/min)	Detection	Limit	Ref.
Bz, MeBz, mXyl	BL			37	1–2 h	Vial, Bottle	MA	10% PEG 400 Celite	70	N_2 70	FID		28, 240, 46
MeBz	BL, LV, BR		EtBz	50	20	Bottle	MA	CS 102 (60/80) 0.375 m × 3 mm	150	N_2 1 kg/cm^2	FID	0.2 mg/g	241
MeBz, AcMe	BL		Bz		30	Syringe	MA	5% APL (80/100) Diasolid M 3 m × 3 mm	50 (75)	N_2 35	FID		242
Bz, MeBz	BL			37	30		MA	10% PEG 400 Celite 545 2 m	80	N_2 70	FID		47
MeBz	UR			50–55 (RT)ᵃ	10ᵃ	Flask	MA	2% PEG 1500 (60/80) CS W 3 m × 4 mm	70	N_2 25	FID		243
MeBz, Xyl	BL			90	30		MA	10% UC-W98 (80/100) CS W AW-DMCS 0.5 m × 3.2 mm	75			0.005 ppm (w/v)	244
MeBz / EtOH, AcOEt	BL, BR, LV		EtBz / nPrOH	50	20	Vial	MA	CS 102 0.555 m × 3 mm / PP Q (50/80) 0.75 m × 3 mm	170 / 180	N_2 1 kg/cm^2	FID		245
Bz	ML			40	10	Flask	MA	PP P (80/100) 1.8 m × 3 mm	165		FID		133
AcOEt	LV, BR	H_2SO_4	nPrOH	50	20	Bottle	MA	PP Q (50/80) 0.75 m × 3 mm	180	N_2 37	FID		107
Aromatic hydrocarbon, ketone	BL, Oil, AQ			37	0.5–2 h	Vial	MA	PEG 400 or SE-30 2 m 0.75 m × 3 mm	80	N_2 70	FID		72
AcOEt / EtOH	BL, BR, LV / FT	H_2SO_4	nPrOH	50	20	Vial	MA	PP QS (80/100) 1 m × 2.6 mm / CS 102 (60/80) 0.8 m × 3 mm	170 / 205	He 20 / N_2 38.8	MS / FID		160
MeBz	UR		EtBz	50	20	Vial	MA	CS 102 (60/80) 0.5 m × 3 mm	200	N_2 30	FID		175
MeBz	BL, UR		EtBz	50	15–30		MA	5% PEG 1500 (80/100) Celite 545 2 m × 3 mm 5% AG L (60/80) CS W 3 m × 3 mm	55	N_2 35	FID		158
Phenyl conjugates	UR	Sulphatase		90	20		PN	10% CW 20M, 2% H_3PO_4 (100/120) CS W 2 m × 2 mm	165	He 35	FID		121
AcEt, MeBz, AciBu, Xyl	BL, LV, BR / LN	NaCl		37	20	Vial	MA	15% SP-1000 (80/100) CS W AW 1.8 m × 2 mm		He 20	MS		246
MeBz	BL, BR, LV / LN, KD		iBuOH	55	15	Vial	MA	25% PEG 6000 (60/80) SL 1 m × 3 mm	80	N_2 30	FID		159
MeBz	BL		EtBz, $CHCl_3$	35	20	Vial	MA	20% DOS (60/80) UP B 1 m × 3 mm	100	N_2 30	FID		247
MeBz	BL, BR, LV		AcOEt, AcMe	60	30	Bottle	MA	15% CW 20M (85/100) 0.2% CW 1500 (80/100) CP C 1.8 m × 1.6 mm	80 80 (130)	N_2 50 20	FID		248
MeBz	LV	Olive oil, NaOH, TX, MeOH	EtBz	35	2.5 h	Vial	MA	20% DOS (60/80) UP B 1 m × 3 mm	100	N_2 30	FID		89
Methyl methacrylate	BL	NaCl		70	20	Vial	LP	SE-54 25 m × 0.23 mm	0 30/min 100		FID	0.02 µg/ml	110

Analyte	Matrix	Additive	I.S.	Temp.	Time	Container	Method	Column	Column temp.	Carrier	Detector	Detection limit	Ref.
iPrOH, AcMe	BL, organ	PCA	tBuOH	65	15	Vial	PN	10% PEG 600 CS W 4 m × 3.2 mm	75	N₂ 40	FID		163
AcEt, sBuOH MeBz	BL, LN, LV, BR		nPrOH EtBz	75 60	10 20	Vial	MA	UB A 2 m × 3.2 mm CS 101 (60/80) 1.5 m × 2.6 mm	90	N₂ 2 kg/cm²	FID		249
THF	LV, KD, BR, UR, FT, SP, MS	NaCl	THP THF-d₈	40	60	Vial	MA	PP P (80/100) 1 m × 3 mm	180 140	N₂ 40	FID	1 µg	125
MeBz	LV		MeBz-d₈	60	30	Vial	MA	PP P (80/100) 1 m × 3 mm	170	He 30	MS	0.1 µg	130
MeBz	BL		EtBz	60	30	Vial	PN	0.3% CW 20M (80/100) CP C 2 m × 1.8 mm	175	He 30 N₂ 30	MS FID		176
Gasoline Kerosene	BL		MeBz-d₈	40	20	Vial	MA	PP P 80/100 1 m × 3 mm 10% OV-17 CS W HP 2 m × 3 mm	180 100	N₂ 40	MS	0.01 µg	250
Bz, MeBz	BL		FBz, oXyl	80	23	Vial	PN	HP-1 + HP-5 (2.65 µm) (30 + 30) m × 0.53 mm	50(6) 10/min 60 30/min 100 10/min 200	He 12.5	PID	5 nM	251
MeBz	Body fluids Tissues		tBuOH				MA	25% PEG 1000 (60/80) SL 2 m × 3 mm 2% OV-17 (60/80) CS W 1.1 m × 2.6 mm	90 90	N₂ 38 He 20	FID MS		252
MeBz	BL		iBuOH	62	40	Vial	PN	5% CW 20M (60/80) CP B 1.8 m × 3.2 mm 0.5% CW 600. 3.8% HC M18 (40/60) CS T 2 m × 3.2 mm	130 85	N₂ 60 N₂ 30	FID		177
MeBz	BL, UR		EtBz	55	20	Vial	MA	BX-20 2.1 m × 3 mm	90	N₂ 40	FID	0.3 µg/g (BL) 0.06 µg/g (UR)	253
Hydrocarbon	BL		MeBz-d₆, indan	60 (RT)	20	Vial	MA	DB-5 (1.5 µm) 15 m × 0.53 mm	40 4/min 90	He 10	MS	0.01	254
MeBz AcOEt iBuOH	BL	NaCl	oXyl	55	20	Vial	MA	10% PEG-1000 (60/80) UP HP 2 m × 3 mm	80	N₂ 60	FID	µg/g 50 ng 50 ng 200 ng	102
Paint thinner	BL, BR, LV KD, LN, MS	NaCl	oXyl	55	20	Vial	MA	10% PEG-1000 (60/80) UP HP 2 m × 3 mm	80	N₂ 60	FID		161
MeBz	BL	NaCl	nPrOH	80	60	Vial	PN	0.2% CW 1500 (80/100) CP C 1.8 m × 2.0 mm HP-1 25 m	80	N₂ 60	FID		178
MeBz, AcOEt EtOH, iBuOH	BL, LN, KD LV, BR, MS	NaCl	oXyl	55			MA	10% PEG 1000 (60/80) UP HP 2 m × 3 mm	70 80	N₂ 60	MS FID		162
Gasoline Kerosene	BL	NaCl	MeBz-d₈	55	20	Vial	MA	HP-1 (2.65 µm) 10 m × 0.53 mm	50 (2) 10/min 200	He 15	MS	50 pg (meBz) 1 ng (TMB)	255
MeCN EtOH	BL, UR, SC		tBuOH nPrOH			Vial	PN	10% CW 20M CP B 15% CW 20M CS W 2 m × 3 mm	80 110		FID		215

[a] After HS incubation as described, the temperature of the HS vial is returned to room temperature, and HS vapour is injected into the GC system.

is important in industrial hygiene (exposure monitoring), environmental sciences (monitoring of contamination), anaesthesiology (pharmaco-kinetic information) and forensic sciences (de-termining cause of death). The analyses are normally done using columns packed with polar materials such as polyethylene glycol, Carbowax and porous polymers, but non-polar and inter-mediate-polarity phases have also been used. Because it is halogen specific and very sensitive, ECD is the most frequently used method of detection. FID, MS and Hall electrolytic conduc-tivity detection (HECD) have also been used as detection methods.

Halogenated aliphatic substances are also sus-ceptible to biological transformation. Trichloro-ethylene and tetrachloroethylene, common en-vironmental pollutants, are metabolized to TCA and TCE. In fact, these compounds are the main substances found after exposure and can be used as indicators [181]. The terminal metabolite, TCA, has been converted into its volatile ester derivatives by addition of dimethyl sulphate [93,94,123,124]. The glucuronide conjugate of trichloroethanol has been determined after hy-drolysis by β-glucuronidase treatment and mea-surement of the resulting TCE by HS-GC [119]. Chloral hydrate has been determined after con-version into TCE [123] and, likewise, TCA has been converted into volatile chloroform by heat-ing [181,256,257]. Detailed HS-GC conditions are presented in Table 5.

4.6. Gases

Gases are obviously the most volatile organic pollutants and in general have only limited water solubility. The use of liquified petroleum gas as a domestic fuel can sometimes lead to gas intoxica-tion and therefore forensic analysis for these lower hydrocarbons may be necessary. Victims of these accidents will retain evidence of exposure in the blood [279]. The analysis involves only warming the samples in a closed HS container (needed owing to the low k values). The only major precautions that must be taken are during the sampling and storage of the samples. Porous polymer adsorbents are used as column packing

materials and FID is commonly used in detec-tion. Detailed HS-GC conditions are presented in Table 6.

4.7. Hydrogen cyanide

Cyanide determination in blood has been conducted as evidence of cyanide intoxication in unnatural deaths and in the diagnosis of nitro-prusside prescription. In fire accidents, blood cyanide is a biological reflection of exposure to hydrogen cyanide gas, as a result of combustion of nitrogen-containing materials. Determination of cyanide levels in biological samples has usual-ly been performed through pretreatment by distillation and microdiffusion followed by the Könic spectrophotometric determination. HS-GC determination is a more rapid analysis and displays high sensitivity and specific detection. The procedure involves the liberation of cyanide from the met-Hb complex and conversion into the volatile protonated form by the addition of acid. Sulphuric acid, phosphoric acid and acetic acid are all used as reagents. The partition coefficient of hydrogen cyanide (HCN) is rela-tively high (ca. 100) [33,286], necessitating sensi-tive GC detection methods such as FTD (NPD) or ECD (after precolumn conversion into cyanogen chloride with chloramine T) [287,288]. Porous polymer adsorbents are used as station-ary phases. Although acetonitrile is often used as an internal standard [289,290], Seto et al. [33] have shown that acetonitrile displays a different HS behaviour to HCN and a small amount of acetonitrile might be present within the back-ground owing to environmental contamination. In addition, the HS-GC method has the un-avoidable disadvantage of thiocyanate interfer-ence; thiocyanate is converted into HCN in the presence of Hb under acidic conditions [33]. Detailed HS-GC conditions are presented in Table 7.

4.8. Endogenous volatile metabolites

Endogenous volatile metabolites are normal by-products of intermediate metabolites, and are therefore compounds of similar chemical struc-

Table 5
Halogenated hydrocarbons

Substance	Specimen	Additive	I.S.	HS temperature (°C)	HS time (min)	HS container	HS injection	Column	Oven temperature (°C)	Carrier gas (ml/min)	Detection	Limit	Ref.
Halothane	BL			30	45	Tube	MA	20% SE-30 CS 0.6 m × 6.4 mm	100	Ar 45	FID		2
CCl₄	BL, UR, ML	Na₂SO₄		60	3		MA	25% CW 1540 Diatoport ST 116 1.8 m × 6.4 mm	132	N₂ 1.1 atm	FID		87
Chlorinated aliphatic hydrocarbon	BR	AmSO₄				Flask	MA	20% AGL CS 1.8 m × 3 mm			FID		258
CH₂Cl₂	BL, UR			75–80	5	Flask	LP	20% CW 20M (80/100) Gas Chrom Q 1.8 m	25	N₂ 90	FID	22 µg/l	259
TCE	BL, UR	Pb, AcOH		60	3 h		MA	10% OV-17 (80/100) Gas Chrom Q 1.8 m × 3 mm	125	N₂ 20	ECD	0.5 µg/ml (CH TCE)	123
CH, TCA TCE, TCA C₂HCl₃	BL	H₂SO₄ H₂SO₄ Me₂SO₄		60	4 h	Vial	MA	5% OV-17 (80/100) CS G 2 m × 3.8 mm	120	N₂ 40	ECD	0.1 µg/ml (TCA) 3 µg/l (C₂HCl₃) 60 µg/l (TCE) 30 µg/l (TCA)	124
CH₂Cl₂ CHCl₃, CCl₄ 1,2-DCE	BL, UR		1-Chlorobutane	90	5	Tube	MA	PP Q (100/120) 1.8 m × 4 mm	175 (2) 5/min 235	He 25	HECD	0.1 mM	260
C₂H₃Cl₃	BL, BR, MF		nPrOH					PP Q (50/80) 0.75 m × 3 mm PP P (80/100) 1 m × 3 mm	180 140	N₂ 37 He 35	FID MS		261
Fron	BL, LV,			37	20	Vial	MA	PP Q (100/120) 1.8 m × 2 mm	150	N₂ 30	FID	2 nM (BL) 10 nM (LV)	262
Chlorinated aliphatic hydrocarbon	BL, BR, FT		cisDCE	40	60	Vial	MA	PP P (80/100) 1 m × 3 mm	155		MS	10–20 pg	263
CCl₄, CHCl₃, CHBrCl₂, CHBr₂Cl, CHBr₃, C₂HCl₃	SR AP			115	30 20		DN	n-Octane (100/120) Porasil C 1.8 m × 6.4 mm	60 7/min 140	N₂ 30	HECD		264
Environmental pollutant	BL, UR, ML AT			106			DN	1% SE-30, 0.32% Tullanox SCOT 80 m × 0.4 mm	30 4/min 220	He 1.5	FID		265
1,2-DCE	BL, LV, LN, SP, BR, KD, AP	Citric acid	CH₂Cl₂	90	30	Vial	MA	Tenax GC 4 m × 4 mm	150	N₂ 43	FID	ng/ml 25 (BL) 50 (Tissue)	266
CHCl₃	BL, BR, LV GC		nPrOH					PP Q	160 175		FID		267
C₂HCl₃	LV, BR, KD AT			60			DN	0.2% CW 1500 (80/100) CP C 1.8 m × 2 mm	60 2/min 100	He 35	HECD	50 pg	268
CCl₄	BL, LV, SR			90	14	Vial	PN	UCON LB 550X (80/100) CS G 3.6 m × 3.2 mm	70	N₂ 30	FID	2 ng/ml	179
1,1-DCE	BL			55	30	Vial	MA	Durapak 1.8 m × 3.2 mm	80	N₂ 25~28	ECD		269
CCl₄, CH₂Cl₂, C₂H₃Cl₃, C₂HCl₃	BL, UR, GC BR, LN, LV			80	15	Vial	PN	BP-1 25 m	45 (5) 10/min 150 (5)		FID MS		270
TCA[a]	PL	MgSO₄		30	60	Vial	MA	10% OV-1 (100/120) Supelcoport 2 m × 2 mm	50	N₂ 15–20	ECD	2 µg/l	181

Table 5 (continued)

Substance	Specimen	Additive	I.S.	HS temperature (°C)	HS time (min)	HS container	HS injection	Column	Oven temperature (°C)	Carrier gas (ml/min)	Detection	Limit	Ref.
C_2HCl_3	FB	SDS	DCPOH	37	60	Vial	MA	GSB/SP 1000 SCOT 27 m × 0.5 mm	100	N_2 10	ECD	0.3 µM	119
TCA[a]	UR		nBuOH	45	20	Vial	MA	10% CW 20M (100/120) CS W AW 2.5 m × 3 mm	130	N_2 70	FID	2 mg/l	256
CF_2BrCl	BL, KD						PN	OV-101 25 m	RT		MS		271
TCA (ester)[b] TCE	UR	H_2SO_4 Me_2SO_4 MeOH		75	30	Vial	PN	PEG 20M 1.8 m	130	N_2 30	ECD		93
TCA (ester)[b] TCE	UR	H_2SO_4 Me_2SO_4 MeOH		75	60	Vial	PN	DB-WAX 30 m split 30:1	140	N_2 1	ECD	0.1 mg/l (TCA) 0.3 mg/l (TCE)	94
$CHCl_3$	BL, BR, KD, LV, Bile, GC		nPrOH	45	30	Vial	MA	5% CW 20M (60/80) CP B 1.8 m × 2 mm	80	He 40	FID		272
Br^- $(CHBr_3)$[b]	AQ	Citrate $KMnO_4$ MnO_2 H_2SO_4 $MgSO_4$		20	40	Vial		20% DC-550 (80/100) CS W AW DMCS 3 m × 3.4 mm	150	N_2 80	ECD		122
$CHCl_3$	PL			30	60	Vial	MA	10% OV-1 (80/100) CS W AW 3 m × 3.2 mm	70	N_2 30	ECD	22.5 ng/l	273
Vinylidene fluoride	BL			55	90	Vial	MA	Unipack IA 1.5 m	40	H_2 30	MS	6 ng/ml	274
Halothane	BL	EDTA		56	30	Vial	MA	PP P (80/100) 2.5 m × 3 mm	150	N_2 35	FID		88
TCA (ester)[b] TCE, C_2HCl_3	BL, UR	βG H_2SO_4	BCE	90	90	Vial	LP	SE-30 (3 µm) 25 m × 0.31 mm split 1:5	70 (1) 20/min 140 (2)	He 2	ECD		257
CH, TCE	LV	βG $AmSO_4$ H_2SO_4	$iC_2H_3Cl_3$	60	90	Vial	LP	SE-30 (3 µm) 25 m × 0.31 mm split 1:5	70 (1) 20/min 140 (2)	He 2	ECD		120
TCA (ester)[b] $CHCl_3$	BL	H_2SO_4	nPrOH iPrOH	90 40		Vial	PN	0.2% CW 1500 (80/100) CP C 1.8 m × 1.6 mm	90	N_2	FID		275
								10% CW 1500 (80/100) CS W 1.8 m × 1.6 mm	85	N_2	FID		
								PP Q 1.5 m	150 (165)	N_2 He	FID MS		
$C_2H_3Cl_3$	BL			80		Vial	PN	FFAP (80/100) CS W-AW 2.4 m × 3.2 mm	85	Ar–CH_4 (1:19) 40	ECD		276
Enflurane	BL, BR, FT		Isoflurane	63	2 h	Vial	PN	15% CW 1500 (80/100) CS W NAW 1.8 m × 3.2 mm	70	N_2 147 kPa	FID MS		277
C_2 halocarbon	Tissue	Isooctane		90 100 55	10		PN LP	10% FFAP 3% OV-17 3% SP-1000 30 m × 3.2 mm	110 150 60	Ar–CH_4 60	ECD		149
C_2H_5Cl	BL, VH	NaCl, NaF	nPrOH	37	15	Vial	MA	PP S 2 m	195		FID		278

[a] TCA is converted into chloroform by heating.
[b] Substances in parentheses are those which are converted by derivatization from non-volatile analytes.

Table 6
Gases

Substance	Specimen	Additive[a]	HS temperature (°C)	HS time (min)	HS container	HS injection	Column	Oven temperature (°C)	Carrier gas (ml/min)	Detection	Limit	Ref.
O_2, N_2	Biological fluids		RT		VS	VE	MS 5A 1.2 m × 0.64 mm	70 100	He	TCD		22
C_2H_2	BL	n-Octanol Pyrogallate	RT			VE	CS P (60/80) 0.51 m × 6.4 mm + 30% BMEEE (60/80) CS P 1.8 m × 6.4 mm 30% BMEEE (60/80) CS P 3.6 m × 6.4 mm		He 50	TCD		23
Cyclopropane C_2H_4 N_2O							Silica gel (60/200) 3.6 m × 4.8 mm 30% HMPA (60/80) CS P 3.6 m × 6.4 mm					
O_2, CO_2	BL	Sapoinin Acid $K_3Fe(CN)_6$	RT			SW	Hexamethylphosphoramide + MS 13X		He 60	TCD		24
Propane	BL, LV, KD		50	60[b]	Bottle	LP	PP Q (80/100) 1.125 m × 3 mm	100	N_2 2 kg/cm²	FID	10 nl	280
Butane	UR, GC		(RT)[b]				PP T (150/200) 0.375 m × 3 mm					281
N_2O	BL		25	30	Vial	MA	PP Q 4.2 m × 2.9 mm	50	H_2 120	TCD		282
Butane	BL, MS,		RT	60	Bottle	LP	PP Q (80/100) 2 m × 3 mm	120	N_2 80	FID	1 nl	283
Propane	MF											
Gasoline	BL	(ClBz)	60	20	Bottle	MA	2% OV-17 (60/80) CS W 2 m × 3 mm	85	He 20	MS		284
Propane	LV, BL, BR	H_2SO_4 (AcMe)	RT	60	Bottle	MA	PP Q (80/100) 2 m × 3 mm	120	He 30	MS		285
Propane Butane	BL, organ	(nPrOH)	75	10	Vial	PN	UB A 2 m × 3.2 mm	90	N_2 40	FID		163
Propane	BL		40	30	Vial	MA	SPB-1 100 m × 0.25 mm split 50:1	40	He 2.3 cm/s	FID	1.5 μl/l	279

[a] Substances in parentheses are internal standards.
[b] After HS incubation as described, the temperature of the HS vial is returned to room temperature, and HS vapour is injected into the GC system.

Table 7
Hydrogen cyanide

Specimen	Additive[a]	HS tempera- ture (°C)	HS time (min)	HS container	HS injec- tion	Column	Oven tempera- ture (°C)	Carrier gas (ml/min)	Detec- tion	Limit (µg/ml)	Ref.
BL	H_3PO_4	60	60	Vial	MA	PP QS 1.8 m × 3.2 mm	80	He 30	FTD	0.05	286
AQ	H_3PO_4	80	10	Vial	PN	PP Q (80/100) 2 m × 3 mm	100	N_2 40	NPD	0.01	291
AQ	H_3PO_4, Br_2, phenol	70	10	Vial	PN	PP Q (80/100) 2 m × 3 mm	120	N_2 50	ECD	0.005	292
BL	AcOH	RT	30	Vial	MA	PP Q (100/120) 1.8 m × 2 mm	110	N_2 20	NPD	0.05	293
BL	AcOH, (MeCN)	RT	30	Vial	MA	PP Q (100/120) 1.8 m × 2 mm	120	He 20	NPD	0.05	289
BL	H_3PO_4, (AcH) (MeCN)	50	30	Vial	MA	PP QS (50/80) 2.1 m × 3 mm	80	N_2 50	FID FTD	0.8 0.2	290
BL	H_2SO_4	50 (RT)[b]	30[b]	Vial	MA	PP O (80/100) 1.5 m × 3 mm	120	He 60	FTD	0.2	294
BL	H_3PO_4(CH_2Cl_2)	50	10	Vial	MA	7% HC M-18 (80/100) CS W AW-DMCS 3 m × 3 mm	55	N_2 30	ECD	0.05	287 288
BL	H_3PO_4, $NaNO_3$	60	30	Vial	PN	PP Q	110	He 4.7	NPD		180
BL	H_3PO_4	50	30	Vial	MA	GS-Q 30 m × 0.53 mm split 1:5	110		NPD	0.0004	33

[a] Substances in parentheses are internal standards.
[b] After HS incubation as described, the temperature of the HS vial is returned to room temperature, and HS vapour is injected into the GC system.

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Table 8
Endogeneous volatile metabolites

Substance	Specimen	Additive	HS temperature (°C)	HS time (min)	HS container	HS injection	Column	Oven temperature (°C)	Carrier gas (ml/min)	Detection	Limit	Ref.
AcH, EtCHO, AcMe, AcEt	ML	Na$_2$SO$_4$	60	3	Vial	MA	20% CW 20M (60/80) CS W-3 m × 3.2 mm	100	N$_2$ 20	FID	0.1 ppm (w/v)	87
EtOH, AcMe, AcEt, AcH, AcPr, Me$_2$S	UR, BL, ML, AQ	Na$_2$SO$_4$	60	15	Vial	MA	20% CW 20M (60/80) CS P AW-HMDS 3 m × 3.2 mm	100	N$_2$ 15.4 (17.4)	FID		83
AcMe, BKB^a	SR		RT		Bottle	MA	20% HC (60/80) CS W 1.8 m × 4 mm	60	N$_2$ 40	FID		297
AcMe, EtOH, MeOH, AcOAc, aldehydes			RT		Vial	MA	20% HC (60/80)	80	N$_2$ 70	FID		99
AcMe, ACA, BHB	BL, LV	KOH PCA K$_2$Cr$_2$O$_4$ H$_2$SO$_4$	65	10	Vial	PN	CS W 1.8 m × 4 mm 15% PEG (60/100) Celite	75	N$_2$ 35	FID	1 μM	295
Volatile metabolites	UR	AmSO$_4$	100			DN	Emulphor ON 870 100 m × 0.5 mm			FID FPD		298
Volatile compounds	UR	Phosphate	80			DN	5% Igepal, 95% SF-96-50 300 m × 0.76 mm	50 (30) 0.5/min 150 (6 h)	He 25	FID		299
Volatile compounds	UR	Phosphate	85	60		DN	5% Igepal CO-880, 95% SF 96(50) 305 m × 0.76 mm	25 0.25/min 30 1.16/min 172 (20)	N$_2$ 20 p.s.i.	MS		300
Volatile organic metabolites	UR	AmSO$_4$	90	60		DN	Emulphor ON-87 100 m × 0.5 mm	60 (16) 2/min 175	N$_2$ 5	MS		301
AcMe, ACA, BHB	UR, PL, body fluid	H$_3$PO$_4$ K$_2$SO$_4$ (butanone) KOH H$_3$PO$_4$ K$_2$Cr$_2$O$_7$ Porasil E	70	15	Bottle	MA	PP R (100/120) 1.2 m × 3 mm	180	N$_2$ 57	FID		296
Organic volatile compounds	Body fluid					DN	Witconel LA-23 100 m × 0.25 mm; Silar 10C 100 m × 0.25 mm	50 (10) 1.5/min 160 (80) 40 (6) 2/min 180 (30)	He 1.5	FID ECD		302

Table 8 (continued)

Substance	Specimen	Additive	HS temperature (°C)	HS time (min)	HS container	HS injection	Column	Oven temperature (°C)	Carrier gas (ml/min)	Detection	Limit	Ref.
AcMe, Me₂S, AcPr, Phenol	UR	NaCl	85			DN	CW 20M 50 m × 0.32 mm	85 (6) 4/min 180 65 (3) 6/min 180	He 1	FID		303
iPrOH AcMe	BL, BR, LV, KD,		37	20	Vial	MA	5% CW 20M (60/80) CP B 1.8 m × 2 mm	85 95	N₂ 30 He 20	FID MS		304
AcMe ACA BHB	PL, KD, LN, LV	NaOH PCA K₂Cr₂O₇ H₃PO₄	60	90	Vial	MA	CW 1500 2 m	75	N₂ 40	FID	1.19 ppm (w/v)	305
EtOH, MeOH, iPrOH, nPrOH, iBuOH, AcH, AcMe, AcEt, AcOEt	BL	NaCl	50	30	Vial	LP	DB-WAX (0.25 μm) 30 m × 0.25 mm	40 (4) 10/min 150	He 18	MS	5 pM (AcOEt) 1.5 nM (MeOH)	306
AcMe	BL	NaF, NaCl	40	18	Vial	PN	0.2% CW 1500 CP C 2 m × 3 mm	100	N₂ 20	FID	0.01 mg/l	91
Short-chain fatty acids	Faeces	HCOOH, Li₂SO₄ (2-ethylbutyric acid)	90	30	Vial	LP	BP-20 (1.0 μm) 25 m × 0.53 mm split 1:7	50 (2) 40/min 100 (0.1) 8/min 215 (1)	He 3	FID	0.7 (C₇)– 17.9 (C₁) μmol per vial	45

[a] After heating at 90°C for 1 h, converted acetone is measured.

Table 9
Unique applications of HS-GC

Substance[a]	Specimen	Additive[b]	HS temperature (°C)	HS time (min)	HS container	HS injection	Column	Oven temperature (°C)	Carrier gas (ml/min)	Detection	Limit	Ref.
Methanethiol	UR		60		Tube	MA	20% DC-200 (60/80) CS P AW 2.4 m × 6.4 mm	50–60	N₂ 75	FID		95
CH₃SH Me₂S₂ EtOH	UR FB	K₂CO₃	60			PN	0.4% CW 1500 (60/80) graphite 2 m × 3 mm	115	N₂ 180 kPa	FID	0.22 mM 0.44 mM 2.4 mM	310
Acetylcholine (Me₃N) EtOH AcOEt, nPrOH	UR	K₂CO₃	60	5	Vial	MA	0.4% CW 1500 (60/80) graphite 2 m × 3 mm	110	N₂ 12	FID	0.22 mM (EtOH) 0.1 mM (Me₃N)	103
MeHgI	LV, KD	IAA, NaSCN	80	5	Vial	PN	10% AT-1000 (80/100) CS W AW 1 m × 3 mm	150	Ar 100	MIPE	1.5 ng/ml	307
Methamphetamine Amphetamine	UR	K₂CO₃ KOH	80	20	Tube	MA	DB-17 (0.5 µm) 30 m × 0.32 mm	130	N₂ 50 cm/s	FID	0.02 µg/ml 0.05 µg/ml	104
Methamphetamine Amphetamine[c]	UR	K₂CO₃	80	20	Tube	MA	DB-1 (0.25 µm) 30 m × 0.32 mm	130	N₂ 50	FID	0.03 µg/ml	105
MeHgI	AQ	IAA	80	4.5	Vial	PN	10% AT-1000 (80/100) CS W AW 1 m × 3 mm	150	Ar 3.5	MIPE	0.5 ppm MeHgI	31
Methamphetamine Amphetamine	UR	K₂CO₃	RT		Tube	MA	DB-17 (0.25 µm) 30 m × 0.32 mm	150	H₂ 3.5	FID	1.0 µg/ml 1.5 µg/ml	106
MeHgI	Dogfish tissue	IAA, H₂SO₄	80	4.5	Vial	PN	10% AT-1000 (80/100) CS W AW 1 m × 3 mm	150	Ar 100	MIPE	20 ng/g	308
MeHgCl, EtHgCl PheHgCl	Mussel	Solvent			Vial	PN	10% AT-1000 CS WAW 2 mm × 2 m CS 101–108 2 m × 2 mm Superox-FA (2 µm) 10 m × 0.53 mm RSL-300 (2 µm) 10 m × 0.53 mm	150 155 150 100	100 20 25 25	MIPE ECD	0.5 µg/l	309
Me₂S	BL AP		60 (RT)[d]	4 h[d]	Vial	MA	10% Polyphenyl ether OS-124 (60/80) SL TPA 3 m × 3 mm	60	N₂ 100	FPD	2.5 µg (BL) 5 µg (AP)	311
EtSH	BL		60 (RT)[d]	11[d]		DN	DB-624 30 m × 0.53 mm	30 (2) 8/min 250	He 27.6 kPa	MS	0.1 µg/l	279
Et₂NH EtOH, AcH	BL, SC, LV. bile	(Bu₂NH) (nPrOH)					20% AGL, 5% KOH CS G 5% CW 20M CP	70 80				312

[a] Substances in parentheses are those which are converted by derivatization from non-volatile analytes.
[b] Substances in parentheses are internal standards.
[c] Trifluoroacetyl derivatives converted through in-column reaction are measured.
[d] After HS incubation as described, the temperature of the HS vial is returned to room temperature, and HS vapour is injected into the GC system.

Table 10
Complex mixtures of analytes

Substance	Specimen	Additive[a]	HS temperature (°C)	HS time (min)	HS container	HS injection	Column	Oven temperature (°C)	Carrier gas (ml/min)	Detection	Limit	Ref.
Carbonyl, sulphide, ester, alcohol	AQ	Na$_2$SO$_4$	60	5	Vial	MA	20% CW 20M (60/80) firebrick AW 3 m × 3.2 mm	100	N$_2$ 20	FID	1 ppm (w/v)	5
Ether, halothane, CHCl$_3$, CCl$_4$, EtOH, AcMe, C$_2$HCl$_3$, paraldehyde	BL	K$_2$CO$_3$	70	1	Vial	MA	10% Silicone oil (60/80) Embacel 3.6 m	RT	H$_2$	FID		4
Sulphide, ketone, alcohol, ester, aldehyde	AQ	Na$_2$SO$_4$	60	5	Vial	MA	20% CW 20M (60/80) CS W AW 2.9 m × 3.2 mm	100	N$_2$ 20	FID	0.01–1 ppm (w/v)	315
Solvents	BL		25			MA	15% Flexol 8N8, 10% DIP, 3% PEG 600 (42/60) C-22 firebrick 1.8 m × 3 mm	95		βID		27
N$_2$O	BL		25	1–2 h	Vial	MA	Active carbon 1 m	35	N$_2$ 100	TCD		316
Cyclopropane							25% PEG–Celite 2 m	24	50	FID		
Ether							25% PEG–Celite 1.5 m	40	50	FID		
Fluothane							25% PEG–Celite 1.5 m	60	50	FID		
Penthrene							25% PEG–Celite 1 m	80	50	FID		
Cyclopropane	BL		25	1–2 h	Vial	MA	25% PEG–Celite 2 m	24	N$_2$ 50	FID		19
Ether							25% PEG–Celite 1.5 m	40	50	FID		
Halothane							25% PEG–Celite 1.5 m	60	50	FID		
Methoxyflurane							25% PEG–Celite 1 m	80	50	FID		
N$_2$O							Activated charcoal 1 m	35	100	TCD		
Hydrocarbon anaesthetics	BL		37	15	Bottle	MA	5% Silicone gum rubber 60/80 CS W 1.5 m × 3.2 mm	100	He 25	FID		20
Anaesthetics	BL		RT	15	Syringe	MA	5% SE 30 (50/80) CS W DMCS 1.5 m × 3.2 mm	85	He 25	FID		21
Volatile compounds	PL		95			DN	10% GE SF-96, 1% Igepal CO 880 9.1 m × 0.5 mm		He 8	FID		317
Volatile organic compounds	BL, UR					DN		−20 (10) 25 (72) 2/min 170		MS		318
	BL, UR, tissue		RT		Jar	MA	PP Q (120/150) 3 m × 3.2 mm	−90 10/min 250	He 300	MS		319

Application	Sample	IS	°C	Time	Vessel	Method	Column	Temp. program	Carrier	Det.	Sens.	Ref.
Paraldehyde Propane MeBz CH$_2$Cl$_2$	BL, PL, UR		60	30	Vial	PN	0.2% CW 1500 CP C 1.8 m × 3.2 mm	120 65 145 80	N$_2$ 15 20 20 15	FID		320
Volatile compounds	SR, UR	(CHClBr) (ClCH$_2$BrHCH$_3$)		15		DN	2% CW 1500 CP C	50 8/min 180	He 25	MS		321
	Tissue Fluid				Vial	PN	5% CW 20M (60/80) CP B 4 m × 2 mm	70 5/min 170				1
Environmental pollutants	ML				Vial	DN	SE-30 WCOT 80 m	30 (2) 4/min 240	He 1.7	MS		322
Volatile organic compounds	BL	(iC$_2$H$_3$Cl$_3$) (EtBz)	65	15	Vial	MA	0.3% CW 20M (80/100) CP C 2 m × 2 mm	35 (2) 5/min 175 (8)	N$_2$ 30	FID ECD		156
Volatile organic compounds	BL, LV, BR	EtBz	65	45	Vial	MA	10% CW 400 (80/100) CS W 1.5 m × 6.4 mm (30/50) Tenax GC	170 140	N$_2$ 40	FID		313
Organic pollutant	BL	(xyl-d$_{10}$)	40–50	15		DN	SE-54 50 m × 0.3 mm	−20 (6) 4/min 130		MS	0.5 ng/ml	323
Solvents	Biological materials		80	30	Vial	LP	5% CW 20M (60/80) CP B 1.3 m × 2 mm	65(3) 5/min 165	He 10	FID		314
Low-molecular-mass volatile compounds	BL, LR, MS, FT		37	1–3 h	Vial	MA	0.3% CW 20M (80/100) CP C 1.3 m × 4(2) mm	50 (2) 8/min 195 (5)	He 15, 4	FID ECD		75
Volatile substance abuse	BL, UR, tissue	(iC$_2$H$_3$Cl$_3$) (EtBz)	65	15	Vial	MA	SPB-1 (5 μm) 60 m × 0.53 mm	40 (6) 5/min 80 10/min 200	He 8.6	FID ECD		59

[a] Substances in parentheses are internal standards.

ture (alcohols, ketones and acids). Clinical or pathological analysis of urine or blood for these substances is important in the diagnosis of disease states. In addition, the occurrence of acetone in blood and breath has interested both forensic toxicologists and clinicians [91]. An example of HS-GC analysis applied to these metabolites is in the determination of ketone bodies. Individual ketone compounds are measured separately. Acetoacetic acid levels are determined after thermal conversion into acetone by acid, and β-hydroxybutyric acid levels are determined after oxidative conversion into acetone by the addition of potassium dichromate. Acetone levels are determined in the presence of potassium hydroxide, to prevent the degradation of the other ketone bodies [295,296]. The concentration of acetone in blood did not differ appreciably among subject groups such as drunk drivers, patients with type I diabetes mellitus and healthy blood donors [91]. Dynamic HS-GC methods have also been used in these types of analyses. Detailed HS-GC conditions are presented in Table 8.

4.9. Unique applications of HS-GC

Several unique applications of HS-GC methodology for the analysis of biological samples have been reported. Levels of certain stimulants (amphetamine and methamphetamine) were determined by the addition of potassium carbonate to convert the amine form of the stimulant into the volatile deprotonated form, thus increasing the vapour pressure [104–106]. The determination of methylmercury is applicable in the study of environmental mercury pollution, and a special GC detector (microwave-induced plasma emission) was used after conversion of this compound into the volatile iodide [307–309]. Detailed HS-GC conditions are presented in Table 9.

4.10. Complex mixture of analytes

The diagnosis of substance abuse can be confirmed by detecting a mixture of volatile substances through the use of HS-GC [59,156,313] as a general screening procedure [254,314]. This system is qualitative, with semi-quantitative evaluation of various constituents. The split detection mode of FID and specific ECD can be utilized as a simple method of screening for a wide range of volatile substances within biological fluids [59]. Mass spectrometry is another potential mode for multiple detection of various kinds of volatile substances [254]. Detailed HS-GC conditions are presented in Table 10.

5. Dynamic HS-GC and other analytical methods

The application of dynamic HS-GC to the determination of volatile substances in biological samples has offered improved sensitivity over static HS-GC methods. This method is used mainly in the determination of endogenous volatile metabolites and various instruments for this technique have been developed [63,298–303,317,318]. Environmental pollutants have also been measured using dynamic HS-GC [264,265,268,321–325]. Michael et al. [265] described a standard procedure for dynamic HS-GC analysis of volatile pollutants in biological samples. Variations of the dynamic HS-GC method include sample purging (where the analyte is bubbled or swept with a carrier gas), sample trapping [where the purged analyte is trapped by physical (cold) or chemical adsorption (using various adsorbents, e.g., Tenax GC)] and the relative temperatures of purging, trapping and desorption.

Early anaesthetic agents, halothane [326] and diethyl ether [327], in the gas phase extracted from blood samples were determined by infrared and mass spectrometry, respectively. Volatile substances extracted from the gas phase of biological samples have also been determined directly by mass spectrometry [328] and Fourier transform infrared spectrometry [319].

6. Acknowledgements

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