We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Determination of Volatile Substances in Forensic Samples by Static Headspace Gas Chromatography

Ernesto Bernal[1,2,3]
[1]University Center Ixtlahuaca,
Ixtlahuaca-Jiquipilco Road, Ixtlahuaca, Mexico,
[2]Forensic Medical Service of the Federal District, Col Doctores, Mexico City,
[3]Institute of Expert Services of the Attorney General of the State of Mexico,
Col San Sebastián, Toluca, Mexico,
Mexico

1. Introduction

The technique of static headspace gas chromatography has great acceptance in the forensic field, especially for the determination of ethanol in biological samples (Macchia et al., 1995; Tagliaro et al., 1992), so most forensic laboratories in the world have this equipment and perform this analysis on a routine basis, but in many of these laboratories, equipment is exclusively employed to determine ethanol, even when this technique can be used to determine many other substances of toxicological interest, volatile substances, without major changes to the equipment (Seto, 1994), thus we can conclude that these laboratories do not exploit all the possibilities of the technique.

The determination of volatile substances is one of the most important tests in forensic toxicology, (Broussard, 2003). Volatile substances can be defined as those organic compounds whose vapor pressures are greater than or equal to 0.1 mm Hg at 20 ° C; the Environmental Proteccion Agency (EPA) defines as volatile substances any compounds of carbon, excluding carbon monoxide, carbon dioxide, carbonic acid, metallic carbides, and ammonium carbonate which participates in atmospheric photochemical reactions. These substances may be involved in forensic cases as toxic agents and because of their use as drugs of abuse; in that context, the most important volatile substance is ethanol, however, there are other volatile substances of forensic interest, such as organic solvents, anesthetics, alkyl nitrites, etc. (table 1) (Moffat et al., 2004).

The determination of volatile substances in forensic samples has been carried out through titrations, spectrophotometric methods and chromatographic methods, as well (Seto, 1994). Titrations and spectrophotometric methods are not specific and usually lack sufficient sensitivity, besides not being able to analyze simultaneously all the volatile substances. In contrast, gas chromatography is qualitative (by the use of retention time) and quantitative (by the use of signal strength), so it is able to analyze simultaneously several volatile substances with the adequate sensitivity and specificity necessary in forensic environments,

therefore, this technique is used to make such determinations. Historically, three types of methods have been used for the preparation of the samples: solvent extraction, direct injection and headspace volume injection; as of late, forensic laboratories prefer the latter over the other two techniques.

Solvents	Aromatic Hydrocarbons	Alkyl Nitrites	Anesthetics
Ethyl Alcohol	Toluene	Amyl Nitrite	Ethyl Ether
Methyl Alcohol	Benzene	Isobutyl Nitrite	Chloroform
Acetone	Xylenes		Fluorocarbons

Table 1. Examples of volatile substances of forensic interest.

The headspace injection technique has great advantages over other methodologies, namely providing a clean injection, resulting in lower spending of gas chromatograph consumables; it is simple, minimizes the possibility of artifacts during the analysis, diminishes the possibility of contamination and accurately quantifies analytes.

This paper will review theoretical aspects of the static headspace technique, as well as complementary additions to it (Kolb, 1999; Slack et al., 2003), the most significant substances from the standpoint of forensic toxicology that can be analyzed by this technique as ethanol (Kugelberg & Jones, 2007; Macchia et al., 1994; Tagliaro et al., 1992), congeners of alcoholic beverages (Iffland & Jones, 2003), inhalants (Angerer & Horsch, 1992; Seto, 1994), anesthetics (Pihlainen & Ojanperä, 1998), carbon monoxide (Boumba & Vougiouklakis, 2005; Vreman et al., 1984) and cyanide (Calafat & Stanfill, 2002; Felby, 2009); in each case, the analytical conditions and modifications necessary for the equipment to perform each analysis will be exposed, advantages and disadvantages of this technique over other existing procedures will be discussed and also, issues relating to development of methods for this technique and their validation.

Finally, newest developments in the area will be mentioned, and how they have replaced headspace injection in some applications.

2. Static headspace

If the components of interest in a solid or liquid sample are volatile, a good way to analyze them is to examine the concentration of these analytes in the gas phase above the matrix (headspace) when in a closed container, either by taking a sample directly from the gas phase or trapping and concentrating the gas prior to analysis. This type of extraction techniques are known as headspace analysis (Smith, 2003); the analysis and subsequent separation of volatile substances is normally carried out by the technique of gas chromatography, which is a mature technology, reliable and supported by a large body of work. The sample can be in contact and in equilibrium with the extractant gas (static or equilibrium headspace), or volatile compounds can be extracted by a steady stream of inert gas (dynamic headspace).

The different headspace sampling techniques can be classified into one-step procedures, such as static headspace, where an aliquot of the vapor phase is transferred in a closed container directly to the gas chromatograph, and two-step procedures, where the volatile analytes are transferred from the matrix of the headspace to a "trap" where they are released

by the action of heat or by a stream of carrier gas, and transferred to the gas chromatograph; dynamic headspace and solid phase microextraction (SPME) fall into this category. Regardless of the kind of headspace used, the sample is always a more or less diluted gas (Kolb, 1999). The technique you choose depends on several factors, such as the type of sample to be tested, if a quantitative or qualitative analysis is desired, the required sensitivity, automation and budget.

Static headspace extraction, also known as equilibrium headspace extraction, is one of the techniques used for qualitative and quantitative analysis of volatile substances in the forensic field, in this technique the sample is placed in a closed vial, the volatile analytes disseminate into the headspace of the vial (figure 1), once equilibrium is reached between the analyte concentration in the headspace and the analyte concentration in the sample, a portion of the headspace is taken and injected into the gas chromatograph; this can be done manually or with an autosampler, this process will be usually carried out at a pressure and temperature above ambient conditions (Slack et al., 2003).
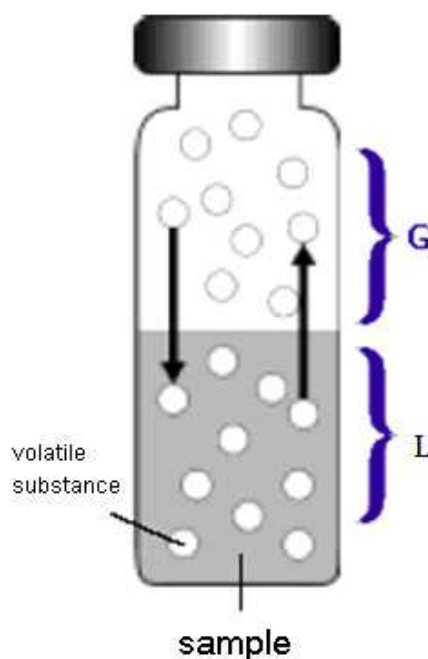

Fig. 1. Headspace extraction fundament. G: Gas, L: liquid

This technique is simple, relatively inexpensive, minimizes the formation of artifacts and can accurately quantify volatile substances with low solubility in water, its only disadvantage being its lower sensitivity with respect to dynamic headspace techniques, although this disadvantage is unimportant in most of its applications to forensic toxicology.

2.1 Instrumentation for static headspace extraction

The equipment for static headspace extraction consists of a container, where equilibrium takes place, a device that heats the container at a constant temperature and an injection device, which transfers a portion of the headspace gas to the gas chromatograph. The container is a glass vial of between 5 ml and 25 ml capacity, which is sealed with a septum coated with polytetrafluoroethylene (PTFE) and an aluminum cap, using a crimp. Injection

can be manual and automatic, however, the first of these techniques have poor reproducibility and there may be contamination between runs, so it is advisable to perform the technique using an autosampler (Seto, 1994).

Current autosamplers work with two different techniques. The first one employs sample loops; in order to fill these loops with headspace gas, the closed vial is pressurized to a pressure level above that present in the vial; the pressurized headspace is then temporarily connected to the sample loop, causing the headspace gas to expand through the sample loop and into the atmosphere; once the sample loop is filled, a valve is changed and its contents are transferred to the chromatographic column. This type of sampling has been commonly used for gas sampling in other areas, such as the petrol industry.

Instead of filling a sample loop, the headspace gas can be expanded directly to the chromatographic column, this type of sampling is called pressure-balance sampling; in this technique, the carrier gas enters the gas chromatograph through a V solenoid valve and is divided before the column. Some of the gas is directed to the column and some to the sampling syringe of the headspace injector. When the syringe enters the vial with the sample, the gas is pressurized; the gas transfer from the headspace to the chromatographic column takes place when the V solenoid valve is closed for a short time, suspending thus the flow of carrier gas; then the headspace gas expands, this time directly to the column, without any loss of headspace gas, as it happens with the previously mentioned procedure. Headspace gas replaces carrier gas during the sampling time, and the volume of headspace gas transferred to the column depends on the sampling time, therefore it is possible to calculate it with accuracy; this volume can be changed by simply changing the sampling time, unlike the previous technique, where the volume of injected gas depends on the sample loop and can only be changed by manually installing another sample loop of a different volume capacity (Kolb, 1999).

2.2 Sample preparation in static headspace extraction

One of the main advantages of static headspace extraction is how easy the sample preparation is; in the case of qualitative analysis, it suffices to place the sample in a vial and seal it with a PTFE septum and an aluminum lid; however, for quantitative analysis, it is necessary to understand and optimize the effects of the matrix, in order to obtain good sensitivity and, above all, accuracy.

2.2.1 Solid samples

For solid samples, it may be necessary to change their physical state. This is achieved by grinding the solid to a powder as fine as possible, or dissolving or dispersing the solid into a liquid. In the first case, the contact volume of the solid is increased in order to establish a better partition between the volatile substance and the headspace gas; however, it is preferred to dissolve the solids in a liquid, because equilibrium is achieved faster this way, and is also more reproducible; on top of that, liquid samples are easier to work with (Slack, et al., 2003).

2.2.2 Liquid samples

Liquid samples are usually simply poured into a vial and sealed immediately after, in order to prevent any evaporation losses (Slack, et al., 2003).

2.3 Optimization of static headspace extraction

Several factors must be optimized in a static headspace extraction in order to obtain a method with the desired extraction sensitivity, reproducibility and efficiency. These factors include the volume of the used vial, the temperature and pressure levels, and how the sample is to be prepared.

2.3.1 Sample

The main factors controlling the sensitivity of the static headspace extraction are the analyte partition coefficient (K), which is the ratio between the analyte concentrations in the liquid phase with the concentration in the gas phase:

$$K=C^L/C^G \tag{1}$$

And the ratio of phases (β), according to the formula:

$$A=C^G=C°/K+\beta \tag{2}$$

Where A is the area of the chromatographic peak obtained for the analyte, C^G is the concentration of analyte in the headspace, C° is the concentration of analyte in the liquid sample, K is the partition coefficient, and β is the ratio volume of the phases. The partition coefficient depends on the extraction temperature, while β is determined by the relative volume between the two phases; In static headspace extraction the sensitivity depends on the solubility of the analyte in the matrix; for analytes with a high partition coefficient, the most important parameter is the extraction temperature, since most of the analyte is in the liquid phase and it can only be passed into the headspace by heating the vial; on the other hand, for analytes with low partition coefficients, they are already present in the headspace even without any heating, so in this case, the most important parameter is the volume relation between the phases. That is, increasing the extraction temperature is only effective in polar volatile analytes, while the sensitivity of non-polar analytes remains essentially unchanged by the increase of the extraction temperature (Slack, et al., 2003).

Quite the opposite, changing the volume of the ratio phases has minimal effect on the sensitivity of polar analytes (high partition coefficients), but affects dramatically the sensitivity of non-polar analytes (low partition coefficients). Another way to increase the sensitivity of the method is by adding a salt, such as sodium chloride, to the sample.

Finally, if an analytical method focused on robustness rather than sensitivity is required, the relation between the partition coefficient and the polarity of the analytes can be used to achieve it; in order to do so, a matrix with high affinity for the analytes is used, so losses due to sample handling or second analyses are prevented. If the values of K are not known, one can simply analyze in the chosen matrix the substance to be determined, as well as determining the area against the sample volume (Slack, et al., 2003).

2.3.2 Chromatographic conditions

Headspace extraction is solely a method of sampling and there are, in principle, no limitations for chromatographic columns and any type can be used, thus it can be selected according the demands of sensitivity resolution and analysis time of a particular analytical

problem. It has been recommended for headpace analysis (Kolb, 1999), to use in the first instance a capillary column of 0.32 mm of internal diameter (I.D.) and 30 m of length. However, if high resolution is required, a longer column with smaller I.D., for example a 50mX0.25 mm I.D. capillary column provides a better separation at the expense of longer analysis time; Nevertheless, in the case of an automated headspace sampler a long analysis time is less unfavorable, because the sample are analyzed anyway unattended overnight. If analysis time is the main parameter, very short capillary columns with I.D.´s of 100 μm or 50 μm can be used. (Kolb, 1999).

Film thickness is an important parameter in gas chromatography because it provides a higher sample capacity for compounds in high concentrations to avoid peak splitting or broadening by overload and consequently poor resolution, However, this is not a problem in headspace analysis, since headspace sample is a diluted gas sample and the amount of analytes is small enough to avoid peak broadening. Film thickness is chosen in headspace analysis to provide good resolution in an adequate time, unless, cryogenic trapping is used, where thick films are preferred.

Column capacity in Headspace gas chromatography has a different meaning and concerns the gas volume which can be introduced in the column without significant band broadening. To avoid any confusions the term headspace capacity has been used (Kolb, 1999). Resuming, peak broadening is determined by injection time, carrier gas flow and the internal diameter of the chromatographic column. Consequently, if high resolution is not required, and a mass spectrometer detector (MSD) is not used, capillary columns with an I.D. of 0.53 mm are preferred, to inject the biggest amount of gas from the headspace and to achieve the best sensitivity.

2.3.3 Enrichment techniques in static headspace extraction

When analytes are under the limit of detection (LOD) of the technique is necessary to use enrichment techniques. In headspace analysis, for this purpose the target analytes must be separated from the headspace gas either by absorption into a liquid or by adsorption onto a solid adsorbent and also by condensation in a cold trap. (Kolb, 1999). Solvent free techniques are particularly desirable in case of trace analysis to avoid problems with solvent impurities. Consequently, cryogenic trapping is the preferred choice to improved detection limits in static headspace analysis

Cold traps are used for two reasons: enrichment purposes and solute band concentration. There are two types of cryogenic trapping: by cryogenic condensation and cryogenic focusing.

In cryogenic condensation the volatile compounds are trapped simply by condensation in traps which usually contain no stationary phase or when the stationary phase has lost is properties as a chromatographic phase, Volatile substances are eluted from the trap by heating and depending on how the cold traps are heated, the analytes can be eluted in a very narrow band that thus, analytes are focused.

In cryogenic focusing, volatile compunds are trapped in the liquid phase of a chromatographic column at a low temperature which, however, preserve its chromatographic properties, In other words cryogenic focusing is based in the same

principles that thermal focusing, commonly used in gas chromatography and the difference in the nomenclature should only indicate the difference in the applied temperature, with cryogenic focusing carried below and thermal focusing above ambient temperature (Kolb, 1999).

Generally, cryogenic focusing is preferred over cryogenic condensation, for several reasons, the first technique needs higher temperatures, consequently its operation is simpler and its more easy to be automated, the rapid heating used in cryogenic condensation may cause decomposition of labile compounds. Moreover, cryogenic condensation has some inherent problems such as breakthrough by aerosol formation., Consequently, trapping may be incomplete by droplet formation, causing peak splitting or distorted peaks, last but not least, in cryogenic focusing, analytes are trapped inside the capillary column, thus, It's a more effective procedure and more simple to achieve than cryogenic condensation; for all these reasons, at the present moment cryogenic focused is favored over cryogenic condensation (Kolb, 1999).

Summarizing, the initial profile of a gas sample depends on sample volume and the inner diameter of the capillary column, film thickness has no effect on the band width causes no focusing effect under isothermal conditions. Only temperature programming helps to elute an originally broad band profile as a sharp peak. However, this approach for highly volatile substances needs low initial temperatures, which leads finally to cryogenic trapping

2.4 Quantitative techniques in static headspace extraction

The four most common approaches to quantitative static headspace gas chromatography calibration are external standard, internal standard, standard addition and multiple headspace extraction (MHE). The choice of technique depends on the type of sample being analyzed (Slack et al., 2003).

2.4.1 External standard calibration

External standard calibration in static headspace gas chromatography is best for analytes in liquid samples where the analytes are soluble in the matrix and the matrix has no effect on the analyte response. In these type of calibration is important to match the standard and sample matrix as closely as possible and to demonstrate equivalence in the response between the standards and the samples. The main difficulty with external standard calibration is that is does not compensate for any variability due to the gas chromatograph injection or due to variation in the analyte matrix.

2.4.2 Internal standard calibration

Internal standard calibration allows compensating for any variation due to matrix effects and gas chromatography injection. Prior to the extraction, a known additional analyte is added to each sample and standard. This compound is the internal standard.

The most important part in internal standard calibration is choosing an appropriate internal standard for any method. This compound must be available in extremely pure form and must never appear in the samples of interest, at least at the analyte concentration expected; it cannot interfere in either the extraction or the chromatography of the analytes. Finally, it

must be structurally similar to the analytes, so that it undergoes similar extraction and chromatography, otherwise the compensation will be lost.

2.4.3 Standard addition calibration

In standard addition calibration, an additional known quantity of the analyte is added directly to the sample, following an initial analysis. In standard addition, the sample is divided into several equal portions, then add increasing levels of standard. In other words, the calibration curve is prepared with the sample thus; all the points of the curve have the same composition, in this way matrix effects are eliminated. This type of calibration is not often used in gas chromatography static headspace.

2.4.4 Multiple headspace extraction

Multiple headspace extraction (MHE) determines the total peak area for an analyte in an exhaustive headspace extraction, so the analyst can calculate the total amount of analyte in the sample.

The main advantage of MHE is that matrix effects are eliminated because it determines the total amount of analyte in the sample; this is achieved by consecutive analysis on the same sample, in the same vial; in each analysis, the amount of analyte in the headspace will decrease until all the analyte is completely extracted.

Currently, it is not necessary to completely extract the analyte; usually, only three or four analyses are performed, and then an analysis by linear regression of the obtained data, in order to mathematically determine the total amount of analyte in the sample (Slack, et al., 2003).

3. Applications in forensic toxicology

In the next sections, the mean applications of static headspace gas chromatography will be described, with an emphasis in methods that could be performed without extensive modification of the equipment commonly present in the forensic toxicology laboratories, in any case, analytical considerations will be discussed, from sampling, materials and reactants needed, analysis, to interpretation of results, method validation and the importance of these test in the legal media, will be reviewed.

3.1 Ethanol

The determination of ethanol is one of the most important analysis in forensic toxicology, either in samples from corpses, or in drivers suspected of driving under the influence; in any case, precise and reliable ethanol determination is necessary. The technique of choice for the analysis of ethanol in biological samples is gas chromatography with a flame ionization detector (FID), using techniques of direct injection or headspace extraction (Kugelberg & Jones, 2007). Gas chromatography allows both a qualitative analysis by retention time, and a quantitative analysis using the area under the curve of the chromatographic peak, which gives it an advantage over older techniques (Seto, 1994).

Currently, the headspace extraction technique is preferred due to the minimal contamination produced to the injector and column of the gas chromatograph; this

technique for the determination of ethanol has been refined over time, to the extent that it is now possible to perform these tests quickly and accurately (Kugelberg & Jones, 2007; Musshoff, 2002).

To apply static headspace gas chromatography to ethanol determination, it is necessary to eliminate or minimize the effects of the matrix; to do so, several factors must be optimized, among which the most important is the calibration of the equipment. In the precise case of gas chromatography, internal standard calibration is recommended: in this type of calibration, a substance (internal standard) in the same concentration is added to all the samples and all the points of the calibration curve, the internal standard is a substance chemically similar to the substance to be analyzed but that does not exist in the sample, so that, in all chromatograms this substance must be identified with a similar intensity; any significant change in this signal will thus indicate errors in the process, and due to the fact that all the obtained signals are normalized according with internal signal standard, these errors can be corrected; in the particular case of the ethanol determination, substances such as n-propanol, t-butanol, i-propanol, i-butanol, etc. have been used as internal standards. It is recommended to use tertiary alcohols because in certain circumstances small amounts of n-propanol are produced during the putrefaction (Kugelberg & Jones, 2007; Musshoff, 2002; Seto, 1994). The determination of ethanol in the Forensic Medical Service of Mexico City is performed by static headspace gas chromatography with isobutanol as internal standard (table 2) with good results (figure 2).
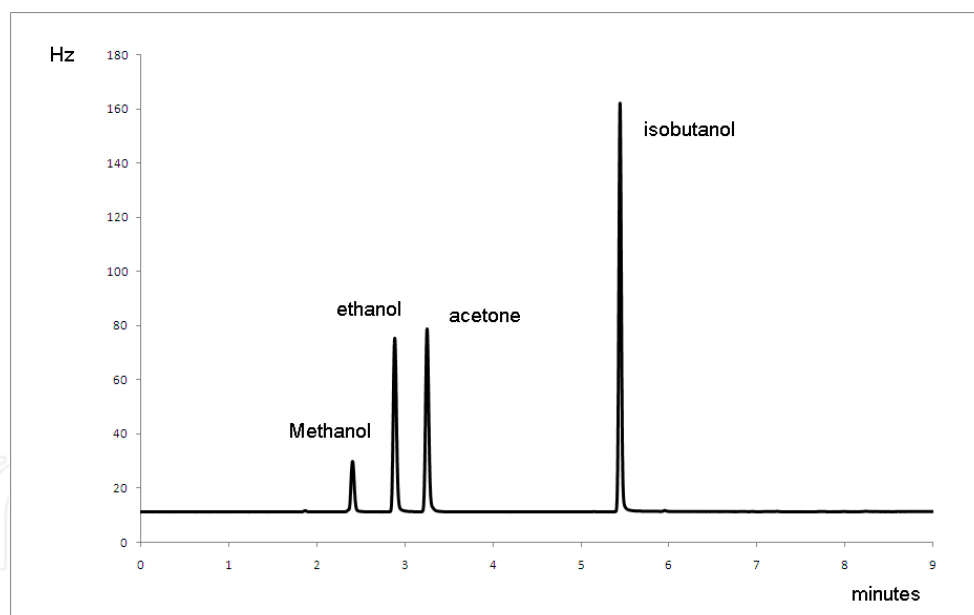
Fig. 2. Chromatogram of volatile substances determination with isobutanol 75 mg/dl as internal standard. Column DB-624, 30.0 m X 0.32 mm, Internal diameter (I.D.), film thickness 1.8 μm, carrier, nitrogen at 1.7 ml/min, Oven initial temperature 60°C for 4 minutes, ramp of 30°C/minute to 120°C final time 3 minutes, headspace temperature 60°C for 10 minutes. Ethanol 150 mg/dl, methanol, 75 mg/dl and acetone 30 mg/dl.

Another factor to consider is the temperature at which the exchange between the liquid and gaseous phase will be carried out and the time it will take; in this case one can choose between favoring the sensitivity of the method (using high temperatures) against its

Specimen (ml)	Additives[1]	Temperature headspace (°C)	Time headspace (Min)	Injection headspace	Column	Temperature Oven (°C)	Carrier gas (ml/min)	Reference
Blood (0.02)	n-propanol, (I.S.) Nitrite, fluoride	60	3	Manual	Porapack Q	150	Nitrogen, 30	Wilkinson, et al., 1975
Blood (0.15)	n-propanol, (I.S.) dithionite, $(NH_4)_2SO_4$	60	30	Automatic	Carbowax 20m on carbopack B, 1.8m X 2mm I.D.	65	Nitrogen, 30	Christmore, et al., 1984
Blood (0.2)	n-propanol, (I.S.) NaCl,	20-40	30	Automatic	Carbowax 1500 on carbopack C, 2mX 2mm I.D.	125	Nitrogen, 20	Penton, 1985
Several	n-propanol (I.S.) NaCl	25	45	Manual	Porapak S 2mX 2mm I.D.	165	Nitrogen, 45	Watts & McDonald, 1987
Blood (0.5)	n-propanol (I.S.)	55	12	Automatic	DB-1 & DB-WAX, 30 m	45	Helium, 75	Brown & Long, 1988
Blood (0.1)	n-propanol (I.S.)	40	18	Automatic	(1) Carbowax 1500 on carbopack C, (2) Carbowax 20M on Carbopack B, (3) Carbowax 20M on chromosorb B, 2mX 3mm I.D. all	100 or 120	Nitrogen, 20	Jones & Schuberth, 1989
Blood (1.0)	n-propanol (I.S.)	30	45	Manual	Porapack Q 2m	160	Ni, 54	Senkowski & Thompson, 1990
Several (0.1)	No	75	30	Automatic	HPFFAP, 5O mX0.2mm I.D.	70	Helium, 200kpa	Macchia, et al., 1995
Blood (1.0)	Isobutanol (I.S.)	55	15	Automatic	Rtx-BAC2, 30mX 0.53 mm I.D.	-60-240	Helium, 3.0	Watanabe-Suzuki, et al., 1999
Blood, vitreous fluid (0.2)	n-propanol, t-butanol (I.S.), NaCl	65	8	Automatic	Rtx-BAC1, RTX-BAC2, 30mX 0.32 mm I.D.	50		Honey, et al., 2005
Several	Isobutanol (I.S.), nitrites	60	10	Automatic	DB-624, 30 mX 0.32 mm I.D.	60-120	Nitrogen, 1.7	Suarez, et al., 2009

[1] I.S. Internal standard

Table 2. Headspace gas chromatography procedures for ethanol determination.

reproducibility, or the other way around, that is, to favor the reproducibility by applying low temperatures, even at room temperature. The reduction in reproducibility of the method is related to oxidation reactions suffered by ethanol in blood at temperatures above 40° C. To avoid such phenomenon, it is recommended to add sodium dithionite to the internal standard solution or applying low headspace temperatures (Christmore, et al., 1984; Musshoff, 2002; Seto, 1994).

Another factor that may decrease the reproducibility of the method is the difference between the amount of soluble proteins and ions present in different specimens of blood, as it alters the partition blood-air of alcohols; for example, ethanol and various volatile substances will not evaporate just the same in a sample of highly diluted blood, coming from a person who suffered a bleeding, than in a regular blood sample. To solve that problem, it is recommended to dilute the samples (usually with the solution of internal standard) in a relation of at least 1 to 5, but preferably of 1 to 10 (Kugelberg & Jones, 2007; Watts & McDonald, 1987). Finally, if it necessary to increase the sensitivity of the analysis, it can be done by adding a salt like sodium chloride, sodium nitrite, etc. (Christmore, et al., 1984), or by cryofocusing techniques (Watanabe-Suzuki, et al., 1999).

There are several columns for alcohol separation available on the market, that can be used for the chromatographic separation, but if it is necessary to determine non-polar volatile substances, it is advisable to choose a column that allows separating all the analytes of interest in a reasonable time; subsequent analyses in two or three chromatographic systems can be performed in order to confirm the obtained results, each one with different retention times for ethanol and internal standard; in some cases, it is even possible to perform a confirmation test with a different internal standard (Brown & Long, 1988; Jones & Schuberth, 1989), finally, FID is the chromatographic detector more commonly used for this kind of analyses. The chromatographic conditions most commonly used for the analysis of ethanol in biological samples are summarized in table 2.

The accuracy and reproducibility of the analysis performed in such a way is high, resulting in inter-laboratory coefficients of variation (CV) of 3% to 5%, and intra-laboratory CV of less than 1%, both with adequate sensitivity (LOD around 1 mg/dl) and high specificity (Jones & Schuberth, 1989; Jones, et al., 1992; Penton, 1985).

3.2 Congeners

Alcoholic beverages contain trace amounts of a wide variety of chemical substances, which are known collectively as congeners. The congener profile of a particular drink depends on the raw materials used in the fermentation process, such as the source of carbohydrates, whether derived from fruits, grape juice, grape mash, malted grain or barley (McAnalley, 2004). Others congeners might be introduced or removed during the distillation process, ageing and final storage of the beverage in special wooden cask; all these congeners helps to impart the special smell and taste of the final product (Iffland & Jones, 2003).

The qualitative and quantitative analysis of congeners have found several applications in forensic toxicology, the presence of those substances in abnormally high concentrations in blood could suggest deliberate criminal intent presumably by addition of these solvents to conventional alcoholic beverages or a failed distillation process. Otherwise, the person might have consumed accidentally denatured alcohol, which is not uncommon in alcoholics

(Jones, et al., 1989). In this particular case the analysis could be done with analytical conditions similar to the common used in ethanol analysis in blood.

Another application of alcoholic beverages congeners is as alcoholism markers (Musshof, 2002); the most associated volatile substances with the abuse of alcoholic beverages are methanol (Brinkmann, et al., 2000; Roine, et al., 1989; Suarez, et al., 2009) and the sum of acetone and isopropanol (Iffland, et al., 1988). Metabolism of methanol via liver alcohol dehydrogenase (ADH) is competitively inhibited by ethanol levels exceeding 20 mg/dl. Consequently, excessive and prolonged drinking results in high blood methanol levels, so methanol can be detected in blood, breath and urine for long after ethanol has returned to its endogenous concentration (Iffland & Jones, 2003; Majchrowicz & Mendelson, 1971; Musshof, 2002). On the basis of these findings methanol levels exceeding 1 mg/dl have been suggested as indicator of alcoholism, while 0.1 mg/dl can be considered a physiological level. On the other hand, join concentrations of acetone and isopropanol higher than 0.9 mg/dl are indicative of heavy drinking, normal levels of isopropanol are less of 0.01 mg/dl and 0.1-0.3 mg/dl for acetone (Iffland, et al., 1988; Iffland & Jones, 2003); this is the result of their reciprocal formation through the alcohol deshydrogenase system.

However, the specificity of isopropanol and acetone as alcoholism markers is low compared to methanol because both substances can be formed in some metabolic disorders or after strenuous exercise (Iffland & Jones, 2003; Musshof, 2002). Finally, has been suggested that these substances can be related to the severity of hangover (Bendtsen, et al., 1998; Calder, 1997; Pronko, et al al., 1997).

The sensitivity of conventional headspace gas chromatography is sufficient for blood alcohol determinations down to 5 mg/dl, but for the detection of congeners as alcoholism markers LOD had to be improved to reach at least the physiological level of these substances several strategies could be used to achieve these, like adding a salt to enhanced the vaporization of the congeners (Bendtsen, et al., 1998; Suarez, et al., 2009) and the cryofocusing technique.

Another interesting forensic application of congener analysis is to evaluate claims of drinking after driving, This kind of defense arises frequently when the drunk driving suspect is not apprehended immediately after an vehicular incident, especially after hit and run incidents, in this situation, the congener profile can be used to identify the consumption of a particular kind of alcoholic beverage from the apprehended driver, comparing the profile present in the blood and urine of the driver with the known congener profile of the alcoholic beverage allegedly consumed after driving (Iffland & Jones, 2003).

The scientific basis of forensic congener analysis depends on the fact that drinks containing different amounts of various congeners produce different low molecular alcohols in blood and urine. Consequently, the qualitative and quantitative analysis of congeners in blood and urine can furnish useful information about the kind of alcoholic drink consumed and also the time of intake relative to the time of blood sampling (Iffland & Jones, 2003).

The main congeners of forensic interest are methanol, n-propanol, isobutanol, 2- butanol, and its metabolite methyl ethyl ketone and n- butanol, while isopropanol and acetone are endogenous substances always detected in blood and urine during a congener analysis by headspace gas chromatography. However, these substances are not ingredients of alcoholic beverages (Iffland & Jones, 2003).

Interpreting the results of congener analysis require to know the congener profile of the drinks consumed before and after driving as well as information about the disposition and fate of these substances in the body and the extent of any metabolic interaction with ethanol.

Congener analysis is a modification of the conventional headspace gas chromatography analysis for measuring blood ethanol concentration: However, the sensitivity of the method must be increased to allow measuring much lower concentrations (Iffland & Jones, 2003) (Table 3).

Congener	Blood concentrations (mg/dl)
Methanol	0.05-5
n-propanol	0.005- 0.3
Isobutanol, n- butanol, 2-butanol and methyl ethyl ketone	0.002-0.2

Table 3. Congener concentrations in blood.

Besides the conditions of the gas chromatographic analysis, the detections limits depend on the particular congener, its molecular weight, the amount of organic matrix in the biological material and the vapor pressure of the water in the sample.

Congener analysis require to enhanced the sensibility, usually by the addition of a salt, usually anhydrous sodium sulfate, various ways of sample pretreatment are used like ultrasonic disintegration prior ultrafiltration, low temperature vacuum distillation, ultracentrifugation, multiple headspace extraction or microdestillation. Headspace gas chromatography is performed with an internal standard calibration, usually with t-butanol as the internal standard and if necessary with a liquid nitrogen freeze trap (cryofocusing) to concentrate the sample, LOD usually achieved are about 0.01 mg/dl for methanol and about 0.001-0.002 mg/dl for n-propanol or isobutanol, Both FID and MSD have been used for the gas chromatography detection. An example of headspace gas chromatography conditions is shown (Iffland & Jones, 2003) (table 4).

Additives[1]	Temperature headspace (°C)	Time headspace (Min)	Injection headspace	Column	Temperature Oven (°C)
t-butanol (I.S.), anhydrous sodium sulfate	70	12	Automatic	Carbowax 20M on Carbopack B, 2mX 2mm I.D.	60-100

[1] I.S. Internal standard

Table 4. Headpace chromatographic conditions for congener analysis.

Finally, the successful application of congener analysis in casework requires considerable experience not only regarding laboratory analysis but also controlled drinking experiments. Studies of this kind furnish the information needed about the pharmacokinetics of specific congener, their interactions with ethanol metabolism and urine/blood relationships. Consequently, much basic research is necessary before embarking in actual casework, this expertise, at the present moment is available at only a few institutes of legal medicine on Germany.

3.3 Inhalants

Inhalants are a diverse group of volatile substances that may be inhaled accidentally or intentionally. Inhalants can be solids and liquids as well as gases. Their common feature is volatility, the property of being or being able to be converted to a form, susceptible to inhalation. Compounds having this property include aliphatic hydrocarbons (butane, hexane, propane, etc.); aromatic hydrocarbons (benzene, toluene, etc.); mixed hydrocarbons (gasoline, lighter fluid, etc.); halogenated hydrocarbons (chloroform, dichloromethane, etc.); chlorofluorcarbons (Freon 11, Freon 12, etc.) and oxygen containing compounds (acetone, nitrous oxide, etc.). Gaseous anesthetics will be studied in detail in the next section (Broussard, 2003).

Inhalants are present in many commercial products (solvents, glue, typewriter correction fluid, gasoline, lighter fluid, refrigerants, propellants for aerosol, etc.), consequently, inhalants are easily available and cheap, these circumstances, have contributed to its use as a drug of abuse, above all, in adolescents. Its use has risen in the last years (Hansen & Rose, 1995), despite legislation that limit its accessibility and to make their use by adolescent illegal. Worldwide, inhalants are one of the most dangerous classes of abused substances and one that is responsible of more deaths annually, that other drugs of abuse (Broussard, 2003).

The best sample for inhalants detection is blood, but urine can be used to detect metabolites, proper collection involves the use of glass tubes with minimal headspace remaining after collection. Volatile organic compounds by definition are highly evaporative, and analytes can be easily lost while samples or standards are being manipulated or stored; on the other hand, because, many of these substances are commonly found in laboratories, it's reasonable that contamination might occur during sample collection or analysis (Ashley, et al., 1996). Specimens should be stored between -5 to 4°C, it's recommended to add sodium fluoride as conservative, under these circumstances samples can be stored for up to forty days (Ashley, et al., 1996). Because of the tolerance that these substances produced, there is not a correlation between the blood inhalants concentrations and the clinical features of toxicity for any of these compounds. Table 5 lists concentration of inhalants found, usually, in postmortem cases (Baselt, 2004).

Inhalant	Fatal Concentrations in blood in mg/dl (Baselt, 2004)	Basal concentrations in blood in ng/dl (Ashley, et al. 1996).
Benzene	Average, 3.6	13.0
Toluene	Average, 2.2	52.0
Propane	1.1 and 110	----------------------
Chloroform	Average, 6.4	----------------------
Cresols	Average, 13.3	----------------------
Dichloromethane	Average, 29.5	----------------------
Diethyl ether	Range 9-375	----------------------
Ethyl chloride	42.3	----------------------
Nitrous oxide	Average, 10.0	----------------------
Tetrachloroethylene	Range 0.45-11.5	19.0
Trichloroethane	Average, 12.6	34.0

Table 5. Toxic and background concentrations of some inhalants in blood

Inhalant	Additives[1]	Temperature headspace (°C)	Time headspace (Min)	Injection headspace	Column	Temperature Oven (°C)	Carrier gas (ml/ min)	Detection	Reference
Freon 11	CH_2Cl_2 (I.S.)	40	30	Manual	HP-5, 12m X0.2 mm I.D.	30	Helium, (1 psi)	MSD	Groppi, et al, 1994
Several	Propanol (I.S.), NaCl	60	3	Automatic	(1) THEED on Carbopack B (2) and Carbowax 20 M on Carbopack B, 2m both	67 or 73	Nitrogen, 30	FID	Logan, et al., 1994
CH_2Cl_2, $CHCl_3$	NaCl, Sec-butanol (I.S.)	55	20	Manual	DB-5, 30 mX 0.25 mm I.D.	70–180	Helium, (4 psi)	MSD	Kim, et al, 1996
Difluro ethane	Propanol (I.S.)	37	15	Manual	RTX-BAC1 30 mX 0.32 mm I.D.	65	Helium, 2	FID	Broussard, et al, 1997
Several	NaCl	80	26	Automatic	DB-1, 30mX 0.25mm I.D.	40-250	Helium, 18	MSD	Schuberth. 1997
C_2Cl_4	C_2HCl_3	60	10	Manual	DB-5, 15 mX 0.25mm I.D. mm Carbowax on carbopack 2m x 0.32 mm I.D.	75 or 110	Helium, Argon 10% methane, 30	MSD, ECD	Isenschmid, et al, 1998
Ethyl chloride	Propanol (I.S.), NaCl	37	5	Manual	Innowax, 15mX0.25 mm I.D.	50	Helium, 1.05	FID	Broussard, et al, 2000
Toluene	citrate, iso-butanol (I.S.)	60	20	Automatic	Innowax, 30mX0.25 mm I.D.	60-140	Helium, 1.0	FID	Kim, et al, 2000
> 50 volatiles	t-butanol (I.S.), nitrite, fluoride	70	30	Automatic	DB1 and DB-WAX (Both 30mX0.25 mm I.D.), DB-624, 30mX0.53 mm I.D	40-150 or 45-90	Helium	FID, MSD	Sharp, 2001
CH_2Cl_2	No	60	20	Automatic	Carbowax 20 M on Carbopack B 2mX 2mm I.D.	90	Helium, 30	FID	Zarrabeitia, et al, 2001
Alkanes	No	80	20	Automatic	CB-624, 30mX0.25 mm I.D	35-150	Helium, (10 psi)	MSD	Gaulier, et al. 2003
$CHCl_3$	Trichloro ethane (I.S.)	60		Automatic	RTX-BAC1 30m.	60-150	----------	FID	Singer & Jones, 2006
MAPP		40	15	Automatic	RTX-BAC1 30 mX 0.53 mm I.D.	40	----------	FID	Avella & Lehrer, 2004
$CHCl_3$	Butanol (I.S.)	90	12	Automatic	Poraplot Q, 25 mX 0.25 mm I.D.	50-230	Helium, 1.0	MSD	Gaillard, et al. 2006

[1] I.S. Internal standard

Table 6. Headspace gas chromatography procedures for inhalants determination.

The analysis of inhalants has been performed for the diagnosis of solvent abuse and monitoring of industrial exposure; In the first case, the determination of the unchanged inhalant in blood is preferred, but in some cases, (trichloroethylene inhalation) metabolites (trichloroethanol and trichloroacetic acid) can be searched in urine; for biological monitoring, the determination of inhalants in blood is preferred too, but the determination of metabolites in urine is commonly done. The technique of choice to perform inhalants analysis is headpace gas chromatography with FID, electron capture detector (ECD) and MSD. FID provides a good linear range detection, while MSD unequivocal identification. ECD is only recommended in trace analysis for halogenated hydrocarbons. Both packed columns such as carbowax and capillary columns such as DB-1 have been used to separate and quantify volatiles. Usually, the headspace gas chromatography method to analyze ethanol in blood can be modified for a preliminary screening before a more specialized and extensive method is performed (Sharp & Dautbegovic, 2001; Sharp, 2001). These procedures have typical LOD´s of 0.01 mg/dl and typical linear ranges to 5 to 10 mg/dl (Broussard, 2003). Analytical conditions of some procedures are given in table 6.

3.4 Anesthetics

Within the group of inhalants, fluorinated inhalants stand out; that is because they can be potentially dangerous, as their lethal doses are only two or four time their therapeutic doses. Careful handling of anesthesia during surgical procedures has succeeded in reducing morbidity and mortality due to overdose, and usually the case of death in surgical procedures is some other reason. However, the analysis of these substances in forensic environments is justified on any case of death during an operation, and due to their use as drugs of abuse, the availability of fluorinated anesthetics is limited to hospital personnel or those engaged in its manufacturing and distribution. At clinics, the determination of fluorinated anesthetics is used for monitoring patients under anesthesia and medical personnel, but under these circumstances the concentrations detected are below those that cause acute poisoning (Pihlainen & Ojanperä, 1998).

Currently, the most used fluorinated anesthetics are halothane, enflurane, isoflurane, sevoflurane and desflurane; isoflurane stands out as the most popular at present times.

These substances are metabolized in the liver and to a lesser extent in the kidneys and lungs. Metabolism is higher with halothane (20-46%), followed by enflurane (2.4-8.5%), sevoflurane (2.5-3.3%), isoflurane (0.2%) and desflurane (0.02%). So their determinations are intended to find drugs, not metabolites. The following concentrations in blood have been found in deaths related with these substances (Table 7).

Anesthetic	Detected concentration (mg/L)
Halothane	45-650
Enflurane	130-710
Sevoflurane	26
Isoflurane	9.9-48

Table 7. Blood concentration in deaths related to fluorinated anesthetics (Baselt, 2004)

Gas chromatography is the technique of choice for the analysis of these substances, column selection is handicapped by the possibility of analyzing other gases used in anesthesia with it, in which case columns with solid adsorbents are to be used; otherwise, capillary columns with silicon phases can be used, detectors such as FID performed well to these compounds, but if it is necessary to decrease LOD of the method, some other, more sensitive detectors can be used, such as ECD (Pihlainen & Ojanperä, 1998; Uyanik, 1997), in order to obtain a structural identification, MSD or infrared detector (IRD) has been used, and the most commonly used preparation technique is static headspace injection. (Pihlainen & Ojanperä, 1998), due to the fact that these compounds are easily transferred to headspace gas, according to blood/gas partition constants. The relation gaseous phase/aqueous phase normally used in the vial is of 5-20, and the sample volume is usually 10% of the gaseous phase; internal standard calibration has been used, with various substances and deuterated standards. The sensitivity of the method can also be improved by increasing the extraction temperature, by decreasing the gaseous phase or through salting techniques (table 8). It is important to avoid losses by evaporation and contamination of the samples throughout the whole analytical procedure. In the analyses of these substances, the chromatographic conditions employed are very similar to those used for the determination of alcohol and inhalants; therefore, it is possible to design a method that can simultaneously analyze these substances (Kovatsi, et al., 2011); our laboratory identified the presence of sevoflurane with the method used to determine alcohol, without changing the chromatographic conditions whatsoever.

Additives[1]	Tempe-rature head-space (°C)	Time head-space (Min)	Injection headspace	Column	Tempe-rature Oven (°C)	Carrier gas (ml/min)	Detection	Figures of Merit[2]	Referen-ces
Enflurane, (I.S.)	75	30	automatic	Carbowax 1500 on carbopack C, 2m	100	----------	FID	---------	Kulhman, et al. 1993
CH$_2$Cl$_2$ (I.S.) Tween	55	15	automatic	DB1 30m X0.53mm I.D.	60	Helium, 15	MSD	---------	Saito, et al. 1995
Enflurane, (I.S.)	41	120	automatic	Poraplot Q 27m X0.25mm I.D.	40-140	Helium, 1.0	MSD	RSD[3]: < 8.06%, RSD[4]: < 6.19%	Accorsi, et al. 2003
n-propanol, (I.S.)	25	60	Manual	Carbowax 1500 on carbopack C, 2m. X 2mm I.D.	100	Helium, 20	FID	CV[3]: 13.7% CV[4]: 3.3%	Burrows, et al. 2004
Aceto-nitrile, (I.S.),NaCl	60	30	automatic	Supelcowa x 10, 30mX 0.25mm I.D.	42-100	Helium, 1.2	FID	LOD: 1.7 mg/dl, RSD[3]:1.3-14.6%	Kovatsi, et al. 2011

[1]I.S. Internal standard; [2]RSD Relative standard deviation; [3]Interday; [4]Intraday

Table 8. Headspace gas chromatography procedures for fluorinated inhalation anesthetics determination

3.5 Carbon monoxide

Carbon monoxide gas is colorless, odorless, tasteless, and is produced by incomplete combustion of organic matter. Carbon monoxide is involved each year in a significant number of deaths around the world, be they accidental or voluntary; the main sources of exposure to carbon monoxide are the exhausts of internal combustion engines, cigarette smoke, heating systems in poor conditions and fires; there can also be endogenous production related to dihalomethane metabolism and heme catabolism (Kunsman & Levine, 2003). Carbon monoxide exerts its toxic effect because it has approximately 220 times greater affinity to hemoglobin than oxygen, therefore, prevents the transport of oxygen to the tissues, and at the same time changes the allosteric structure of hemoglobin, increasing its affinity with oxygen, this way preventing the exchange of oxygen in the tissues (Walch et al., 2010).

The compound formed by carbon monoxide and the reduced form of hemoglobin, carboxyhemoglobin, is therefore a measure of carbon monoxide poisoning, and its determination is performed in all forensic toxicology laboratories in the world on a routine basis. While carboxyhemoglobin saturation levels greater than 50% are indicative of carbon monoxide poisoning as a cause of death, levels between 10% and 50% are indicative of exposure to this toxic and can cause various symptoms.

Different methods have been developed to determine the percentage of carboxyhemoglobin saturation in blood. These methods are based on colorimetry, infrared spectrophotometry, visible ultraviolet spectrophotometry and gas chromatography (Boumba & Vougiouklakis, 2005). Currently, the most widely used methods are those based on ultraviolet-visible spectrophotometry, utilizing conventional spectrophotometers, usually making two readings at two different wavelengths (Maehly, 1962) or by specialized spectrophotometers: oximeters (Mahoney, et al., 1993); these methods are fast and simple, however, they are unreliable in certain circumstances, such as in putrefied samples, where the putrefaction process produces substances that generate spectral interference or the spontaneous formation of methemoglobin and sulfhemoglobin, this way preventing the determination of carboxyhemoglobin, or in blood samples from fire deaths, where methemoglobin is spontaneously produced (Lewis, et al., 2004; Seto, 1994; Walch, et al., 1984). In this regard, static headspace gas chromatography is not affected by these circumstances, because this technique separates the carbon monoxide from blood, and is thus considered a highly specific and sensitive technique, which makes it the referential technique for determining carbon monoxide (Boumba & Vougiouklakis, 2005); however, static headspace gas chromatography has not been usually used for this type of analyses because it takes longer than established techniques and requires skilled personnel (Mahoney, et al., 1993). Nonetheless, that last disadvantage does not apply to forensic environments, where this technique is widely known.

Carbon monoxide determination by static headspace gas chromatography involves mixing the blood with a substance that lyses erythrocytes and releases the carbon monoxide of blood, either by adding an acid (denatures hemoglobin) or ferrocyanide potassium (oxidizes hemoglobin to methemoglobin); in some cases, a reducing agent can also be added to ensure the methahemoglobin that may be present in the sample is reduced to hemoglobin, which is

important in samples from aviation accidents (Lewis, et al., 2004; Walch, et al., 1984). The gas released into the headspace is analyzed by gas chromatography using different detectors. The detection has been carried out using a FID, after a catalytic reduction of carbon monoxide to methane (Cardeal, et al., 1993; Czogala & Goniewicz, 2005; Walch et al., 2010), a thermal conductive detector (TCD) (Lewis, et al., 2004; Van Dam & Daenens, 1994), by the release of mercury vapor resulting from the combination of carbon monoxide with mercuric oxide (Vreman, et al., 1984) and by MSD (Oritani et al., 2000). The separation is usually done through molecular sieve columns.

In order to determine the percentage of carboxyhemoglobin saturation in blood, it is necessary to quantify the total amount of hemoglobin and a calibration curve must be prepared; to do so, different approximations are used. The most commonly used, parts from a blood sample to which carbon monoxide is passed until the carboxyhemoglobin saturation reaches 100%, the other points of the calibration being obtained by diluting this blood in a blood sample with 0% carboxyhemoglobin, passing oxygen to it (Canfield, et al., 1998). Due to the complexity of the above mentioned procedure, other calibration forms have been implemented, using certified gas standards (Czogala & Goniewicz, 2005) or the stoichiometric liberation of carbon monoxide from the reaction between formic acid and hot sulphuric acid (Cardeal, et al., 1993). Detection limits obtained by this technique are less than 0.1% of carboxyhemoglobin saturation in blood, which is much lower than the normal levels reported (Kunsman & Levine, 2003) and less than the detection limits of the spectrophotometric techniques, reported in 1% (Boumba & Vougiouklakis, 2005). Table 9 describes in detail, the chromatographic conditions for carbon monoxide determination by this technique.

In conclusion, the determination of carbon monoxide by static headspace gas chromatography is a very specific method, as it is not influenced by sample conditions; besides, it has an accuracy and sensitivity which surpasses other, more conventional techniques. However, the fact that it is time-consuming, expensive, requiring of excessive sample handling, and, above anything else, the need to measure total hemoglobin and preparing standards (Canfield, et al., 1998), has prevented this technique from being used on a routine basis in most forensic toxicology laboratories.

3.6 Cyanide

Cyanide is a potent, fast-action toxin, which effects by reacting with trivalent iron of cytochrome oxidase, inhibiting the respiratory system, resulting in a quick deterioration of vital functions. Blood concentrations of 2 to 3 ug/ml are already considered not compatible with life, while basal levels of cyanide in non smoker's blood of 16 ng/ml have been detected (Baselt, 2004). Cyanide exposure is relatively common; it is known for being ingested with suicidal intentions or homicidal purposes; but accidental intoxications in fires have also been reported, where the pyrolysis of nitrogen-containing synthetic materials, such as polyurethane and polyacrylonitrile produces hydrogen cyanide, exceeding the mortal concentration in some cases (Seto, et al., 1993; Moriya & Hashimoto, 2001), so even if the most common causes of death in fires are burns, injuries and carbon monoxide intoxication, hydrogen cyanide can also be involved. Concentrations above the normal levels have been detected in smokers, but the toxic effects of these concentrations are yet to be fully clarified; other sources of cyanide exposure are cyanogenic glycosides, present in

Additives[1]	Temperature[2] headspace (°C)	Time headspace (Min)	Injection headspace	Column	Temperature Oven (°C)	Carrier gas (ml/ min)	Detection	Figures of Merit	Reference
$K_3Fe(CN)_6$, saponin, phosphates	0	30	automatic	Molecular sieve 5A, 90cmX0.53 cm I.D.	110	Air, 50	Mercury vapor	LOD: 0.005%. CV[3]:<13%, CV[4]:<10%	Vreman, et al, 1984
H_3PO_4, n-octanol	T.A.	70	automatic	Porapak Q, 3 mX0.9 mm I.D.	80	Nitrogen, 30	FID		Cardeal, et al, 1993
H_2SO_4, saponin	T.A.	40	Manual	Molsieve 5A, PLOT, 25mX0.32 mm I.D	80	Helium, 3.0	TCD	LOD: <0.02% CV[3]:13.7%, CV[4]:3.3%	Van Dam & Daenens, 1994
$K_3Fe(CN)_6$, t-butanol			automatic	DB-624 60mX 0.32mm I.D.	40-80	Helium, 28 cm/ s	MSD	-----------	Oritani, et al, 2000
Dithionite, H_2SO_4 saponin	T.A.	40	automatic	Molsieve 5A 20mX0.32 mm I.D.	120	Helium, 35 psi	TCD	CV:< 10%	Lewis, et al, 2004
$K_3Fe(CN)_6$	T.A.	30	automatic	Molsieve 5A, PLOT 30mX0.53 mm I.D.	30	Helium, 3	FID	-----------	Czogala & Goniewicz, 2005
H_2SO_4, saponin	50	30	automatic	Molsieve 5A, PLOT 50mX0.53 mm I.D.	80-300	Helium, 12	FID	CV[3]:<11.0%, CV[4]:<5.0%	Walch, et al, 2010
HNO_3,	60	30	Automatic	Molsieve 5A, 25mX0.53 mm I.D.	80		FID	----------------	Felby, 2009

[1] I.S. Internal standard; [2]T.A. Ambient temperature; [3]Interday; [4]Intraday

Table 9. Headspace gas chromatography procedures for carbon monoxide determination.

untoasted almonds, bay leaves, apple seeds, etc. Cyanide exposure also results from its use as fumigant, as a metabolite of sodium nitroprusside and in chemical industry, where cyanide has found applications in the metallurgical, oil, photographic and plastic industries (Kunsman & Levine, 2003). Because of this and the presence of basal cyanide levels, it is necessary to count with methods to quantitatively determine cyanide in biological samples.

Traditionally, this determination is carried out by spectrophotometric techniques, preceded by a distillation or microdiffusion pretreatment (Seto, 1994); the technique of gas chromatography with headspace injection provides a faster analysis, susceptible to be automated, with high sensitivity, good recoveries (around 90%) and specific detection The procedure involves the release of cyanide from the biological sample in the form of hydrogen cyanide by the addition of an acid, usually into the previously sealed vial, through the septum; different acids have been used, such as phosphoric acid, sulfuric acid, nitric acid or acetic acid, although the most used is phosphoric acid, as it does not form any blood clots; in the reaction mix within the vial, acetonitrile is usually added when internal standard calibration is used (Calafat & Stanfill, 2002; Moriya & Hashimoto, 2001), and ascorbic or acetic acid, with the purpose of avoiding the production of cyanide in blood by action of its metabolite, thiocyanate, which could result in problems with samples by smokers or with chronic intoxications (Seto, 1996); heating times are usually longer than half an hour and temperature below 63°C are recommended in order to prevent the formation of cyanide from blood. Regarding the detection by gas chromatography, due to the small amounts of cyanide to determine and its relatively high partition coefficient, it is necessary to use more sensitive detectors; pertaining this, two strategies are commonly followed, in the first one, cyanide is detected in the gas chromatograph using a nitrogen-phosphorus detector (NPD) (McAuley & Reiver, 1983; Moriya & Hashimoto, 2001; Seto et al., 1993), or cyanide can be detected as cyanogen chloride after a derivatization with chloramin-T using a ECD (Felby, 2009; Odoul, et al., 1994) but a MSD with an isotope as internal standard has also been used (Dumas, et al., 2005). Finally, it is important to consider the rapid disappearance of cyanide from the blood, which was calculated is up to 30% on a day, in refrigerated blood at 4°C (Calafat & Stanfill, 2002), so it is necessary to perform this analysis as soon as possible in order to obtain meaningful results. Table 10 describes the most important chromatographic parameters.

3.7 Ketone bodies

Endogenous volatile metabolites are normal by products of intermediate metabolites. Analysis of these metabolites is important in the diagnosis of certain disease states. An example of headspace gas chromatography analysis applied to these metabolites is the determination of ketone bodies (Seto, 1994). Ketone bodies are present in biochemical altered states known as diabetic ketoacidosis, related to diabetics and alcoholic ketoacidosis, a consequence of chronic abuse of alcohol. Studies (Iten & Meier, 2000; Pounder, et al., 1998; Thomsen, et al., 1995) indicate that alcoholic ketoacidosis could be the cause of death in alcoholics, in cases of sudden and unexpected death, where alcohol determination was negative and that an alcoholic ketoacidosis state can be diagnosed with the determination of ketone bodies in postmortem blood samples. Ketone bodies (acetone, acetoacetate and β-hydroxybutyrate) can be measured separately by gas chromatography headspace. Acetone are determined at a headspace temperature below 60°C to avoid decarboxilation of

Additives[1]	Temperature[2] headspace (°C)	Time headspace (Min)	Inyección headspace	Column	Temperature Oven (°C)	Carrier gas (ml/min)	Detection	Figures of Merit[3]	Reference
1-octanol, acétic acid	T.A	30	Automatic	Porapak Q, 180cmX2mm I.D.	110	Nitrogen, 20	NPD	LOD 0.05 mg/L, CV[5]:3.3%	McAuley & Reive, 1983
H_3PO_4	50	30	Manual	GS-Q, 30mX0.53 mm I.D.	90	Helium, 4.7	NPD	LOD 0.1 µg/L RSD[5]:6.1%	Seto, et al., 1993
H_3PO_4	50	30	Manual	GS-Q, 30mX0.53 mm I.D.	90	Helium, 4.7	FID	LOD 0.02 mg/L RSD[5]:3.3%	Seto, et al., 1993
H_3PO_4	55	60	Automatic	CP-Sil 8B 50m X 0.23 mm I.D.	60	Helium, 2.0	ECD	LOQ: 0.1 mg/L RSD[5]:1-8%	Odoul, et al., 1994
H_3PO_4, ascorbic acid I.S.	70	15	Manual	SupelQ Plot 30mX 0.32 mm I.D.	-30-160	Helium, 3.0	NPD	LOD: 2 µg/L RSD[5]:12%	Ishii, et al., 1998
H_3PO_4 I.S.	55	15	Manual	GS-Q, 30mX0.53 mm I.D.	100 to 140	Helium	NPD	-------------	Moriya & Hashimoto, 2001
H_3PO_4, ascorbic acid, I.S.	60	5	Automatic	HP-PLOT Q, 15 m X 0.32mm I.D.	30 to 190	Helium, 3.0	NPD	LOD 13.8 mg/L, CV[5]:16%	Calafat & Stanfill, 2002
H_3PO_4, ascorbic acid	50	30	Manual	HP-PLOT Q, 30mX0.53 mm I.D.	170	Helium, 5.0	NPD	LOD: 0.7 µg/L, CV[5]:3.4-5.3%	Shibata, et al. 2004
H_3PO_4, ascorbic acid	60	15	Manual	GS-GASPRO, 30m X 0.32 mm I.D.	40-250	Helium,	MSD	LOD: 0.3 µmol/L CV[4]:3.9%, CV[5]:4.4%	Dumas, et al. 2005
HNO_3,	60	30	Automatic	CPSIL-19 CB, 50m X 0.32 mm I.D.	80	Nitrogen, 20 psi	ECD	LOD: 0.01 mg/L CV 3.3-7.2%	Felby, 2009

[1]I.S. Internal standard; [2]T.A. Ambient temperature; [3]LOQ, limit of quantitation, RSD Relative standard deviation; [4]Interday; [5]Intraday

Table 10. Headspace gas chromatography procedures for cyanide determination.

acetoacetate or in the presence of potassium hydroxide (Felby & Nielsen, 1994; Seto, 1994); for acetoacetate determination, headspace temperature is set at 100°C to promote acetoacetate decarboxylation to acetone and β-hydroxybutyrate levels are determined after oxidative conversion into acetone with potassium dichromate (Seto, 1994) or enzymatic reduction to acetoacetate by β-hydroxybutyrate hydrogenase and then thermal conversion to acetone (Felby & Nielsen, 1994). The most used sample to performed this test is blood but it was found a good correlation between blood:spinal fluid and blood:viteous humor ketone bodies concentration, consequently, spinal fluid and vitreous humor can be used as an alternative specimens in this analysis (Felby, et al., 2007).

3.8 Other applications

Finally, there are analyses within forensic science, where static headspace gas chromatography can be used as a part of a more complex analysis; as fire debris analysis (Ren & Bertsch, 1999; Sandercock, 2008), where the static headspace analysis provides complementary information to that provided by dynamic headspace techniques, for the determination of amphetamines and methamphetamines by the addition of potassium carbonate to transform the amine of the stimulant in its unprotonated, volatile form (Seto, 1994), and more recently, the study to substances produced during corpse decomposition (Statheropoulos, et al., 2005; Swann, et al., 2010), where different types of separation techniques are used to characterize the compounds produced in the decomposition and determine how they are produced.

4. Conclusions

Static headspace gas chromatography is a mature and reliable technique; it is considered the technique of choice for the analysis of ethanol in biological samples, and is therefore present in the vast majority of forensic laboratories around the world with the qualified personnel to operate it; however, the applicability of this technique is not limited to this test and can be used for the analysis of various substances with minimal modifications, providing proper calibration and proper handling of matrix effects, excellent validation parameters, along with a clean injection. So, with this technique, various substances can be analyzed without the need of additional methods, and that would allow forensic laboratories to expand the number of cases they can take care of, with a minimal investment.

To accomplish that, it is necessary to know the fundamentals of this technique, the different chemical and physical phenomena involved, and the potential occurrences in the analysis of a particular substance, in order to develop a method with the required sensitivity, specificity and reproducibility.

In this respect, specialized methods for the analysis of substances have been developed, such as the determination of ethanol, cyanide or carbon monoxide; but other methods can also be designed, methods capable of analyzing large numbers of volatile substances (Sharp, 2001), or systems designed for specific forensic situations, such as the analysis of toxic gases produced during a fire (Felby, 2009), the determination of ethanol in drivers (Jones & Schubert, 1989), the diagnosis of alcoholic ketoacidosis (Felby & Nielsen, 1994), etc. In some of its applications, static headspace gas chromatography faces competition from SPME (Furton, et al., 2000; Snow, 2000), a more versatile method which has begun to substitute

older methodologies in some applications. However, the broad acceptance of static headspace gas chromatography for ethanol determination has consequently caused for such equipment to be present in most forensic laboratories, which can also be used for analyzing other volatile substances in a reliable way, without investing in other technologies.

5. Acknowledgments

6. References

Accorsi, A.; Valenti, S.; Barbieri, A.; Raffi, G.B. & Violante F.S. (2003) Enflurane as an internal standard in monitoring halogenated volatile aneasthesics by headspace gas chromatography – mass spectrometry. *Journal of chromatography A*. 985,259-264

Angerer, A. & Hörsch, B. (1992) Determination of aromatic hydrocarbons and their metabolites in human blood and urine. *Journal of chromatography*. 580, 229-255.

Ashley, D.L.; Bonin, M.A.; Cardinali F.L.; McCraw, J.M. & Wooten, J.V. (1996). Measurement of volatile organic compounds in human blood. *Environmental health perspectives.* 104 (suppl. 5) 871-877.

Avella, J. & Lehrer, M. (2004). Fatality due to methyl acetylene-propadiene (MAPP) inhalation. *Journal of forensic science.* 49, 6, 1-3.

Baselt, R.C. (2004). *Disposition of toxic drugs and Chemicals in man*. 7° Ed. Biomedical Publication, Foster City, California

Bendtsen, P.; Jones, A.W. & Helander, A. (1998). Urinary excretion of methanol and 5-hydroxytryptophol as biochemical markers of recent drinking in the hangover state. *Alcohol & alcoholism*. 33,4,431-438.

Boumba, V.A. & Vougiouklakis T. (2005). Evaluation of the methods used for carboxihemoglobin analysisis in postmortem blood. *International Journal of toxicology.*24, 275-280.

Brinkmann, B.; Köhler, H.; Banaschak, S.; Berg, A.; Eikelmann, B.; et al. (2000) ROC analysis of alcoholism markers -100% specificity. *International Journal of legal medicine*. 113, 293-299.

Broussard, L.A. (2003) Inhalants. In Levine, B. *Principles of forensic toxicology*. 2° Edition, AACC press, Washington D.C.

Broussard, L.A.; Broussard, A.K.; Pittman, T. & Lirette, D.K. (2000) Death due to inhalation of ethyl cloride. *Journal of forensic science* 45, 6, 223-225.

Broussard, L.A.; Brustowicz, T; Pittman, T.; Atkins, K.D. & Presley, L. (1997) Two traffic fatalities related to the use of difluoroethane. *Journal of forensic science* 42, 6, 1186-1187.

Brown, D.J. & Long, W.Ch. (1988). Quality control in blood alcohol analysis: simultaneous quantitation and confirmation. *Journal of analytical toxicology*. 12, 279-283.

Burrows, D.L.; Nicolaides, A.; Stephens G.C. & Ferslew K.E. (2004) The distribution of sevoflurane in a sevoflurane induced death. *Journal of forensic science*, 49, 2, 1-4.

Burrows, D.L.; Nicolaides, A.; Stephens G.C. & Ferslew K.E. (2004) Sevoflurane analysis in serum by headspace gas chromatography with application to various biological matrices. *Journal of analytical toxicology*, 28, 418-421.

Calafat, A.M. & Stanfill, S.B. (2002) Rapid quantitation of cyanide in whole blood by automated headspace gas chromatography. *Journal of chromatography B*. 772, 131-137.

Calder I. (1997) hangover Not the ethanol perhaps the methanol. *British Medical Journal* 314, 2-3.

Canfield, D.V.; Smith, M.D.; Ritter, R.M. & Chaturvedi, A.K. (1998). Preparation of carboxyhemoglobin standards and calculation of spectrophtometric quantitation constants, *Federal aviation administration*. Report DOT/FAA/AM-98/21. 1-8.

Cardeal, Z.L.; Pradeau, D.; Hamon, M.; Abdoulaye, I.; Paillier, F.M.; et al. (1993). New calibration method for gas chromatography assay of carbon monoxide in blood. *Journal of analytical toxicology*. 17, 193-195.

Christmore, D.S.; Kelly, R.C. & Doshier, L.A. (1984). Improved recovery and stability of ethanol in automated headspace analysis. *Journal of forensic science*. 29, 4, 1038-1044.

Czogala, J. & Goniewicz, M.L. (2005). The complex analytical method for the assessment of pasive smokers´ exposure to carbon monoxide. *Journal of analytical toxicology*. 29, 830-834.

Dumas, O., Gingras, G. & Leblanc, A. (2005). Isotope dilution-mass spectrometry determination of blood cyanide by headspace gas chromatography. *Journal of analytical toxicology*. 29, 71-75.

Felby, S. & Nielsen E. (1994) Determination of ketone bodies in postmortem blood by headspace gas chromatography. *Forensic Science International*. 64, 83-88.

Felby, S. (2009) Determination of cyanide in blood by reaction headspace gas chromatography. *Forensic Science Medicine and Pathology*. 5, 39-43.

Felby, S.; Nielsen E. & Thomsen, J.L. (2007) The postmortem distribution of ketone bodies between blood, vitreous humor, spinal fluid and urine. *Forensic Science Medicine and Pathology*. 4,2, 100-107.

Furton, K. G.; Wang, J.; Hsu, Y.-L.; Walton, J. & Almirall, J.R. (2000). The Use of Solid-Phase Microextraction-Gas Chromatography in Forensic Analysis. *Journal of chromatographic science*. 38 ,7, 297-306.

Gaillard, Y.; Masson Seyer, M.F.; Goroud, M.; Roussot, J.F. & Prevosto, J.M. (2006). A case of drug facilitated sexual assault leading to death by chloroform poisoning. *International Journal of legal medicine*. 120, 241-245.

Gaulier, J.M.; Tonnay, V.; Faict, T.; Sayer, H.; Marquet, P. & Lachatre, G. (2003) Analytical aspects of volatile substance abuse (VSA). *Journal of forensic science* 48, 4, 1-3.

Groppi, A.; Polettini, A.; Lunetta. P.; Achille, G. & Montagna, M. (1994) A fatal case of trichlorofluoromethane (freon 11) poisoning. Tissue distribution study by gas chromatography –mass spectrometry. *Journal of forensic science* 39, 3, 871-876.

Hansen, W.B. & Rose, L.A. (1997) Recreational use of inhalants drugs by adolescents: a challange for family physicians. *Family Medicine*. 27,6, 383-387.

Honey, D.; Caylor, C.; Luthi, R. & Kerrigan, S. (2005) Comparative alcohol concentrations in blood and vitreous fluid with illustrative case studies. *Journal of analytical toxicology*. 29, 365-369.

Iffland, R. & Jones, A.W. (2003) Evaluating alleged drinking after driving –The hip flask defence. *Medicine, Science & the Law*. 43,1, 39—68.

Iffland, R.; Schmidt V.; Ochmichen M.; Schmidtmann U. & Norpoth, T. (1988). Acetone and isopropanol concentrations in blood. Dependency of acute and chronic ethanol consumption. *Blutalkohol*. 25, 80-96

Isenschmid, D.S., Cassin, B.J.; Hepler, B.R. & Kanluen, S. (1998). Tetrachloroethylene intoxication in an autoerotic fatality. *Journal of forensic science* 43, 1, 231-234.

Ishii, A.; Seno, H.; Watanabe Suzuki, K. & Suzuki, O. (1998). Determination of cyanide in whole blood by gas chromatography with cryogenic oven trapping. *Analytical chemistry*. 70,4873-4776.

Iten, P.X. & Meier, M. (2000). β-Hidroxybutyric acid. An Indicator for an alcoholic ketoacidosis as cause of death in deceased alchol abusers. *Journal of forensic Science*. 45, 3, 624-632.

Jones, A.W. & Schuberth, J. (1989) Computer aided headspace gas chromatography applied to blood alcohol analysis: importance of online process control. *Journal of forensic science*. 34, 5, 1116-1127.

Jones, A.W.; Beylich, K.M.; Bjorneboe, A.; Ingum, J. & Morland, J. (1992) Measuring ethanol in blood and breath for legal purposes: variability between laboratories and between breath test instruments. *Clinical Chemistry*. 38, 5, 743-747.

Jones, A.W.; Lund, M. & Andersson, E. (1989). Drinking drivers in Sweden who consumed denatured alcohol preparations.: An analytical toxicology study. *Journal of analytical toxicology*. 17, 182-185.

Kim, N.Y. & Park, S.W. (2000) The comparison of toluene determination between headspace solid phase microextraction and headspace methods in glue-sniffer´s blood and urine samples. *Journal of forensic science* 45, 3, 702-707.

Kim, N.Y.; Park, S.W. & Suh, J.K. (1996) two fatal cases of dichlorometane or chloroform poisonings. *Journal of forensic science* 41, 3, 527-529.

Kolb, B. (1999). Headspace sampling with capillary columns. *Journal of Chromatography A*. 842, 163-205

Kovatsi, L.; Giannakis, D.; Arzoglu, V. & Samanidou V. (2011) Development and validation of a direct headspace GC-FID method for the determination of sevoflurane, desflurane and other volatile compounds of forensic interest in biological fluids: Application on clinical and postmortem samples. *Journal of separation Science*. 34, 1004-1010.

Kugelberg, F.C. & Jones A.W. (2007) Interpreting Results of ethanol analysis in postmortem specimens: A review of literature. *Foresic Science International*. 165, 10-29.

Kulhman, J.J.; Magliulo, J.; Levine, B. & Smith, M.L. (1993). Two deaths involving isoflurane abuse. *Journal of forensic Sciences*. 38, 4, july, 968-971.

Kunsman, G.W. & Levine, B. (2003) Carbon monoxide/Cyanide. In. Levine, B. *Principles of Forensic Toxicology*. 2° Edition, AACC press, Washington D.C.

Lewis, R.J.; Johnson, R.D. & Canfield, D.V. (2004). An accurate method for the determination of carboxihemoglobin in postmortem blood using GC-TCD. *Journal of analytical Toxicology* 28, 59-62

Logan, B.K.; Case, G.A. & Kiesel, E.L. (1994) Differentiation of diethyl ether/acetone and ethanol/acetonitrile and other common volatiles by dual column gas chromatography. *Journal of forensic science*. 39, 6, 1544-1551.

Macchia, T.; Mancinelli, R.; Gentili, S.; Lugaresi, E.C.; Raponi, A. & Taggi F. (1995). Ethanol in biological fluids: Headspace GC measurement. *Journal of analytical Toxicology* 19, 241-246

Maehly, A. (1962). Quantitative determination of carbon monoxide, In *Methods of forensic Science*, volume 1. Lundquist, F. 539-592, Interscience Publisher,

Mahoney, J.J.; Vreman, H.J.; Stevenson, D.K. & Van Kessel, A.L. (1993). Measurement of carboxihemoglobin and total hemoglobin by five specialized spectrophotometers (CO-oximeters) in comparison with reference methods. *Clinical Chemistry*. 39/8, 1693-1700.

Majchrowicz, E. & Mendelson, J.H. (1971). Blood methanol concentrations during experimentally induced ethanol intoxication in alcoholics. *The journal of pharmacology and experimental therapeutics*. 179, 2, 293-300.

McAnallay, B.H. (2004). The chemistry of alcoholic beverages. In Garriott, J.C. *Medico-legal aspects of alcohol*. Lawyers & Judges Pub. Co. Tucson.

McAuley, F. & Reive, D.S. (1983). Rapid quantitation of cyanide in blood by gas chromatography. *Journal of analytical toxicology*. 7, 213-215.

Moffat, A.C., Osselton, M.D. & Widdop, B. (2004). *Clarke´s Analysis of drugs and Poisons*. Pharmaceutical Press, London.

Moriya, F. & Hashimoto, Y. (2001). Potencial for errors when assessing blood cyanide concentrations in fire victims. *Journal of forensic science*. 46, 6, 1421-1425.

Musshoff, F. (2002). Chromatographic methods for the determination of markers of chronic and acute alcohol consumption. *Journal of chromatography B*. 781, 457-480.

Odoul, M.; Fouillet, B.; Nouri, B.; Chambon, R. & Chambon, P. (1994). Specific determination of cyanide in blood by headspace gas chromatography. *Journal of analytical toxicology*. 18, 205-207.

Oritani, S.; Zhu, B.L.; Ishida, K.; Shimotouge, K.; Quan,L. et al. (2000). Automated determination of carboxyhemoglobin contents in autopsy material using headspace gas chromatoghraphy/mass spectrometry. *Forensic Science International*. 113, 375-379.

Penton, Z. (1985). Headspace measurement of ethanol in blood by gas chromatography with a modified autosampler. *Clinical chemistry*. 31, 3, 439-441.

Pihlainen, K. & Ojanpëra, I. (1998) Analytical Toxicology of fluorinated inhalation anaesthetics. *Forensic Science International*. 97,117-133.

Pounder, D.J.; Stevenson, R.J. & Taylor, K.K. (1998). Alcoholic ketoacidosis at autopsy. *Journal of forensic science*. 43, 812-816.

Pronko, P.S.; Velichko, M.G.; Moroz, A.R. & Rubanovich, N.N. (1997) Low molecular weight metabolites relevant to ethanol metabolism: correlation with alcohol withdrawal severity and utility for identification of alcoholics. *Alcohol & alcoholism*. 32,6,761-768.

Ren, Q. & Bertsch, W. (1999) A comprehensive sample preparation scheme for accelerants in suspect arson cases. *Journal of forensic science*. 44, 3,504-515

Roine, R.P.; Eriksson, J.P.; Ylikahri, R.; Penttilä, A. & Salaspuro, M. (1989) Methanol as a marker of alcohol abuse. *Alcoholism: clinical and Experimental research*. 13,2,172-175.

Saito, K.; Takayasu, T.; Nishigami, J.; Kondo, T.; Ohtsuji, M.; et al. (1995). Determination of the volatile anesthetics halothane, enflurane, isoflurane and sevoflurane in biological specimens by pulse heating GC-MS. *Journal of Analytical toxicology*, 19, 115-119

Sandercock, P.M.L. (2008). Fire investigation and ignitable liquid residue analysis. A review: 2001-2007. *Forensic science international*. 176,93-110

Schuberth, J. (1997). Gas residues of engine starting fluid in postmortem sample from arsonist. *Journal of forensic science* 42, 1, 144-147.

Senkowski, C.M. & Thompson K.A. 1990). The accuracy of blood alcohol analysis using gas chromatography when performed on clotted samples. *Journal of forensic science*. 35, 1, 176-180.

Seto, Y,; Tsunoda, N.; Ohta, H. & Shinohara, T. (1993). Determination of blood cyanide by headspace gas chromatography with NPD and using a megabore capillary column. *Analytica Chimica Acta*. 276, 247-259.

Seto, Y. (1994) Determination of volatile substances in biological samples by headspace gas chromatography. *Journal of Chromatography A*. 764, 25-62.

Seto, Y. (1995). Oxidative conversion of thiocyanate to cyanate by oxyhemoglobinduring acid denaturation. *Archives of Biochemistry and Biophysics*. 321, 245-254.

Sharp, M.E. & Dautbegovic, T. (2001). Ether stability in preserved blood samples and a case of ether assisted suicide. *Journal of analytical toxicology*. 25, 628-630.

Sharp, M.E. (2001). A comprehensive screen for volatile organic compounds in biological fluids. *Journal of analytical toxicology*. 25, 631-635.

Shibata, M.; Inoue, K.; Yoshimura, Y.; Nakazawa, H. & Seto, Y. (2004). Simultaneous determination of hydrogen cyanide and volatile aliphatic nitriles by headspace gas chromatography, and its application to an in vivo study of the metabolism of acrylonitriles in the rat. *Archives of toxicology*. 78, 301-305.

Singer, P.P. & Jones, G.R. (2006). An unusual autoerotic fatality associated with chloroform inhalation. *Journal of analytical toxicology*. 30, 216-218.

Slack, G.C.; Snow, N.H. & Kou, D. (2003) extraction of volatile organic compounds from solids and liquids, in: Sample *Preparation Techniques in analytical chemistry*, Mitra, S., 183-225. John Wiley & Sons, Canada.

Smith, R.M. (2003) Before the injection Modern methods of sample preparation for separation techniques. *Journal of chromatography A*. 1000, 3-27.

Snow, N.H. (2000) Solid phase microextraction of drugs from biological matrices. *Journal of chromatography A*. 885, 445-455.

Statheropoulos, M.; Spiliopoulou, C. & Agapiou, A. (2005). A study of volatile organic compounds evolved from the decaying human body. *Forensic Science International*. 153, 2-3, 147-155.

Suarez, L.A.; Arellano, R. & Bernal, E. (2009). Metanol como marcador de abuso en el consumo de alcohol en muestras forenses. *Revista de toxicología*. 26, 56-59.

Swann, L.M.; Forbes, S.L. & Lewis, S.W. (2010). Analytical separations of mammalian decomposition products for forensic science. A review. *Analytica chimica acta*. 682, 1-2, December, 9-22.

Tagliaro, F.; Lubli, G.; Ghielmi, S.; Franchi D. & Marigo, M. (1992). Chromatographic methods for blood alcohol determination. *Journal of Chromatography* 580, 161-190.

Thomsen, J.L.; Felby, S.; Theeilade, P. & Nielsen, E. (1995). Alcoholic ketoacidosis as a cause of death in forensic cases. *Forensic Science International*. 75, 2-3, 163-171.

Uyanik, A. (1997). Gas chromatography in anesthesia I. A brief review of analytical methods and gas chromatographic detector and column systems. *Journal of chromatography A*. 693,1-9.

Van Dam, J. & Daenens, P. (1994). Microanalysis of carbon monoxide in blood by headspace capillary gas chromatography. *Journal of forensic science*. 39, 2, 1421-1425.

Vreman, H.J.; Kwong, L.K. & Stevenson, D.K. (1984) Carbon Monoxide in blood: An improved microliter blood sample collection system, with rapid analysis by gas chromatography. *Clinical Chemistry*. 30/8, 1382-1386.

Walch, S.G.; Lachenmeier, D.W.; Sohnius, E.M.; Madea, B. & Musshof, F. (2010). Rapid determination of carboxihemoglobin in postmortem blood using fully automated headspace gas chromatography with methaniser and FID. *The open toxicology journal*. 4, 21-25.

Watanabe-Suzuki, K.; Seno, H.; Ishii, A.; Kumazawa, T. & Suzuki, O. (1999). Ultrasensitive method for the determination of ethanol in whole blood by headspace capillary gas chromatography with cryogenic oven trapping. *Journal of chromatography B*. 727, 89-94

Watts, M.T. & McDonald, O.L. (1987) The effect of biologic specimen type on the gas chromatograpic headspace analysis of ethanol and other volatile compounds. *American Journal of clinical Pathology*. 87, 79-85

Wilkinson, P.K.; Wagner, J.G. & Sedman, A.J. (1975) Sensitive headspace gas chromatography method for the determination of ethanol utilizing capillary blood samples. *Analytical Chemistry*, 47, 9, 1506-1510.

Zarrabeitia, M.T.; Ortega, C.; Altuzarra, E.; Martinez, M.A.; Mazarrasa, O. & Calvet, R. (2001) Accidental dichloromethane fatality: a case report.. *Journal of forensic science* 46, 3, 726-727.

**Gas Chromatography in Plant Science, Wine Technology,
Toxicology and Some Specific Applications**
Edited by Dr. Bekir Salih

ISBN 978-953-51-0127-7

Hard cover, 346 pages

Publisher InTech

Published online 29, February, 2012

Published in print edition February, 2012

The aim of this book is to describe the fundamental aspects and details of certain gas chromatography applications in Plant Science, Wine technology, Toxicology and the other specific disciplines that are currently being researched. The very best gas chromatography experts have been chosen as authors in each area. The individual chapter has been written to be self-contained so that readers may peruse particular topics but can pursue the other chapters in the each section to gain more insight about different gas chromatography applications in the same research field. This book will surely be useful to gas chromatography users who are desirous of perfecting themselves in one of the important branch of analytical chemistry.

INTECH
open science | open minds