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Breath Testing by

Fourier Transform Infrared Spectroscopy for

Solvent Intoxication Diagnostics

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Academic dissertation

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List of original publications

This thesis is based on the following original articles, which will be referred to in the text by their Roman numerals.

I   Laakso O, Haapala M, Jaakkola P, Laaksonen R, Nieminen J, Pettersson M, Räsänen M, and Himberg J-J. The use of low resolution FT-IR spectrometry for the analysis of alcohols in breath. Journal of Analytical Toxicology 24: 250–6, 2000.

II   Laakso O, Haapala M, Jaakkola P, Laaksonen R, Luomanmäki K, Nieminen J, Pettersson M, Päivä H, Räsänen M, and Himberg J-J. FT-IR breath test in the diagnosis and control of treatment of methanol intoxications. Journal of Analytical Toxicology 25: 26–30, 2001.

III   Laakso O, Haapala M, Kuitunen T, and Himberg J-J. Screening of exhaled breath by low-resolution multicomponent FT-IR spectrometry in patients attending emergency departments. Journal of Analytical Toxicology 28: 111–7, 2004.

IV   Laakso O, Pennanen T, Himberg K, Kuitunen T, Himberg J-J. Effect of eight solvents on ethanol analysis by Dräger 7110 Evidential breath analyzer. Journal of Forensic Sciences 49: 1113–6, 2004.

V   Laakso O, Haapala M, Pennanen T, Kuitunen T, and Himberg J-J. Fourier-transformed infrared breath testing after ingestion of technical alcohol. Journal of Forensic Sciences (Submitted for publication).

Abbreviations

ANOVA	analysis of variance	EtOH	ethanol
BBR	blood-breath ratio	MeOH	methanol
CNS	central nervous system	1-propanol	n-propanol
DUI	driving under the influence	2-propanol	isopropyl alcohol
ER	emergency room	MEK	methyl ethyl ketone
FT-IR	Fourier transform infrared (spectroscopy)	MIBK	methyl isobutyl ketone
		MTBE	methyl tert-butyl ether
FVC	forced vital capacity	N_2	nitrogen
IR	infra red	O_2	oxygen
LLOQ	lower limit of quantification	CO_2	carbon dioxide
LOD	limit of detection	CO	carbon monoxide
ppb	parts per billion (by volume)	H_2O	water
ppm	parts per million (by volume)	N_2O	nitrous oxide
SD	standard deviation		
SNR	signal to noise ratio		
VOC	volatile organic compound		

Definitions

alcohol: any organic compound in which a hydroxyl group (–OH) is bound to a carbon atom, which in turn is bound to other hydrogen and/or carbon atoms

solvent: liquid that dissolves a solid, liquid, or gaseous solute, resulting in a solution

matrix: environment from which a given sample is taken (e.g., exhaled breath)

beamsplitter: a dichroic mirror in the interferometer (Figure 2, page 25)

MCT detector: The MCT detector is composed of a thin layer (10 to 20 µm) of mercury, cadmium and telluride (HgCdTe). Photons excite electrons into the conduction band, thereby increasing the conductivity of the material. The change in conductivity is thus proportional to the light intensity.

Peltier cooling: Peltier devices are small solid-state devices that function as heat pumps. A typical unit is a few millimetres thick sandwich formed by two ceramic plates with an array of small Bismuth Telluride cubes in between. When a DC current is applied, heat is moved from one side of the device to the other—where it must be removed with a heatsink. The "cold" side is commonly used to cool an electronic device.

mass-flow meter/controller: The operating principle of the mass flow meter is thermodynamic. Resistance temperature measuring elements are built in the sensor tube. A precise amount of heat is directed to the sensors (Aalborg), or between them (Brooks). With no flow, the heat reaching each temperature element is equal. With increasing flow, the flow stream carries heat away from the upstream element, and an increasing amount towards the downstream element. An increasing temperature difference develops between the two elements, and this difference is proportional to the amount of gas flowing or the mass flow rate. A bridge circuit interprets the temperature difference and an amplifier provides the output signal. In a mass-flow controller, the signal from a mass flow meter is further used to position the precision solenoid control valve to control the gas flow rate.

Abstract

Technical or contaminated ethanol products are sometimes ingested either accidentally or on purpose. Typical misused products are black-market liquor and automotive products, e.g., windshield washer fluids. In addition to less toxic solvents, these liquids may contain the deadly methanol.

Symptoms of even lethal solvent poisoning are often non-specific at the early stage. The present series of studies was carried out to develop a method for solvent intoxication breath diagnostics to speed up the diagnosis procedure conventionally based on blood tests. Especially in the case of methanol ingestion, the analysis method should be sufficiently sensitive and accurate to determine the presence of even small amounts of methanol from the mixture of ethanol and other less-toxic components. In addition to the studies on the FT-IR method, the Dräger 7110 evidential breath analyzer was examined to determine its ability to reveal a coexisting toxic solvent.

An industrial Fourier transform infrared analyzer was modified for breath testing. The sample cell fittings were widened and the cell size reduced in order to get an alveolar sample directly from a single exhalation. The performance and the feasibility of the Gasmet FT-IR analyzer were tested in clinical settings and in the laboratory. Actual human breath screening studies were carried out with healthy volunteers, inebriated homeless men, emergency room patients and methanol-intoxicated patients. A number of the breath analysis results were compared to blood test results in order to approximate the blood-breath relationship. In the laboratory experiments, the analytical performance of the Gasmet FT-IR analyzer and Dräger 7110 evidential breath analyzer was evaluated by means of artificial samples resembling exhaled breath.

The investigations demonstrated that a successful breath ethanol analysis by Dräger 7110 evidential breath analyzer could exclude any significant methanol intoxication. In contrast, the device did not detect very high levels of acetone, 1-propanol and 2-propanol in simulated breath. The Dräger 7110 evidential breath ethanol analyzer was not equipped to recognize the interfering component.

According to the studies the Gasmet FT-IR analyzer was adequately sensitive, selective and accurate for solvent intoxication diagnostics. In addition to diagnostics, the fast breath solvent analysis proved feasible for controlling the ethanol and methanol concentration during haemodialysis treatment. Because of the simplicity of the sampling and analysis procedure, non-laboratory personnel, such as police officers or social workers, could also operate the analyzer for screening purposes.

1. Introduction

More than a thousand intoxication deaths occur annually in Finland. Ethanol is the most common single causative chemical: it explains nearly half of the deaths (Vuori *et al.* 2006). In the year 2003, there were 457 hospital care periods due to alcohol poisoning, 9,777 due to alcohol intoxication (STAKES 2006). In 2004, 544 deaths occurred due to ethanol poisoning—methanol caused 26 and ethylene glycol 14 deaths. The proportion of methanol deaths has remained high since the mid-1990's, mainly due to methanol-based windshield washer fluids (Vuori *et al.* 2006).

Prompt treatment of methanol and ethylene glycol intoxication is a prerequisite for a good outcome. The clinical signs and symptoms of solvent intoxication are unspecific, at least at an early stage, and medical history is often missing or incomplete. Fast diagnostic methods for non-laboratory personnel use are needed not only in hospital emergency rooms, but also in out-of-hospital settings.

Specific blood tests are in many ways the golden standard where solvent analysis is concerned. Gas chromatography and enzymatic immunoassay are commonly used methods for the determination of serum alcohols. The turn-around time of a gas chromatography analysis or an enzymatic procedure is approximately 1 h, when an on-site laboratory is available to carry them out. In many hospitals, specific methods for methanol and ethylene glycol analyses are not available and the samples must be sent to an off-site laboratory, which markedly extends the delay in diagnostics (Church *et al.* 1997).

Faster (non-specific) laboratory tests, such as the serum osmolal and anion gap tests, are available to support the diagnosis of a toxic alcohol poisoning until the blood levels are available. However, a normal osmolal gap does not exclude toxicity from methanol or ethylene glycol (Glaser 1996), and increases in the osmolal gap can also occur in patients with multiple organ failure and other unmeasured osmolal entities (Church *et al.* 1997). Profound anion gap metabolic acidosis suggests toxicity with methanol or ethylene glycol (Church *et al.* 1997), but anion gap acidosis becomes evident only when the parent alcohol products are metabolized to their toxic acidic byproducts (Mycyk *et al.* 2003). There have also been a few reports on analyzing ethanol from saliva and sweat (Buono 1999, Smolle *et al.* 1999), but the method has not been validated for other solvents. Urine microscope analysis for crystals caused by ethylene glycol poisoning requires expertise and is by no means a quantitative method.

The analysis of exhaled air provides a non-invasive method for estimating the concentrations of volatile components in blood. Ethanol breath testing has been used in intoxication diagnostics for decades. However, rapid commercial breath assays aimed particularly for detecting toxic solvents are not available.

The present series of studies was carried out to develop a new FT-IR method for solvent intoxication breath diagnostics. The new method should be faster and easier than the old methods without compromising the analytical accuracy, sensitivity and selectivity. Additionally, a commercially available evidential breath ethanol analyzer was examined to find out its capability to reveal a coexisting toxic solvent.

2. Review of the literature

2.1. Composition of normal exhaled breath

The most abundant matrix components in the human breath (Table 1) are nitrogen (N_2), oxygen (O_2), water (H_2O) and carbon dioxide (CO_2). Nearly 3,500 different volatile organic compounds (VOC) were detected by gas chromatography and mass spectrometry in a study of breath samples from fifty normal humans (Phillips *et al.* 1999). Sorbent traps were used in order to concentrate these very-low concentration compounds. Half of the VOCs were of endogenous origin. An average breath contained approximately 200 VOCs. Only 27 VOCs were detected in every breath sample, nine of which were of endogenous origin. The most abundant endogenous VOCs found in more than half of the breath samples were isoprene and acetone. The analyzer in that study was not configured to detect the small compounds like ammonia, methane and carbon monoxide. The absolute concentrations of the components were not determined in this study.

Interference from endogenous or exogenous compounds may be an important consideration when breath analysis is adopted as a monitoring technique for solvents. The above-mentioned breath components are usually present in low concentrations, when compared to the level of solvents found in breath following solvent intoxication. However, it is essential to be aware of possible interferences when low concentrations of solvents are being examined. In the case of acetone, carbon monoxide or methane, quite high "normal" breath concentrations may occur, and interference is thus possible.

In the following, the most abundant breath components are discussed from the perspective of analyzing techniques based on infrared spectroscopy.

2.1.1. Oxygen, nitrogen, water and carbon dioxide

Exhaled breath contains approximately 5 vol% of carbon dioxide in its last (alveolar) fraction. In addition, exhalation is fully saturated with water vapour, which means 5.2 vol% in the breath temperature of 34 ºC (Hlastala *et al.* 1988, Lide 2000). Water vapour may cause problems in analysis due to its non-linear absorption in the wide range of the infrared spectrum. The strong infrared spectrum of water overlaps the spectra of other breath components (Figure 11, page 43). Symmetric diatomic molecules such as oxygen and nitrogen are not detectable with the infrared techniques (Hollas 1996).

2.1.2. Isoprene

Isoprene is a by-product of cholesterol synthesis during the conversion of mevalonate to mevanolate-5-pyrophosphate and isopentenyl pyrophosphate (Sharkey 1996). Isoprene has been reported by many authors to be the main endogenous hydrocarbon in exhaled human breath. In these studies, the mean breath isoprene concentrations varied from 89 to 370 ppb in healthy volunteers (Cailleux *et al.* 1989, Davies *et al.* 2001, Hyspler *et al.* 2000, Karl *et al.* 2001). Breath isoprene concentrations may fall when cholesterol synthesis is suppressed, for example, by treatment with simvastatin (Karl *et al.* 2001, Sharkey 1996). According to Cailleux and co-workers (1993), the blood isoprene concentration is approximately 3 µg/l in healthy adults.

2.1.3. Acetone

Acetone occurs as a metabolic component in blood, urine and human breath. It can be

Table 1. *Major components in normal end-exhaled breath.*

Component	Concentration		Comments (references in brackets)
	[vol%]		
Oxygen	< 21		
Nitrogen	~ 70		
Carbon dioxide	4–6		
Water vapor	< 5.2		in 34 ºC, 1 atm (Lide 2000)
	[ppm]	[µg/l]	
Methane	0–120	0–76	mean ~ 20 ppm in producers (Bjorneklett *et al.* 1982, Corazza *et al.* 1994, Florin *et al.* 2000, Rumessen *et al.* 1994)
Carbon monoxide	1–3	1–3	in non-smokers (Archbold *et al.* 1995, Irving *et al.* 1988, Middleton *et al.* 2000, Uasuf *et al.* 1999, Yamaya *et al.* 1998, Zayasu *et al.* 1997)
	4–8	4–9	during airway inflammation (Uasuf *et al.* 1999, Yamaya *et al.* 1998, Zayasu *et al.* 1997)
	14–24	16–27	mean in smokers (Archbold *et al.* 1995, Irving *et al.* 1988, Middleton *et al.* 2000, Yamaya *et al.* 1998, Zayasu *et al.* 1997)
Acetone	0.2–1.8	0.5–4	in healthy (Jones 1987, Kundu *et al.* 1993, Smith D *et al.* 1999)
	15–68	35–157	in adults after fasting 36 h (Jones 1987)
	14–168	32–287	in children on ketogenig diet (Musa-Veloso *et al.* 2002)
	148–868	343–2000	in dead diabetics (extrapolated from blood concentrations) (Brinkmann *et al.* 1998)
Isoprene	0.1–0.4	0.3–1.1	mean in healthy (Cailleux *et al.* 1989, Davies *et al.* 2001, Hyspler *et al.* 2000, Karl *et al.* 2001)
Ammonia	0.2–1	0.14–0.7	in healthy (Kearney *et al.* 2002, Smith D *et al.* 1999, Spanel *et al.* 1998)
	0.8–1.8	0.5–1.2	5 h after a liquid protein meal (Spanel *et al.* 1998)
	1	0.7	mean in chirrosis with hyperammonemia (Shimamoto *et al.* 2000)
	1.5–2	1–1.4	in uremic before dialysis (Narasimhan *et al.* 2001)

formed endogenously from fatty acid oxidation. The endogenous acetone concentration is low in healthy, normally nourished people. Fasting and unbalanced diabetes mellitus increase the endogenous generation of acetone. Brinkman and co-workers (1998) measured a significantly higher mean endogenous blood acetone concentration due to diabetic ketoacidosis. Blood samples taken during the autopsy of seven men had a mean concentration of 270 mg/l acetone. It corresponds with 478 ppm in breath, when 245 is used as the blood-breath ratio (Table 2, page 20).

2.1.4. Ammonia

Ammonia derived from the catabolism of proteins and amino acids is normally present in breath in low concentrations. Breath ammonia is increased in hepatic disease (Shimamoto *et al.* 2000) and uremic patients (Narasimhan *et al.* 2001). It also increases during a Helicobacter pylori urea breath test (Kearney *et al.* 2002). A breath ammonia concentration of 2.0 ppm has been reported in a uremic patient before dialysis treatment (Narasimhan *et al.* 2001). In blood, ammonia is present mainly in ionized form. Normally, the total ammonia blood concentration is below 50 µmol/l .

2.1.5. Methane

Methanogenous bacteria are the principal hydrogen-consuming bacteria in the large intestine of methanogenic humans ("methane producers"). They use hydrogen to produce methane and reduce flatulence and bloating by the conversion of four volumes of hydrogen gas to one volume of methane gas (Florin *et al.* 2000).

In the international literature, the prevalence of methane producers varies from 10% to 54% of the population (Le Marchand *et al.* 1993, McKay *et al.* 1985, Peled *et al.* 1987). Possible factors affecting excretion status are age, sex, diet, bacterial flora, ethnic origin and intestinal transit time (Florin *et al.* 2000). Patients suffering from gastrointestinal diseases, such as Crohn's disease and ulcerative colitis, have been found to produce less methane than healthy controls (McKay *et al.* 1985).

2.1.6. Carbon monoxide

Smoking, either passive or active, is the main source of carbon monoxide (CO), since inhaled tobacco smoke contains 4–5 vol% of CO (Kirkham *et al.* 1988). Exhaled CO correlates well with the blood carboxyhaemoglobin level (Guyatt *et al.* 1988, Jarvis *et al.* 1980). The wide range of exhaled CO concentrations in smokers is due to varying numbers of cigarettes smoked per day and variation in the interval between CO measurement and the latest cigarette (Woodman *et al.* 1987). Due to increased oxidative stress, exhaled carbon monoxide concentrations are slightly increased in inflammatory respiratory diseases, as well. For example, exacerbation of asthma has been shown to raise the exhaled CO to 8.4 ppm and upper respiratory tract infection to 3.8–5.6 ppm (Yamaya *et al.* 1998, Zayasu *et al.* 1997).

2.2. Common intoxicating solvents

2.2.1. Ethanol

Ethanol is the most common intoxicating solvent. The small intestine extracts roughly 80% of an oral ethanol dose; the stomach absorbs the remainder. Since ethanol is poorly absorbed from the stomach, factors that delay gastric emptying decrease the absorption. In healthy adults, 80%–90% of the absorption occurs within 30–60 minutes, but food may delay complete absorption for 4–6 hours (Ellenhorn 1997, Jones *et al.* 2003). Most of the ethanol is metabolized. Conversion of ethanol to acetaldehyde by alcohol dehydrogenase is the rate-limiting step. The rate of metabolism varies to a large extent, from 100 to 200 mg/kg/h (Jones *et al.* 2003).

Ethanol is a central nervous system (CNS) depressant. Variations in ethanol toxicity occur as a result of the concurrent presence of hypoglycemia and drug interactions. A blood concentration over 3.5 g/l is considered lethal (Winek *et al.* 2001). It corresponds with 830 ppm in breath, when the blood-breath ratio of 2,300 is used (Table 2, page 20). Chronic alcoholics can develop a marked tolerance to ethanol and cope with blood levels considered potentially fatal for non-tolerant individuals (Ellenhorn 1997). Treatment of acute ethanol intoxication is mostly symptomatic.

2.2.2. Methanol

Methanol is a common industrial chemical used for synthetic reactions or as a solvent. For the general public, exposure may occur through consumer products such as paint removers, automotive fluids (cleaners, windshield washer antifreezes) and fuels as well as copying fluids. Poisonings from methanol are relatively infrequent, but can be lethal or very severe in morbidity, possibly resulting in permanent blindness or death (Davis *et al.* 2002, Jacobsen *et al.* 1997).

A methanol blood concentration over 6 mmol/l [20 mg/l] is considered toxic (Winek *et al.* 2001). Nevertheless, there is little correlation between blood levels of methanol and the severity of the poisoning, because it is the metabolites that are toxic. In many cases, subjects poisoned do not seek treatment until the syndrome has developed into an advanced stage. At that point, the blood methanol level may be low (Jacobsen *et al.* 1997).

To date, the exhaled methanol concentration in intoxicated patients has not been reported in literature. Even though the *in vitro* blood-air partition coefficient is not equal to the *in vivo* blood-breath ratio (see Chapter 2.3), it can be used to roughly approximate the blood-breath relationship. If a median *in vitro* blood-air partition coefficient of 2,650 (Table 2, page 20) were used for blood-breath conversion, the methanol blood concentration of 6 mmol/l would correspond to 60 ppm in breath.

The methanol toxicity includes an initial CNS depression similar to but much weaker than that produced by ethanol, followed by a latent period of 10–30 h. The latent period is generally shorter when larger amounts are consumed and longer when ethanol is also consumed. During the latent period, methanol is metabolized by alcohol dehydrogenase into toxic compounds, formaldehyde and formic acid (Ellenhorn 1997). After the asymptomatic period, symptoms such as nausea, vomiting, weakness, abdominal pain and respiratory difficulties begin to appear. At this stage, patients often report visual defects ranging from blurring to total loss of vision. The presence of deep metabolic acidosis is common. In severe cases, the result may even be coma or death (Davis *et al.* 2002, Jacobsen *et al.* 1997).

Treatment of severe methanol intoxication necessitates prompt haemodialysis and metabolic inhibitors (fomepizole or ethanol) in addition to supportive treatment (Jacobsen *et al.* 1997, Lushine *et al.* 2003).

2.2.3. Propanols

1- and 2-propanols are commonly used industrial solvents. 2-propanol is often added to ethanol-based windshield washer fluids and cooker fuels. These products are used by some alcoholics. 1- and 2-propanols are roughly twice as toxic as ethanol (Dreisbach *et al.* 1987). 2-propanol is generally less toxic than methanol or ethylene glycol, and the toxicity is due to 2-propanol itself and to acetone, its primary metabolite (Church *et al.* 1997). 1-propanol may be slightly more toxic than 2-propanol, but it seems to induce many of the same biological effects (Gosselin *et al.* 1984). The toxic blood concentration for propanols is 0.4–0.8 g/l (Maynard 2001). It would correspond with 160–400 ppm in breath, if median *in vitro* blood-air partition coefficients (Table 2,

page 20) were used for blood-breath conversion.

The principal manifestation of acute 1- or 2-propanol poisoning is CNS depression. Chronic alcoholics may tolerate very high levels of 2-propanol without developing significant CNS depression (Gosselin *et al.* 1984). The treatment of isopropyl alcohol toxicity is primarily symptomatic, with haemodialysis reserved for refractory hypotension (Church *et al.* 1997, Dreisbach *et al.* 1987).

2.2.4. Ethylene glycol

Ethylene glycol is widely used in industry and can be readily obtained by the consumer, mostly as radiator antifreeze for automobiles. Ethylene glycol is rapidly absorbed orally and produces a CNS depression roughly similar to that induced by ethanol. Peak levels occur 1 to 4 hours post ingestion. The liver oxidizes ethylene glycol primarily into glycoaldehyde, glycolic acid and finally to glyoxylic acid. Depending on the cofactors thiamine and pyridoxine, the metabolism of glyoxylic acid follows several pathways which may end in oxalic and formic acids. An ethylene glycol blood concentration over 8–24 mmol/l [0.5–1.5 g/l] is considered toxic (Anderson 2004, Winek *et al.* 2001). The acidic metabolites are more toxic than the parent compound (Ellenhorn 1997). Due to its physicochemical properties, the ethylene glycol concentration in breath is very low, even in the case of severe intoxication (see Chapter 2.3.1.).

Typically, 4–12 h elapse before nausea, vomiting, hyperventilation, elevated blood pressure, tachycardia, muscular tetany and convulsions appear after ingestion of ethylene glycol. More specific signs are hypocalcaemia and a severe metabolic acidosis. As the syndrome develops, the outcome may be cardiac failure, acute oliguric renal failure, secondary CNS depression and coma (Jacobsen *et al.* 1997). In addition to supportive procedures, haemodialysis and metabolic inhibitors are used to treat severe ethylene glycol intoxications.

2.2.5. Acetone

Acetone is used as a chemical intermediate and a solvent for paints, plastics and adhesives. Exogenous acetone is rapidly absorbed via respiratory and gastro-intestinal tracts or through dermal contact. Exhalation is the major route of elimination for acetone (Baselt 2004). Ingested 2-propanol is readily metabolized into acetone. High concentrations of acetone have been detected in alcoholics who had drunk technical ethanol products containing a few percent 2-propanol (Zuba *et al.* 2002).

Acetone is less toxic than many other industrial solvents. However, a high acetone concentration can cause CNS depression, cardiorespiratory failure and death (Baselt 2004). According to Maynard (2001), the threshold concentration for toxicity is 200 mg/l in blood. It would correspond with 354 ppm in breath, if a median *in vitro* blood-air partition coefficient 245 were used for blood-breath conversion (Table 2, page 20).

2.2.6. Methyl ethyl ketone and methyl isobutyl ketone

In addition to acetone, methyl ethyl ketone (MEK) and methyl isobutyl ketone (MIBK) are the ketone solvents with the widest range of application in industry (Kawai *et al.* 2003). They are also added in low concentrations to ethanol-based cooker fuels and windshield washer fluids. The absorption of MEK and MIBK is rapid via inhalation and ingestion, and these compounds are moderate skin penetrants, as well (Baselt 2004). Both chemicals irritate mucous membranes and have some CNS effect (Kawai *et al.* 2003). Toxic plasma levels for MEK and MIBK have not been defined. The occupational threshold limits for under-15-min airborne exposure are 300 ppm for MEK and 75 ppm for MIBK.

2.2.7. Abused inhalants

Inhalant abuse is defined as deliberate inhalation of a volatile substance to achieve a change in mental state (Kurtzman *et al.* 2001). A typical volatile solvent abuser inhales the vapour directly from a household product container or places the product in a paper bag or on a piece of cloth which is then placed over the nose and mouth (Bowen *et al.* 1999). Inhaled solvents enter the bloodstream directly from the lungs and rapidly reach the brain and other body organs. Blood levels of most volatiles peak within a few minutes of exposure and then decrease rapidly as the substance is distributed to the central nervous system and absorbed by fat (Kurtzman *et al.* 2001). The symptoms of an acute intoxication with solvents are quite similar to those of alcohol intoxication, but they are more rapid in onset and briefer in duration (Bryson 1989). An experienced user may prolong the effects by concentrating the drug inside a plastic bag and continuing to sniff (Kurtzman *et al.* 2001).

In a recent study, the most commonly used inhalants were glue, shoe polish, gasoline and lighter fluid (Wu L-T *et al.* 2004). Glues contain different mixtures of easily volatile compounds. In addition to toluene, these products may contain xylenes, heptane, methyl ethyl ketone, among other substances (Chao *et al.* 1993, Midford *et al.* 1993). Toluene blood concentrations among inhalant abusers have been found to range from 0.1 to 92 mg/l (Chao *et al.* 1993, Park *et al.* 1998). Median concentrations were below 10 mg/l. This corresponds with 130 ppm in breath, when a blood-breath ratio of 20.6 is used. There are no reports on breath butane concentrations during or after lighter gas abuse.

2.3. Pulmonary excretion of a solvent

The results of breath testing are often interpreted to correspond with blood concentrations. Nevertheless, breath and blood are two physiologically distinct samples, and it may not be appropriate to conclude that a breath sample provides information which equates directly with a peripheral venous blood sample (Wilson 1986). The term "blood-breath ratio" (BBR) has been used to represent the ratio of solvent (most often ethanol) concentration in the blood to that in the exhaled breath (Hlastala 1998) (Equation 10.1). For example, a blood-breath ratio of 2,100 is traditionally used to convert the result of breath ethanol analysis into the corresponding blood ethanol concentration for medico-legal purposes (Jones *et al.* 2003). Instead of a single value of 2,100, the ethanol blood-breath ratios derived from simultaneous measurements of blood and breath ethanol concentrations in numerous human studies ranges from 2,160 to 2,475 (median 2,300) (Alobaidi *et al.* 1976, Dubowski *et al.* 1979, Haffner *et al.* 2003, Jones 1978, 1985, Jones *et al.* 1996a, 2003). In February 2003, the Finnish legal breath-ethanol concentration limits for DUI (driving under the influence) were lowered to correspond with the blood-breath ratio of 2,300 instead of 2,100: 0.22 mg/l (drunken driving) and 0.53 mg/l (aggravated drunken driving).

The blood-breath ratio of a volatile compound is affected by several factors; the most important of these are discussed in the following.

2.3.1. Blood-air partition coefficient

The partition coefficient (or partition ratio) defines the distribution of a substance (such as ethanol) between two media (such as blood and air) at equilibrium (Equation 10.2). It is a physicochemical property of the gas and the liquid at thermodynamic equilibrium of the two phases involved at the interface between the two materials (Hlastala 1998). This equilibration obeys Henry's law in the case of low concentrations (Equation 10.3). A partition coefficient for a given compound is the ratio of molar concentrations achieved between the two compartments at equilibrium. To describe the pulmonary excretion, in vitro blood-air partition coefficients have been determined for the most common volatile compounds (Table 2).

A blood-air partition coefficient has not been determined for ethylene glycol. The Henry's law coefficient for ethylene glycol at 25 ºC is 20,000 times higher than for ethanol or methanol. The corresponding water–air partition coefficient at 34 ºC is more than 10,000 times higher than that for methanol, even if a quite high temperature effect were assumed (Table 3). According to these data, the ethylene glycol concentration in breath will be very low, even in the case of severe intoxication.

Table 2. Physical properties of solvents.

Component	Molecular weight	Relative density	Boiling point	Vapour pressure at 20 ºC	Solubility in water at 20 ºC	Blood-air partition coefficient at 37 ºC (*BBR in brackets*)	
	g/mol	g/l	ºC	Pa		median	range
Ethanol	46.1	790	79	5800	miscible	1566	1332–2516
						(2300)	*(2160–2475)*
Methanol	32.0	790	65	12300	miscible	2650	1626–2874
1-propanol	60.1	800	97	2000	miscible	1038	955–1120
2-propanol	60.1	790	83	4400	miscible	848	719–1426
Ethylene glycol	62.1	1100	198	7	miscible	n.d.	n.d.
Acetone	58.1	790	56	24000	miscible	245	196–330
MEK	72.1	810	80	10500	29 g/100ml	202	125–215
MIBK	100.2	800	117	2100	1.9 g/100ml	88	86–90
MTBE	88.2	740	55	27000	4.2 g/100ml	20	20
Diethyl ether	74.1	710	35	58600	6.9 ml/100ml	12.2	12.2
Ethyl acetate	88.1	900	77	10000	very good	98	77–120
Butane	58.1	600	-1	213700 *	6.1 µl/100 ml	n.d.	n.d.
Toluene	92.1	870	111	3800	none	15.2	15.6–14.7
						(20.6)	*(18.2–23)*

* at 21.1 ºC

BBR = *in vivo* blood-breath ratio at 34 ºC; MEK = methyl ethyl ketone; MIBK = methyl isobutyl ketone; MTBE = methyl tert-butyl ether; n.d. = not defined

(Alobaidi *et al.* 1976, Dubowski *et al.* 1979, Fiserova-Bergerova *et al.* 1986, Foo *et al.* 1991, Gargas *et al.* 1989, Garriott *et al.* 1981, Haffner *et al.* 2003, Harger *et al.* 1950, Imbriani *et al.* 1997, IPCS, Jones 1978, 1985, Jones *et al.* 1990, 1996a, 2003, Kaneko *et al.* 1994, Pezzagno *et al.* 1983, Sato *et al.* 1979)

2.3.2. Water solubility

Interaction in the conducting airway plays a very significant role in soluble gas exchange. For a gas of very high water solubility, such as ethanol, practically all of the exchange occurs in the airways. During inspiration, the soluble gas concentration in the incoming air becomes gradually equilibrated with the concentration in the airway mucosa, according to the partition ratio. By reaching the alveoli, additional soluble gas is then taken up from the alveolar blood. On exhalation, the air initially loses the soluble gas to the alveolar end of the airways, and progressively more loss to the airway mucosa occurs along the entire length of the airways. Exhaled alcohol or other soluble gas leaving the mouth therefore comes from the airway mucus and underlying tissue rather than the alveoli and blood in the pulmonary circulation (Schrikker *et al.* 1989, Tsu *et al.* 1991). The amount of soluble gas reaching the mouth greatly depends on the loss in the airways, which depends on the breathing pattern and the air and airway surface temperatures. This contributes to the very large variation in the breath test readings obtained from actual subjects (Hlastala 1998). Due to the variation, breath ethanol concentration has been adopted as a basis for justification *per se*, without attempts to convert it to blood levels.

As the blood-air partition coefficient of the exchanging gas decreases, the importance of airway surface exchange diminishes, and more of the gas exchange occurs in the alveoli. For the normal respiratory gases, O_2 and CO_2, only an insignificant amount of exchange occurs across the airways (Tsu *et al.* 1991).

2.3.3. Molecular size

Robertson and co-workers (1986) compared the elimination of three inert gases with similar partition coefficients but with different molecular weights (26 to 184.5 g/mol). They discovered an order of 10% consistent impairment of exchange in the higher molecular weight gases. This

Table 3. *Henry's law constants (Sander 1999).*

Component	literature at 25 ºC [M/atm]	calculated* at 21 ºC [M/atm]	$\dfrac{-\Delta_{soln} H}{R}$ [K]	Water-air partition ratio
Ethanol	1.90 E+02	2.57 E+02	6600	6197
Methanol	2.25 E+02	2.85 E+02	5200	6884
1-propanol	1.30 E+02	1.83 E+02	7500	4418
2-propanol	1.30 E+02	1.83 E+02	7500	4418
Ethylene glycol	4.00 E+06	n.d.	n.d.	n.d.
Acetone	3.00 E+01	3.70 E+01	4600	893
MEK	2.00 E+01	2.51 E+01	5000	606
MIBK	2.20 E+00	2.22 E+00	170	54
Butane	1.10 E-03	1.27 E-03	3100	0.03
Toluene	1.50 E-01	1.80 E-01	4000	4.3

* according to equation 10.3

MEK = methyl ethyl ketone; MIBK = methyl isobutyl ketone; n.d. = not defined

was assumed to be due to an impaired diffusion and a different distribution between plasma and red cells.

2.3.4. Temperature

The normal human core temperature is in the region of 37 ℃. The deep alveolar temperature presumably equals the core temperature. The temperature in the airways decreases towards the mouth and nose. The end-expiratory breath temperature in normal circumstances is approximately 34.5 ℃. According to Jones (Jones 1982a), this temperature is reached only when 90% of the forced vital capacity (FVC) is exhaled. At 50% of the FVC, it was 0.5 ℃ lower. According to laboratory and human studies, a rise in temperature lowers and a decrease increases the blood-air partition coefficient in the order of 6.5%–8.6% / 1 ℃ (Fox *et al.* 1987, 1989, Harger *et al.* 1950). Therefore, even mild hypothermia or hyperthermia may considerably distort the breath solvent concentration and lead to an inaccuracy of predicted blood solvent concentration.

2.3.5. Ventilation-perfusion ratio

In addition to the blood-gas partition ratio, the fraction of the compound excreted to breath is dependent on the corresponding rate of alveolar blood flow and pulmonary ventilation in different lung regions (Equation 10.4). The ventilation is more homogenous during light exercise than at rest. Nevertheless, the effect of differences in the pulmonary ventilation-perfusion ratio is unlikely to affect the breath test results by more than 3% for the majority of industrial solvents that have blood-gas partition coefficients greater than 10 (Wilson 1986).

2.3.6. Solvent distribution between blood and other tissues

Ethanol is completely miscible with water and distributes into the water compartment of body fluids and tissue. During the absorption of ethanol from the intestine, the concentration of ethanol in the arterial blood is higher than in the venous blood. The magnitude of this arterio-venous difference depends in part on the rate of absorption of ethanol from the intestine: rapid absorption exaggerates the difference and slow absorption minimizes it. During the post-absorptive state, the ethanol concentration gradients between arterial and venous blood are reversed. In a study of twelve healthy men, the post-absorption period began 6 to 77 min after a 30-minute alcohol drinking period (Jones *et al.* 1989).

Due to the quite rapid absorption of solvents, the breath testing generally takes place in the post-absorptive phase (Jones *et al.* 2003).

The breath ethanol concentration reflects the arterial blood concentration more closely than it does the venous blood concentration. In the absorption phase, the apparent (venous) blood-breath ratio is therefore lower than in the post-absorption phase. Hence if the 2,100 blood-breath ratio were used for the breath-to-blood conversion in the absorption phase, the blood concentration would be over-estimated (Jones *et al.* 1989). This effect of distribution on the blood-breath ratio is most probably similar for all highly water-soluble components, but thus far no studies have been conducted to verify it.

2.4. Breath sampling

The breath profile for exhaled gaseous components is divided into three phases (Figure 1) (Hlastala 1998). At the beginning of the expiration, phase I appears as a horizontal line representing the airway dead space (approximately 150 ml) which contains little or no gases derived from the blood circulation. The hosing of the analyzer and the volume of the measuring cell adds up the dead space. The first phase is followed by a rapid rise in concentration of volatile components (phase II). The rise slows down to the "alveolar plateau" (phase III) as the

alveolar air is reached (Hlastala 1998, Lubkin *et al.* 1996).

Because of the airway exchange, phase I is much shorter for water-soluble solvents such as ethanol than it is for components changing mainly in the alveoli (CO_2, N_2). The concentration of volatile components will still have an upward gradient after reaching phase III, if a subject continues to exhale at a constant rate. In the case of CO_2, the rise is slight and mainly attributed to the continued liberation of solvent gas from the alveoli. The slope for readily water-soluble solvents is somewhat steeper and caused by a complex interactive process of heat and gas exchange in the bronchial tree, as well as the gas diffusion properties of the peribronchial tissues. The rise in exhaled solvent concentration will end only after deceleration of the air flow at the end of the exhalation. Consequently, the larger the volume exhaled, the higher the measured breath solvent concentration will be. (Hlastala 1998)

A standardized and reproducible breath sample is important for quantitative breath analysis. If the breathing and collection technique are not standardized, the proportion of alveolar air and dead space air will vary, leading to highly variable data (Manolis 1983). A few mathematical models have been developed for describing the breath alcohol exhalation profiles (George *et al.* 1993, Lubkin *et al.* 1996, Tsu *et al.* 1991). These models can provide a useful basis for designing a breath solvent sampling method. Usually, the aim is to obtain a representative alveolar sample (Wilson 1986). A few topics of breath sampling are presented in the following.

2.4.1. Breathing technique before sampling

Jones (1982b) determined the influence of the breathing technique on the temperature and ethanol-content of the breath. With 30-second breath-holding before expiration, he found the concentration of

ethanol to increase by 16% and the temperature of breath by 0.6 °C. Hyperventilating for 20 seconds immediately before the analysis of breath decreased the concentrations of ethanol by 11% and produced a 1.0 °C fall in breath temperature. After a slow (20 s) exhalation, expired ethanol was increased by 2%, with no changes in breath temperature. In addition to temperature changes, the duration of contact between the breath and the mucous membranes covering the respiratory tract was concluded to represent the main reason for the observed effects. Theoretically, these findings are applicable to any very water-soluble solvent.

2.4.2. Re-breathing

Breathing in and out of a reservoir for several breaths (re-breathing), has been used to obtain equilibrated alveolar gas samples (Ohlsson *et al.* 1990). In the case of water-soluble components such as ethanol, the air within the lung and reservoir system equilibrates after several breaths, as the air passes back and forth over the airways, warming the airways to body temperature and equilibrating the airways with the alveoli. After equilibration, the reservoir air alcohol
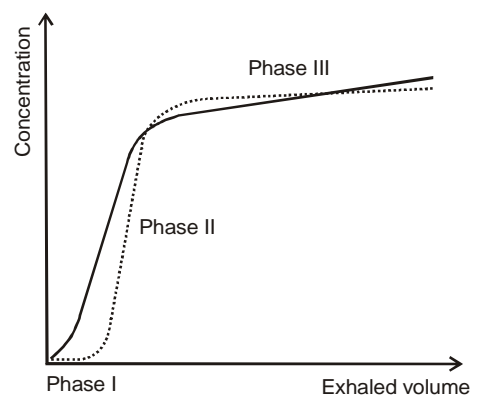
Figure 1. Exhaled profile of a water-soluble solvent (ethanol, solid line) compared to a less soluble component (CO_2, dashed line) (Hlastala 1998).

concentration should be equal to alveolar air alcohol concentration. The isothermal re-breathing could also counteract the impact of altered pre-test breathing or inspired air temperature (Ohlsson *et al.* 1990). Hypoxia limits the duration of the re-breathing manoeuvre and thus prevents a complete equilibration.

2.4.3. Methods for standardizing the breath sample

The carbon dioxide concentration in alveolar air is stable and more or less constant in resting healthy subjects. A minimum CO_2 concentration could be required in order to ensure the collection of an appropriate alveolar breath sample. In some studies, the concentration of breath compounds from healthy subjects in a fasting state has been normalized by respiratory CO_2 (i.e., the concentration in breath is expressed as a fraction of the total CO_2 expired). It has been estimated that normalization halves the standard deviation for determining the concentration of breath compounds (Cheng *et al.* 1999). Nevertheless, it has to be kept in mind that exhaled concentration/time profiles are different for CO_2 and water-soluble solvents (Figure 1, page 23) (George *et al.* 1993, Hlastala 1998, Lubkin *et al.* 1996).

Because the temperature and the total exhaled volume affect the water-soluble solvent breath concentration, these parameters can be used for sample standardization. The demand of a minimum volume (e.g. 1.5 l (OIML 1998)) in the breath test may not be sufficient by itself, because the vital capacity varies markedly between subjects. In order to get a correct result, the expiration volume should be more than 70% of the subject's vital capacity (Schoknecht *et al.* 1990). In case of an expired volume remarkably lower than 50% of the vital capacity, the measured values can be more than 10% below the expected values (Schoknecht *et al.* 1990). If the breath analyzer has a very fast response time for the analyte, the

plateau phase of the exhalation can be verified during sampling. An appropriate protocol to reducing variation is to obtain at least duplicate breath samples (Lubkin *et al.* 1996).

2.4.4. Control of volatile components in the inspired air

Since a volatile compound in the breath may have originated either from the body or the inspired air, the collection method should allow the determination of its source. The amount of the compound originating from the body can be determined by calculating an alveolar gradient (concentration in alveolar breath minus concentration in room air). The alveolar gradient is generally positive for compounds produced by the body and negative for environmental pollutants (Phillips 1997).

2.4.5. Prevention of condensation

Breath is saturated with water. It should not condense in the collecting apparatus, since volatile compounds could be lost by partitioning into the aqueous phase. All of the tubing and reservoirs of the analyzing system should be heated in order to prevent any condensation (Phillips 1997).

2.5. Gas phase infrared spectroscopy

Infrared (IR) spectroscopy is a chemical analytical technique, which measures the infrared intensity versus wave number (wavelength) of light. Based upon the wave number, infrared light can be categorized as far infrared (4–400 cm^{-1}), mid-infrared (400–4,000 cm^{-1}) and near infrared (4,000–14,000 cm^{-1}) (Hollas 1996). The Gamet FT-IR analyzer used in the present studies operated in the mid-infrared region.

Mid-infrared spectroscopy detects the vibration characteristics of chemical functional groups in a sample. When an infrared light interacts with the matter,

chemical bonds will stretch, contract and bend. As a result, a chemical functional group tends to adsorb infrared radiation in a specific wave number range. For example, the stretch of the C=O group appears at approximately 1,700 cm^{-1} and that of O–H at roughly 3,600 cm^{-1}. According to Beer's law, the absorbance of infrared radiation is directly proportional to the concentration of the sample and the path length (Equation 10.5). The total absorbance of the sample is the sum of the absorbances of its components. (Hollas 1996)

2.5.1. Fourier transform infrared spectroscopy

There are alternative ways to record a frequency domain spectrum. It can be recorded by scanning through the frequency range and recording the signal at the detector. Alternatively, the time domain spectrum can be recorded first and then transformed to the frequency domain spectrum. The process of proceeding from the time domain spectrum to the frequency domain spectrum is known as Fourier transformation. (Hollas 1996)

There is an important advantage in recording the time domain spectrum: all the frequencies in the spectrum are recorded all the time. This is known as the multiplex or Fellgett advantage and results in a comparable spectrum being obtained in a much shorter time. An interferometer (Figure 2) is used to modulate the infrared spectrum before leading it through the sample. The interferometer utilizes a beamsplitter (B) to split the incoming infrared beam (S) into two optical beams. One beam reflects off of a flat mirror (M2) fixed in place. The other beam reflects off of a flat mirror (M1) which travels a very short distance (typically a few millimetres) away from the beamsplitter. The two beams reflect off of their respective mirrors and are recombined when they meet at the beamsplitter. The re-combined signal (D) results from the "interference" of the beams with each other. The

resulting signal is called the interferogram, which has every infrared frequency "encoded" into it. When the interferogram signal is transmitted through the sample, the specific frequencies of energy are absorbed by the sample. The uniquely characteristic infrared signal is measured by the detector and digitized. The digitized signal is decoded by Fourier transformation to a spectrum, a plot of raw detector response versus wave number. (Hollas 1996)

A spectrum obtained without a sample (background spectrum) is induced by the instrument and the environments. A background spectrum must always be run when analyzing samples by FT-IR. A sample spectrum looks similar to the background spectrum, except for the fact that the sample peaks are superimposed upon the instrumental and atmospheric contributions to the spectrum. To eliminate these contributions, the sample spectrum must be normalized against the background spectrum (Equation 1.1). The final absorbance spectrum should be devoid of all instrumental and environmental contributions and only present the features of the sample (Smith BC 1995).

The FT-IR analyzer provides a spectrum of a mixture of yet unidentified gases with unknown partial pressures. A computer with analysis software is
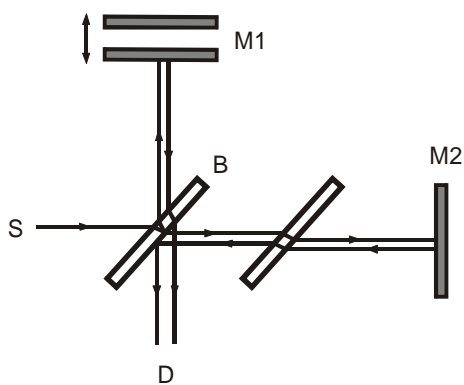

Figure 2. Michelson interferometer (Hollas 1996).

required to calculate the partial pressures of the pure gases in the mixture (Ahonen *et al.* 1996).

2.5.2. Advantages of low resolution

The resolution of the analyzer is determined by the extent to which the interferogram can be observed, and depends on the maximum displacement of the moving mirror. Slight mirror movement leads to a low resolution (Equation 10.7) (Hollas 1996). The resolution in a low-resolution instrument is defined as being more than 4 cm^{-1}. The most obvious advantages of low resolution over high resolution are the simpler design of the instrument, shorter measurement time and higher signal-to-noise ratio (Jaakkola *et al.* 1997).

For quantitative analysis, the most important property of the spectrum is the signal-to-noise ratio (SNR). The uncertainty of the analysis results is directly proportional to the baseline noise in the spectrum and to the square root of the spectral resolution. Thus, the SNR of the spectrum has a stronger effect on the uncertainty of the quantitative analysis results than the instrumental resolution. The resolution and SNR are inter-dependent: increasing the resolution also increases the noise, if the instrumental conditions remain the same. Therefore, in order to optimize the performance, it is practical to maximize the SNR by lowering the resolution (Jaakkola *et al.* 1997).

Non-linearity of a low-resolution instrument can be an advantage in increasing the dynamic range of quantitative analysis, because in low resolution, the absorbances measured at high concentrations are lower than in high resolution. This is a significant advantage in the case of a low concentration component required to be measured in the presence of strongly absorbing components with a high degree of spectral overlap (Saarinen *et al.* 1991).

2.6. Existing solvent breath tests

Portable and bench-top breath ethanol devices have been available for many years and are widely used for traffic law enforcement. The earlier breath ethanol analyzers based on single wavelength IR-detection were not specific to ethanol. Co-existing acetone, for example, had some effect on analysis results (Sutton 1989). Current evidential breath analyzers for ethanol are more accurate and precise. They are typically based on multiple-wavelength IR-detection or a combination of an electrochemical sensor and IR-detection (Jones *et al.* 1996b, Lagois 2000). In the case of co-ingestion of ethanol and another volatile solvent having effect on ethanol analysis results, these analyzers are designed to either reject the ethanol analysis or subtract the erroneous effect. None of these analyzers are designed for identifying the interfering component. The requirements for these analyzers are registered in an international recommendation (OIML 1998).

The simpler breath ethanol analyzers are still used for screening purposes. Many emergency departments have adopted these breath meters for determining bedside alcohol concentrations in intoxicated patients. The clinical breath alcohol testing should meet the same quality-assurance and quality-control requirements as any point-of-care test (Wu AH *et al.* 2003).

Besides the original articles included in this thesis, few studies have been published on breath tests for poisoning due to toxic alcohols or other intoxicating solvents. Nishiyama and co-workers (2001) examined an FT-IR analyzer for the monitoring of solvent poisonings. In their first (and so far the only published) study, they studied ethanol intoxications in volunteers. Breath acetone has been measured by gas chromatography in epileptic patients treated with ketogenic

diets (Musa-Veloso *et al.* 2002), and commercial breath acetone measurement kits are available for dieting purposes (Kundu *et al.* 1993).

Breath testing of employees in order to determine occupational exposure has been studied quite extensively, but it has not yet found its way to routine biological monitoring programmes (Wilson *et al.* 1999). The solvent concentrations in the exhaled breath after an airborne exposure diminish rapidly, and the remaining "steady" concentrations are very low (Liira *et al.* 1988, Lindstrom *et al.* 2002). These low concentrations have been measured mainly with gas chromatography and mass spectrometry in laboratory settings. Franzblau measured methanol in ambient air and exhaled breath with an FT-IR analyzer (Franzblau *et al.* 1992). These (non-toxic) concentrations correlated well with each other as well as with blood concentrations. However, measuring occupational exposure to solvents in low concentrations falls out of the scope of this thesis.

In conclusion, breath testing has been previously used in solvent intoxication diagnostics practically solely for ethanol.

3. Aims of the study

The general objective of the study was to develop an FT-IR breath test for solvent intoxication diagnostics. The specific aims were:

1. To modify the hardware and analysis software of the Gasmet FT-IR analyzer for breath testing.
   (Studies I–III, V)

2. To evaluate the performance of the Gasmet FT-IR analyzer for solvent breath testing.
   (Studies I, V)

3. To evaluate the feasibility of the Gasmet FT-IR analyzer in clinical settings.
   (Studies I–III, V)

4. To evaluate the ability of Dräger Alcotest 7110 MK III FIN evidential breath ethanol analyzer to reveal the presence of other intoxicating solvents.
   (Study IV)

4. The apparatus

4.1. Gasmet FT-IR analyzer

Study I was performed with a desktop Gasmet FT-IR spectrometer (Temet Instruments Oy, Helsinki, Finland). It was equipped with a modified Genzel interferometer (GICCOR, Temet Instruments Oy, Helsinki, Finland) and a multi-reflection gas cell. The breath sample was collected directly into the 0.2 l gas cell heated to 50 °C (Figure 3). The gas cell fittings were narrow (inner diameter 4 mm), causing a considerable resistance to blow. Prior to hitting the detector, the IR radiation passed 2.0 m by reflecting repeatedly from gold-coated mirrors at both ends of the gas cell. The material of the gas cell windows and the beamsplitter was BaF_2. The IR radiation source was silicon carbide. A Peltier-cooled MCT detector was operated in the wave number range of 4,000–900 cm^{-1} (2.5–11 µm). All spectra were measured at 8 cm^{-1} resolution and the scan rate was 10 scans/s.

A portable Gasmet FT-IR spectrometer (Gasmet DX2000, Temet Instruments Oy, Helsinki, Finland) was used in the remaining four studies. It was pilot-case-sized and weighed 18 kg. As a distinction from the desktop model, this point-of-care analyzer was equipped with a Temet Carousel Interferometer (Temet Instruments Oy, Helsinki, Finland), and the cell fittings were widened (inner diameter 9 mm), in order to reduce the resistance to blow. During the study conducted in the emergency rooms, the device was placed on a pushcart for easy bedside access and run on a 12 V battery. Theoretically, an eight-hour analyzing time was possible without charging. In the other studies, the analyzer was connected to mains.

Single-use bacterial filters (Pall BB25, Pall Industries Ltd, CA, USA) connected to the sampling hose were used as a mouthpiece and to protect the analyzer from contamination. The dead space before the measuring cell (consisting of the sampling hose and the bacterial filter) was 60 mL.

4.1.1. Analysis software
Both of the Gasmet FT-IR analyzers were equipped with multicomponent analysis software (Calcmet, Temet Instruments Oy, Helsinki, Finland). This software quantifies the sample components simultaneously by using a modified classical least squares fitting algorithm. It uses a maximum amount of pre-computed information to make the analysis simple and fast (Jaakkola *et al.* 1997). The analysis is based on Beer's law and assumes that the absorbances of the components in the gas phase are directly proportional to their concentrations. The multicomponent analysis algorithm resolves the composition of the measured unknown spectrum, using a set of single component reference library spectra. The baseline of the unknown spectrum is generated mathematically to account for baseline fluctuation (Jaakkola *et al.* 1997).
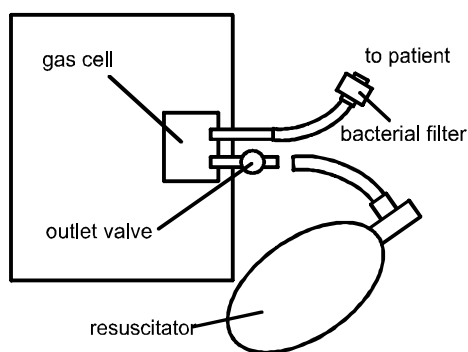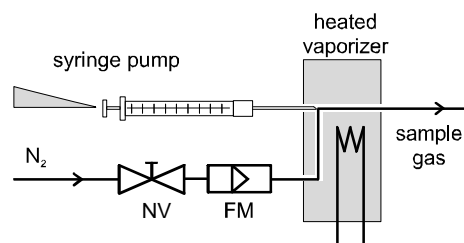

Figure 3. *Gasmet FT-IR analyzer configuration for breath testing.*

The residual spectrum is the difference between the original sample spectrum and the linear combination of the single component library spectra in concentrations determined by the analysis results (Jaakkola *et al.* 1997). The reliability of the analysis can be characterized from the residual spectrum. Ideally, it should be random noise (Saarinen *et al.* 1991). A residual spectrum different from random noise may be due to a detectable sample component not included to the reference library (Saarinen *et al.* 1991). The original FT-IR spectrum is automatically recorded on the hard disk for possible post-examination.

4.1.2. Preparation of the reference library spectra (calibration)

Before each study, the Gasmet FT-IR analyzer was inspected and calibrated in cooperation with the manufacturer. The infrared spectra of each single component were measured in appropriate concentrations and stored in the reference library. *Pro analysi* grade reagents and certified gases were used for calibration.

The reference library contained several reference spectra for components in varying concentrations throughout the measuring range in order to minimize the effect of possible non-linearity. The Calcmet software is able to calculate the non-linearity factors automatically during the calibration process. This option was used in the present study.

The reference spectra for liquids were made by a Gasmet Calibrator (Temet Instruments Oy, Helsinki, Finland). It contained a syringe pump (Cole-Parmer 74900 series, Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA), a manual needle valve, a mass flow meter (Aalborg GFM17, Aalborg Instruments & Controls, Orangeburg, New York, USA) and a stainless steel injection chamber (Figure 4). The syringe pump injected precise amounts of liquid into a heated N_2 gas flow in the injection chamber. Hamilton 25, 50 or 100µL syringes (Hamilton 1700-series, Hamilton Company, Reno, NV, USA) were used depending on the target concentration. The injected liquid was vaporized rapidly, and a continuous flow of a sample gas was produced. The chamber was heated to temperatures 2 °C below the boiling point of the component in question. The maximum error in preparing a reference dilution was calculated to be ± 2.5% on the basis of flow rate errors and injection rate errors.

Different reference concentrations of gaseous substances (for example CO, CO_2, methane and butane) were prepared by diluting certified reference gases with nitrogen (Figure 5). The flow of gases was controlled by Brooks SL5850 mass-flow controllers (Emerson Process management, Brooks Instrument, Hatfield, PA, USA).

Figure 4. *Gasmet calibrator design. NV, needle valve; FM, mass-flow meter.*
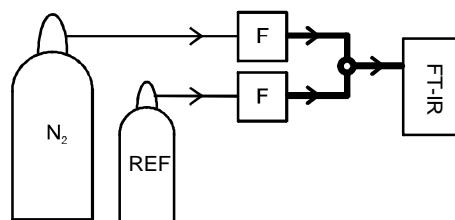
Figure 5. *Generation of reference samples for gaseous components. REF, reference gas; F, mass-flow controller; FT-IR, Gasmet FT-IR analyzer. Heated lines are drawn in bold.*

4.1.3. Breath sampling procedure

Background measurements were conducted with N_2 or O_2, because these diatomic symmetric molecules do not absorb IR-light. The background measurement was carried out daily before the measurement session.

Before each assay, the measuring cell of the analyzer was flushed with ambient air, and the analysis was performed (0-sample). Paired samples of exhaled breath were taken in order to minimize random error. A carbon dioxide concentration greater than 3 vol% was used as a marker of alveolar sample. Samples were accepted if the exhaled CO_2 was over 3 vol% and the variation less than 10%. Sampling was repeated when necessary. The sample with the highest CO_2 level was used in the final analysis, except in the cases of methanol intoxication, where the mean of the samples was used.

Participants were requested to inhale deeply and then blow the entire lung volume through the analyzer's gas cell. Samples from unconscious patients were collected by ventilating the patient through the gas cell of the analyzer with a revivator bag. The sample collecting system can be fitted to an intubation tube as well as a face mask (Figure 3, page 29). An alveolar sample was trapped in the gas cell at the end of the expiration by sealing off the collecting system with a manual valve.

4.2. Dräger Alcotest 7110 MK III FIN evidential breath analyzer

An evidential breath analyzer has been defined as an instrument which measures the mass concentration of ethanol by analyzing deep lung air, and is usable for evidential purposes (OIML 1998). These breath analyzers are designed to be able to cope with an extra solvent without any significant interference on ethanol analysis. The Dräger Alcotest 7110 MK III FIN evidential breath analyzer (Dräger Safety AG & Co. KGaA, Lübeck, Germany) was selected for the present study, because it is the make used by the Finnish Police. It will be referred below as "Dräger 7110 evidential breath analyzer".

The Dräger 7110 evidential breath analyzer is a semi-portable device which is commonly kept at police stations. It determines the breath alcohol concentration using an electrochemical sensor in addition to an infrared sensor. The infrared sensor operates in the wavelength of 9.5 µm, in order to avoid the effect of acetone in particular. The idea is that as two different measuring systems are used, the analyzer is able to detect an interfering component, discard the analysis and display an "interfering compound" message. Measurements are considered acceptable only if the results provided by both sensors are within tight limits. (Lagois 2000)

In order to ensure the alveolarity of the samples, only a small volume of end-expiratory breath is required. Flow sensors measure the volume of air blown into the instrument and make sure that the dead space air will not be analyzed. Two temperature sensors record the temperature of the exhaled airflow at the mouthpiece end of the breath hose. Results from the breath-ethanol analysis are standardized to a fixed exhalation temperature of 34°C. The analyzer measures the ambient air ethanol concentration and automatically checks the calibration with a reference gas sample in the course of each measuring event. (Lagois 2000)

The Dräger 7110 evidential breath analyzer was serviced and calibrated before the laboratory-testing in accordance with the manufacturer's instructions.
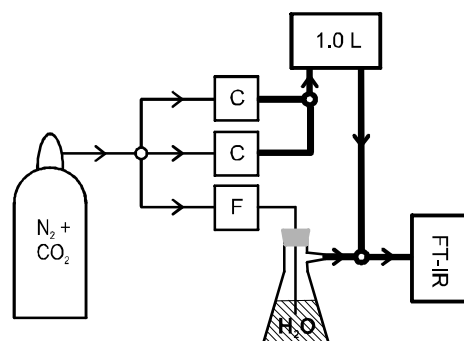
4.3. Breath simulators

Two different types of breath simulators were used in order to generate standardized samples for the evaluation of the analyzers' properties.

4.3.1. Bubbling system

For ideal dilute solutions, Henry's law predicts a linear relationship between the partial vapour pressure of the solute and its concentration in the aqueous phase. A gaseous sample containing water vapour and a solvent was generated by bubbling nitrogen gas through a water-solvent mixture. The nitrogen flow was controlled by a Brooks SL5850 mass-flow controller (Figure 6). In addition to a mixture of a single component in water, dilute mixtures of two or more solvents in water
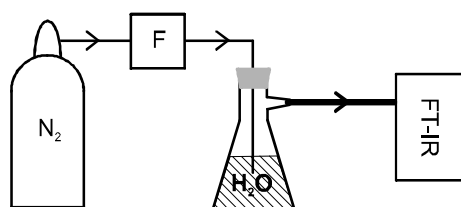
Figure 6. Breath simulator based on a bubbling system. F, mass-flow controller; FT-IR , Gasmet FT-IR analyzer. Heated lines are drawn in bold.

Figure 7. Breath simulator based on calibrators. C, Temet calibrator; F, mass-flow controller, 1.0 L, heated integrator; FT-IR, Gasmet FT-IR analyzer.

were also prepared. The temperature of the liquid and ambient air was monitored. These samples contained water vapour in biological amounts (3.0 vol%), but differed from human breath by containing no carbon dioxide.

4.3.2. Calibrator system

Due to the possibility that chemical interference in the liquid phase may affect the vaporization in the bubbling system, a calibrator system was developed for breath simulation.

The calibrator system consisted of two parallel Gasmet Calibrators and a water bubbling system (Figure 7). A close imitation of exhaled breath was achieved by using 5 vol% carbon dioxide in nitrogen as a carrying gas (AGA, Espoo, Finland). Water was vaporized into the system by bubbling CO_2-N_2 gas through water warmed to 37 °C. The final sample water vapour content was 2.5 vol%. Ethanol or methanol and possible interfering compounds were injected to the system from separate calibrators in order to avoid the possible interference in the liquid phase. In order to avoid swaying in the final sample concentration, the syringe pumps were operated at a high speed and the sample gas was conducted through a heated 1.0 l integrator. The final concentration of the sample components was calculated on the basis of the injection rates and the total gas flow.

5. Study subjects

Six hundred and fifty-eight persons participated in the studies. The demographics of the study subjects are shown in Table 4. All of the study subjects were informed about the study protocol, and they gave their informed written or verbal consent. In cases of unconsciousness, the consent was obtained from the patient's relatives. No reward was given to any of the study subjects.

5.1. Ethical considerations

The Ethics Committees of the Departments of Medicine at Helsinki University Central Hospital and at Tampere University Hospital approved the study on methanol intoxications (96/1997 and 26 Aug 1997, respectively). The Ethical Committee of the Hospital District of Helsinki and Uusimaa approved the study conducted at the emergency rooms (5/2000). The Study Committee of the Social Services Department of the City of Helsinki approved the breath testing of the inebriated homeless men (18 May 1998 and 7 Sep 1999).

Table 4. *Study subjects*

Study subjects (Study)	Number participated (refused)	Sex m / f	Age median (range)	Comatose	Informed consent	Interventions	Main study purposes
Healthy volunteers (I, V)	9	9 / 0	32 (29–47)	0	written	Ethanol per os Breath tests Blood tests	Modifying the hardware Evaluating the performance
Methanol intoxications (II)	6	6 / 0	41 (31–60)	5	written	Breath tests Blood tests	Evaluating the feasibility Evaluating the performance
ER patients (III)	609 (146)	286 / 323	44 (15–96)	4	written	Breath tests	Screening the breath volatiles Evaluating the feasibility Evaluating the performance
Inebriated homeless men (V)	35	35 / 0	50 (35–65)	0	verbal	Breath tests	Screening the breath volatiles Evaluating the feasibility

6. Study protocols

6.1. Modifying the Gasmet FT-IR analyzer for breath testing

The Gasmet FT-IR gas analyzer was initially intended for the analysis of industrial exhaust fumes (Ahonen *et al.* 1996), not for breath testing. During the study period, both the hardware and software of the analyzer were modified for breath testing purposes.

6.1.1.1. Analyzer hardware
Prior to the initiation of the studies, the size of the gas cell was reduced from 1 l to 0.2 l in order to achieve alveolar samples. The patient interface was constructed from a simple plastic hose and a bacterial filter used in the anaesthesia circuits (Figure 3, page 29). The impact of the sampling system components on the results of the analysis was evaluated in a few small experiments.

6.1.1.2. Gas cell size
As the sample is blown to the measuring cell, it becomes turbulent and gradually displaces the gas previously existing in the cell. The gas changing in the cell was investigated by leading nitrous oxide (0.5– 1 l/min) into the gas cell previously flushed with plain nitrogen (unpublished data). Simultaneous non-stop FT-IR measurements were carried out to determine the sufficient volume in which the gases could be adequately exchanged.

Further tests were conducted with a Datex S/5 anesthesia monitor (Meriläinen *et al.* 1993) in order to determine the exhaled gas behaviour in the measuring cell during authentic breath sampling (unpublished data). A healthy volunteer subject (male, 40 yrs, 192 cm, 90 kg) gave breath samples. The flow, pressure and carbon dioxide concentration were measured simultaneously at 0.1 s intervals before the bacterial filter and at the outlet of the measuring cell. The carbon dioxide concentration of nine good-quality exhalations was averaged in relation to exhaled volume. The gas mixing in the sampling system was estimated on the basis of the difference in the carbon dioxide concentration at the two measuring points.

6.1.1.3. Gas cell fittings
The fittings for the new portable version of the analyzer were made markedly wider (inner diameter 9 mm) in order to decrease sample flow resistance. A specific study was performed to measure the effect of the sample flow resistance on the results of the analysis (Study I). In the beginning of the protocol, the test subject (male 32 yrs, 192 cm, 87 kg) ingested a weight-adjusted dose of ethanol in grapefruit soda (final ethanol concentration 20 vol%) in 10 minutes to achieve a blood ethanol concentration of 1 g/l. Measurements started immediately after the ingestion of the ethanol and lasted until the late postabsorptive phase. Four different resistance levels were generated by blocking the outlet opening so that the resulting mean pressures during sampling ranged from 1.3 kPa (13 cmH$_2$O, full open) to 4.1 kPa (41 cmH$_2$O, corresponding to the opening in the previous version).

6.1.1.4. Filter and sample hose
Unheated hosing is a condensation focus for warm exhalation saturated with water. The filter is intended to protect the analyzer from particles and bacteria, but it might also trap some water vapour. The effect of the hosing and the bacterial filter on the results of the water, CO$_2$ and ethanol analysis was determined in an experiment with one volunteer (male 40 yrs, 192 cm, 90 kg, unpublished data). The subject blew repeated samples, either directly to the cell or through the filter and

hose. The effect on ethanol results was tested in the postabsorptive phase after the volunteer had ingested a weight-adjusted dose (0.6–0.8 g/kg) of ethanol in grapefruit soda.

6.1.2. Analysis software settings

The adjustment of the analysis software settings included the selection of the appropriate components into the reference library and of the suitable analysis options for each of the components.

Hundreds of breath tests were run to find out the most significant breath components detectable by the Gasmet FT-IR analyzer, and also to determine their concentration distribution in the Finnish population. Exhaled breath was systematically screened in two important populations: patients attending the emergency rooms and men intoxicated with technical ethanol products (Studies III, V). On the basis of the data collected, the detectable normal breath components and possible exogenous solvents were selected into the reference library.

The whole FT-IR spectrum in the wave number range of 4,000–900 cm^{-1} was not used for the analysis. The regions of the spectrum used were selected for each component separately. The principle was to select regions containing the typical absorption bands of the component and to avoid the regions of extensive absorbance of the bulk components (water and carbon dioxide). To ensure the linearity of the analysis, the regions with saturated absorption peaks were not selected. In addition, the software was configured to automatically ignore absorbance peaks over the limit defined by the user (mostly 0.4 absorbance units).

6.1.2.1. Screening the breath of patients attending emergency rooms

All of the patients attending the emergency rooms (ER) were included in the study, with the exception of children under 15 years of age. This study was carried out in two hospitals and two municipal health care centres in Hyvinkää and Porvoo, Finland. The study was performed in eight or sixteen-hour periods appropriately distributed to cover every hour of one week. These periods were spread over eight weeks from September to November 2000, avoiding public holidays.

During the study period, the 609 participants were inquired about their reason for coming to the ER, alcohol usage, and smoking habits. Height, weight, sex and age were recorded. The breath test was performed as soon as possible after the admission. The analyzer was run on a battery, and it was brought to the bedside for breath sampling. Before each assay, the measuring cell of the analyzer was flushed with ambient air, and the analysis was performed (0-sample). Paired samples of exhaled breath were taken. The sample with the highest CO_2 level was used in the final analysis.

6.1.2.2. Breath testing after ingestion of technical ethanol products

Thirty-five men living in a dormitory supported by the City of Helsinki were included. Most of these men had consumed low-priced technical ethanol products, such as windshield washer fluids and cooker fuels. The breath tests were performed in order to determine the special characteristics of exhaled breath in this population. Forty-six breath tests were run during five afternoons in 1998 and 2000 (three men participated three times and five twice).

The participants were interviewed for the type of ethanol products used as well as for their medical conditions and current medication. Their height, weight and the time of the latest proper meal were recorded. The study was conducted in a dormitory on Sahaajankatu, Helsinki, Finland.

The breath sampling was carried out according to the procedure described above. As a rule, 10 to 30 minutes had elapsed between the latest alcohol dose and breath testing. Ethanol vaporizing

from the mucous membranes of the mouth due to prior drinking was accounted for by taking two or more breath samples. A rapid decrease in breath ethanol in subsequent measurements would have revealed a mouth alcohol effect. The breath test results were not correlated with blood concentrations, because appropriate conditions for controlling the volume of the solvent and time of ingestion were not available.

6.2. Evaluating the performance of the Gasmet FT-IR analyzer in solvent breath testing

It is generally accepted that at least the following parameters should be evaluated for quantitative procedures: calibration model (linearity), accuracy (bias, precision), limit of quantification (sensitivity), selectivity and stability (Peters *et al.* 2002).

6.2.1. Calibration model (linearity)
Linear range is the interval between the upper and lower concentration of the analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity (Shah *et al.* 2000).

The calibration model and linearity of the Gasmet FT-IR analyzer is based on Beer's law. To confirm the linearity, nine ethanol and eight methanol reference spectra were prepared with the Temet calibrator (Study I). The measurement time was 3 minutes, according to the normal calibration procedure. The reference concentrations ranged from 22 to 2,010 ppm for ethanol and from 33 to 2,000 ppm for methanol. The largest absorbances for ethanol (at 1049 cm^{-1}, 9.533 µm) and methanol (at 1057 cm^{-1}, 9.461 µm) were measured manually from the IR-spectra. The linear regression line equation between the absorbance and the vapour concentration was calculated. The

residuals were examined to verify the linear fit (unpublished data).

In order to avoid measuring the absorbance at high, non-linear absorbance levels, the linearity check was later repeated at 2,972 cm^{-1} (3.365 µm) for ethanol and at 2,979 cm^{-1} (3.357 µm) for methanol (unpublished data). The concentration range was 10 to 960 ppm for ethanol (5 steps) and 10 to 1,440 ppm for methanol (6 steps).

To verify the validity of the linear calibration model in the matrix of human breath, simulated breath samples spiked with ethanol were generated with the calibrator system (Study I). Four samples at each of the six levels (from 144 to 1733 ppm) of ethanol were analyzed. An automated spectral analysis protocol (Calcmet analysis software) was used instead of manually measuring the peaks from the FT-IR spectrum. The linear regression line equation between the analysis results and the theoretical vapour concentration was calculated. The residuals were examined to verify the fit (unpublished data).

6.2.2. Accuracy
Accuracy is defined as the degree of closeness of the determined value to the nominal or known true value under prescribed conditions (Shah *et al.* 2000). It consists of random and systematic error components, i.e., precision and bias.

The precision expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. In the present study, the precision was divided into within-day and between-day repeatability. The precision was calculated according to Bookbinder and co-workers (1986) (Equation 10.8).

The bias is defined as the difference between the expectation of the test results and an accepted reference value (Shah *et al.* 2000). It is caused by one or more

systematic error components. The bias of analysis results was calculated for ethanol and methanol (Equation 10.9).

Samples for the preliminary accuracy testing were made by the bubbling method, at 21 ± 1 °C, 1 atm (Study I). Nine ethanol and ten methanol water solutions were prepared. The ethanol and methanol concentrations in the liquid ranged from 0.2 to 32 g/l and 0.1 to 32 g/l, respectively. The measurement time was 1 minute, and each sample was analyzed 10 times. The precision and bias of the Gasmet FT-IR analysis results were determined by comparing the results with the expected values calculated according to the median of the Henry's law literature values (Sander 1999).

Linear regression equations were calculated for the relationship between the analysis results and the liquid concentrations. The coefficient of this equation was further converted into the units of Henry's law. The values obtained were compared to the values in the literature (Table 3, page 21).

The precision and bias of ethanol and methanol analyses were further investigated by analyzing simulated breath samples generated with the calibrator system (Study V). The results of the analysis were compared with the theoretical nominal values calculated on the basis of the calibrator system settings. The precision and bias were determined on ethanol levels of 150–1,730 ppm [0.27–3.17 mg/l] and on methanol level of 50 ppm [63 µg/l]. Four to sixty-six one-minute measurements were conducted on each level.

6.2.3. Sensitivity

The sensitivity of an analytical method is described in terms of the limit of detection (LOD) and the lower limit of quantification (LLOQ). The limit of detection is determined as the lowest concentration of an analyte which the bioanalytical procedure can reliably differentiate from background noise. The

lower limit of quantification is determined as the lowest amount of an analyte in a sample which can be quantitatively determined with suitable precision and accuracy (Shah *et al.* 2000). The limits of detection for ethanol and methanol were determined in laboratory settings by analyzing plain matrix (Equation 10.10).

In Study I, the analyzed matrix contained either pure nitrogen or nitrogen with 3.0 vol% water vapour. The samples were prepared by leading a N_2 flow through the measuring cell or by using the bubbling method (for generating the water vapour). Thirty one-minute measurements were carried out at 21.0 ± 1.0 °C, 1 atm.

The limits of detection and the lower limits of quantification for fourteen components were systematically further determined in laboratory settings in five different matrixes: plain nitrogen, simulated breath (water vapour 2.5 vol% and CO_2 5.0 vol% in N_2), simulated breath spiked with 500 ppm ethanol or 500 ppm methanol, and simulated breath spiked with 440 ppm ethanol, 250 ppm acetone, 41 ppm 2-propanol, 53 ppm MEK and 24 ppm MIBK (Study V). The latter mixture resembles exhaled breath after the ingestion of Marinol-100 (cooker fuel, Berner ltd, Helsinki, Finland). In addition to ethanol (over 80% w/w), Marinol-100 contains MEK (2% w/w), MIBK (2% w/w) and 2-propanol (1%–5% w/w) (Berner 2005). The samples were generated with the calibrator system. Thirty samples of each mixture of components were analyzed, and LOD and LLOQ were calculated for the components not present in the matrix.

6.2.4. Selectivity

Selectivity is defined as the ability of a bioanalytical method to measure unequivocally and differentiate the analyte(s) in the presence of components which may be expected to be present. Typically, these might include metabolites, impurities, degradants, matrix components, etc. (Shah *et al.* 2000).

Interference is considered unacceptable when it affects the accuracy of quantization at concentrations to the extent that it may result in erroneous study conclusions. Small interferences can be accepted as long as precision and bias remain within certain acceptable limits (Dadgar *et al.* 1995).

The selectivity of the Gasmet FT-IR analyzer was preliminarily evaluated by determining the effect of ethanol on methanol analysis (Study I). The study was conducted in laboratory settings with the bubbling method at 21.0 ± 1.0 °C, 1 atm. The effect of high ethanol concentration (300 ppm in vapour) on the results of methanol analysis was tested at seven methanol levels ranging from 14 to 2,000 ppm in vapour. The bias caused by the matrix ethanol was calculated for each concentration level by comparing the results of the methanol analysis with ethanol to those without ethanol. Ten one-minute measurements were run at each analyte level.

The selectivity of the analyzer for ethanol and methanol was investigated more systematically at clinically significant concentrations in a matrix resembling human breath (Study V). The sample was spiked with a number of possible interferents, one at a time. Samples were prepared with the calibrator system. The concentration of ethanol in the simulated breath was set to 150 ppm [0.27 mg/l] in order to be close to the legal DUI limit. The sample methanol concentration was set to 50 ppm [63 µg/l]. It corresponds with a blood concentration of 6.1 mmol/l, when a blood-breath ratio of 3,063 is applied (Study II) (Ellenhorn 1997). In order to determine the possible effect on analysis results, three one-minute measurements were taken before and after adding the interfering compound. At least two concentration levels of each interferent were investigated. The interferent concentrations used mostly exceeded their highest likely levels in humans. The bias caused by the interferent

in the matrix was calculated for each concentration level by comparing the results of the analysis for ethanol or methanol with the interferent to those without an interferent (= nominal value). The procedure was performed twice for each interferent concentration level. Mean bias was calculated from the results of the six measurements. The overall effect of compounds tested was evaluated by calculating the average absolute bias (Equation 10.11).

6.2.5. Stability

Stability means the chemical stability of an analyte in a given matrix under specific conditions for given time intervals (Shah *et al.* 2000). The stability was evaluated by keeping a breath sample in the measuring cell for three hours and analyzing it repeatedly (Study V). In addition to normal breath components, the sample contained 100–1,000 ppm [0.2–1.9 mg/l] ethanol. During the test, the manual valve was closed and the sampling hose connected to the analyzer.

6.3. Evaluating the feasibility of the Gasmet FT-IR analyzer in clinical settings

Toxicological thresholds for intoxicating solvents are mainly determined as blood concentrations. Therefore, the solvent breath tests are mostly used for estimating blood concentrations of intoxicating agents instead of using the exhaled concentrations *per se*. Several patient-related factors in the respiratory system and blood circulation affect the gas exchange between the blood and exhaled breath, which may cause misinterpretation of the results of the analysis, regardless of the accuracy of the breath analysis method. Simultaneous blood and breath samples were taken in Studies I and II in order to evaluate the feasibility of the Gasmet FT-IR analyzer in the clinical settings. The principal aim of the studies was not to

investigate the blood-breath transport of volatile compounds.

6.3.1. Correlation of blood and breath concentrations

In Study I, six healthy volunteer subjects were tested. They fasted for three hours before the experiment. At the beginning of the protocol, each of the men ingested a weight-adjusted dose (0.6–0.8 g/kg) of ethanol in grapefruit soda (final ethanol concentration 200 ml/l) in 10 minutes to achieve a blood ethanol concentration of 1 g/l. After the ingestion, the subjects' mouths were rinsed with water, and 8–28 minutes elapsed before sampling. Breath and venous whole blood samples were taken at three points: blood ethanol concentration 1) rising (absorption phase), 2) at its peak (plateau phase), and 3) declining (postabsorptive phase). At each point, a blood sample and three breath samples were taken. Whole blood ethanol concentration was measured with headspace gas-liquid chromatography.

In Study II, the objective was to catch all methanol intoxication patients treated at Helsinki University Central Hospital and Tampere University Hospital during the study period from April 1998 to June 1999. Repeated blood and breath ethanol and methanol samples were taken from six male patients. The first breath samples were taken 1.5–12 hours after the arrival of the patient. Further breath samples were taken at 15–30 min intervals during the treatment. Blood samples were taken simultaneously with breath samples whenever the concentration of methanol had changed considerably. Whole blood ethanol and methanol concentrations were measured with headspace gas-liquid chromatography.

The suspected linear blood-breath correlation based on Beer's and Henry's laws was evaluated in clinical settings by calculating a linear regression line for the pairs of blood and breath ethanol and methanol measurements. Relative residuals were observed to evaluate the goodness of the linear fit. In addition, a blood-breath ratio was calculated for ethanol and methanol (Equation 10.1). The accuracy of the clinical analysis results was estimated by comparing the blood-breath ratios to the values in the literature.

6.3.2. Sampling and analyzing

The transportability, robustness and easiness of use of the analyzer have been evaluated by the study team throughout the entire study period. The easiness of use was evaluated more specifically during Study III. Four nurses with no previous laboratory experience were employed as study assistants to take the breath samples. Before the study, they had a two-hour training session. The nurses were inquired about the easiness of the sampling in each of the 609 patients' data form.

6.4. Evaluating the ability of Dräger 7110 evidential breath analyzer to reveal the presence of other intoxicating solvents

Breath testing is widely used to determine the degree of ethanol intoxication. Dräger 7110 evidential breath analyzer's response to a solvent other than ethanol was investigated to discover if any hint could be achieved in the case of co-ingestion of toxic solvents (Study IV). This study was carried out at the Laboratory of Physical Chemistry at the University of Helsinki.

Simulated breath samples were generated with the calibrator system (Chapter 4.3.2). The ethanol concentrations used in this study were determined according to the legal breath-ethanol concentration limits for DUI (driving under the influence) enforced in Finland at that time: 0.25 mg/l (drunken driving) and 0.60 mg/l (aggravated drunken driving).

Eight potentially interfering solvents were selected for *in vitro* testing: acetone, methanol, 1-propanol, 2-propanol, methyl ethyl ketone, methyl isobutyl ketone,

diethyl ether and ethyl acetate. Prior to the measurement with an interfering solvent, plain ethanol was measured in simulated breath in order to calculate the specific interference effect. The concentration of the interfering compound was raised until the "interfering compound" message was displayed, and the analysis was rejected. The Gasmet FT-IR analyzer was attached to the system to indicate stable concentrations before measurements with the Dräger 7110 evidential breath ethanol analyzer. Measurements at threshold levels were repeated with the intention of reducing random errors.

In addition to the absolute and relative errors in the results of the ethanol analysis at the maximal interferent level, coefficient C_x was determined to describe the biasing power of an interfering compound (Equation 10.12).

6.5. Statistical analyses

The Mann-Whitney U test was used to compare two groups with non-normal distribution (age: participants with successful breath samples vs. participants with failed breath samples, Study III). The Student's t-test was used to compare two groups with normal distribution (exhaled CO in non-smokers: men vs. women, Study III).

The Kruskal-Wallis test was employed to compare multiple groups with non-normal distribution (proportion of methane producers: multiple groups classified according to age, sex, smoking and gastro-intestinal disease, Study III). Analysis of variance (ANOVA) was used to compare multiple groups with normal distribution (exhaled CO in smokers: multiple groups classified according to the time of the latest cigarette and the number of cigarettes smoked per day, Study III). The ANOVA was also used for precision calculations (Study V).

Linear regression line equations and squared Pearson correlation coefficients (R^2) were calculated to estimate linear relationship between two parameters (e.g., blood and breath methanol concentration in Study II). To evaluate the scatter of the results around the linear fit, the residuals were observed and relative standard deviation calculated.

Positive and negative predictive values were calculated for a CO breath test designed to differentiate smokers from non-smokers (Study III). Single Grubb's test was employed to identify outliers in the linearity test (Study V).

The statistics were calculated with SPSS for Windows and MS Excel software. $P < 0.05$ was considered statistically significant.

7. Results

7.1. Configuration of the Gasmet FT-IR analyzer for breath testing

The use of a low-resolution FT-IR method for breath testing had not been reported previously. In the beginning of the study period, the Finnish and European patents were applied for. They were granted in 2004 and 2006, respectively (Himberg *et al.* 2004, 2006).

### 7.1.1. Impact of sampling system components on analysis results

According to the N_2O test, the volume required for a more than 90% or 95% change in the cell gas was 0.4 l or 0.5 l, respectively.

When CO_2 was measured before and after the cell during an authentic breath sampling, there seemed to be a 0.4–0.7 l difference in the steep part of the CO_2 curves (Figure 8). This difference represents the volume required to exchange the gas in the sample hose and the measuring cell. A CO_2 concentration of over 3 vol% behind the cell was reached when 1.5 l was exhaled. The CO_2 concentration of 3 vol% corresponded with 70% of the maximum CO_2 concentration measured before the bacterial filter.

The patient-analyzer interface of the early version was not satisfactory. Blowing into the gas cell with 4 mm fittings caused a more than 30 cmH_2O pressure (peak pressure over 40 cmH_2O), while the flow remained mostly below 15 l/min. The mean volume exhaled was 4 l and the mean duration of blowing 20 s.

Blowing into the gas cell with 9 mm fittings was considerably easier than with the previous version. The sample flow even exceeded 100 l/min, and the pressure remained mostly below 1.5 kPa. The exhaled ethanol concentration measured did not change even though different resistance levels were generated by blocking the outlet opening. The concentration of CO_2, however, increased from 3.1 to 4.2 vol%, as the increasing resistance induced a longer blowing time (3.4 s and 8.3 s, respectively).
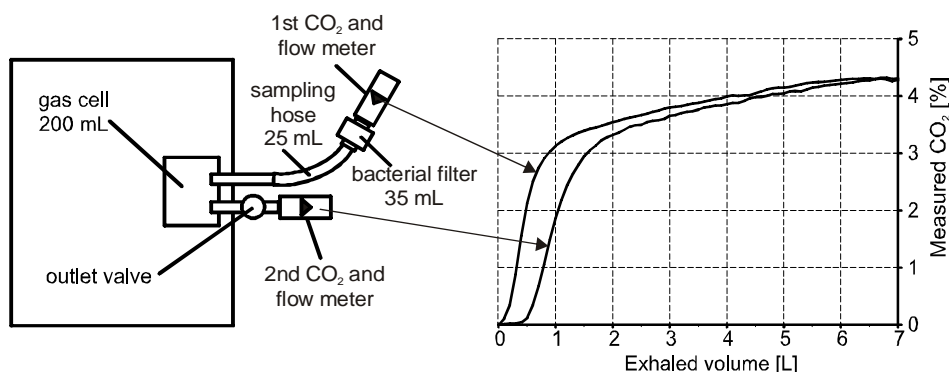
Figure 8. The increase in the carbon dioxide concentration during breath sampling. The CO_2 and flow was measured by Datex S/5 anaesthesia monitor during nine exhalations and averaged. The upper line represents the measurements before the filter and the lower in the outlet of the measuring cell.
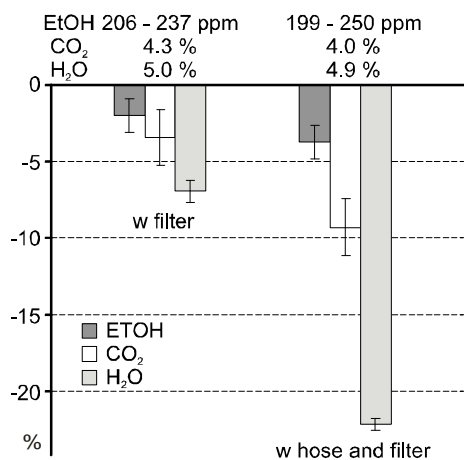
Figure 9. The relative effect of filter and sampling hose on breath analysis results [%]. Means and 95% confidence intervals for the means are displayed. Concentrations without filter and hose are shown on the top of the figure.

The effect of an unheated 50 cm sampling hose and bacterial filter on the results of the analysis for water, carbon dioxide and ethanol is shown in Figure 9. The nominal ethanol concentration varied from 199 to 250 ppm due to absorption and metabolism during the study.

7.1.2. Reference library composition and analysis software settings

During the studies, we selected a reasonable combination of library components for solvent breath testing as well as the best analysis regions for each of them. The most up-to-date combination of the reference library components, their IR spectra and the wave number ranges used in the analysis are shown in Figures 10 and 11. The results of the two breath screening experiments are described in the following.
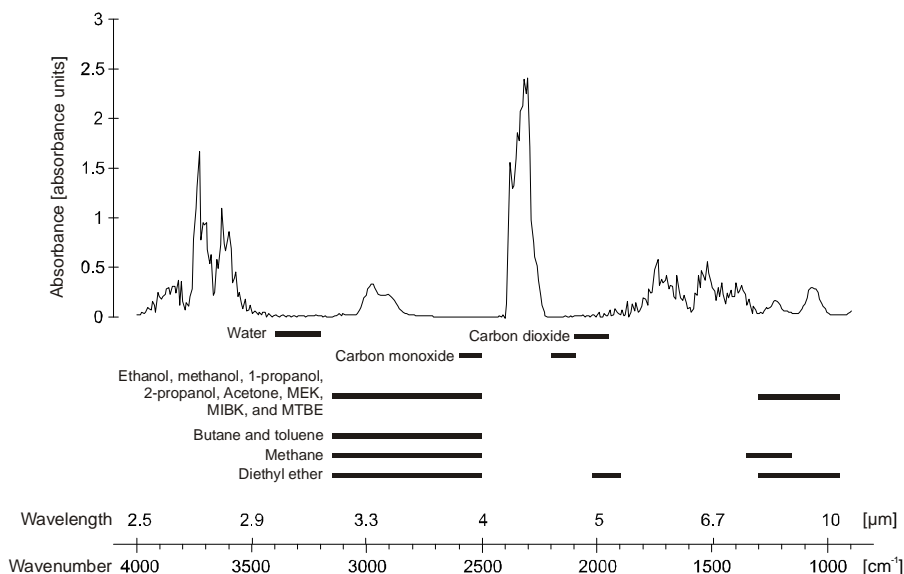
Figure 10. An FT-IR spectrum of a breath sample after ingestion of Marinol-100, which contains ethanol. methyl ethyl ketone, methyl isobutyl ketone and 2-propanol. The bars under the spectrum represent the wave number range used in the analysis of the breath components.
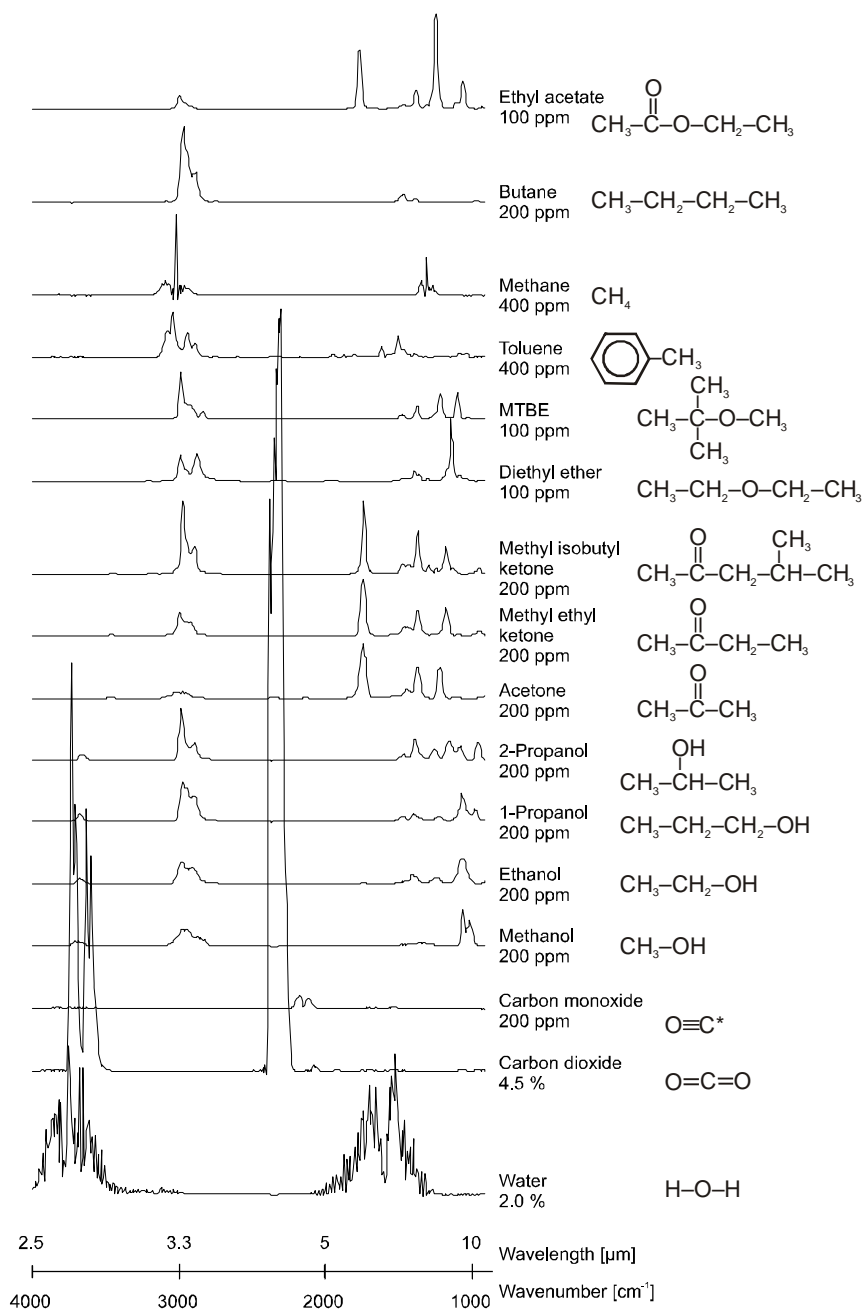
Figure 11. FT-IR spectra and chemical structures of the reference library components.

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7.1.2.1. Breath composition of patients attending emergency rooms

The concentration distributions of the main detected components are shown in Figure 12.

The self-reported smoking rate among participants was 36.3%. Exhaled CO of more than 4 ppm [4.5 µg/l] had a positive predictive value of 0.92 and negative predictive value of 0.96 for smoking. Among smokers, the time elapsed from the latest cigarette best explained the variation in exhaled CO (ANOVA: $p < 0.01$, $R^2 = 0.23$). No CO intoxications occurred during the study.

The proportion of patients exhaling more than 2 ppm [1.3 µg/l] of methane over the ambient air concentration (methane producers) was 31.6%. Age was the best predictor of exhaled methane. Increasing age was associated with a greater proportion of methane producers and a higher absolute exhaled methane concentration (Kruskal-Wallis test: $p < 0.01$). Sex, smoking and acute gastro-intestinal disorder all became non-significant factors after age consideration.

Acetone (0.7 ppm or over) was detected in the breath of 298 participants. Elevated exhaled acetone (10–76 ppm [23–175 µg/l]) was detected in ten patients.

A patient exhaling more than 25 ppm [46 µg/l] of ethanol, corresponding with more than 0.1 g/l in blood, was classified as "ethanol positive". Fifty-four (9.5%) patients were ethanol positive. With night-time admissions, 30% of all and 63% of trauma patients tested ethanol positive. Men were ethanol positive 3.7 times more frequently than women. The ethanol concentrations measured were high: median exhaled ethanol concentration was 435 ppm [795 µg/l], corresponding with a blood ethanol concentration of 2.0 g/l.

Methyl ethyl ketone (66 ppm [189 µg/l]) was detected in one breath sample. This patient had used windshield washer fluid (Lasol 100, Berner ltd, Helsinki, Finland). No other intoxicating solvents were detected.
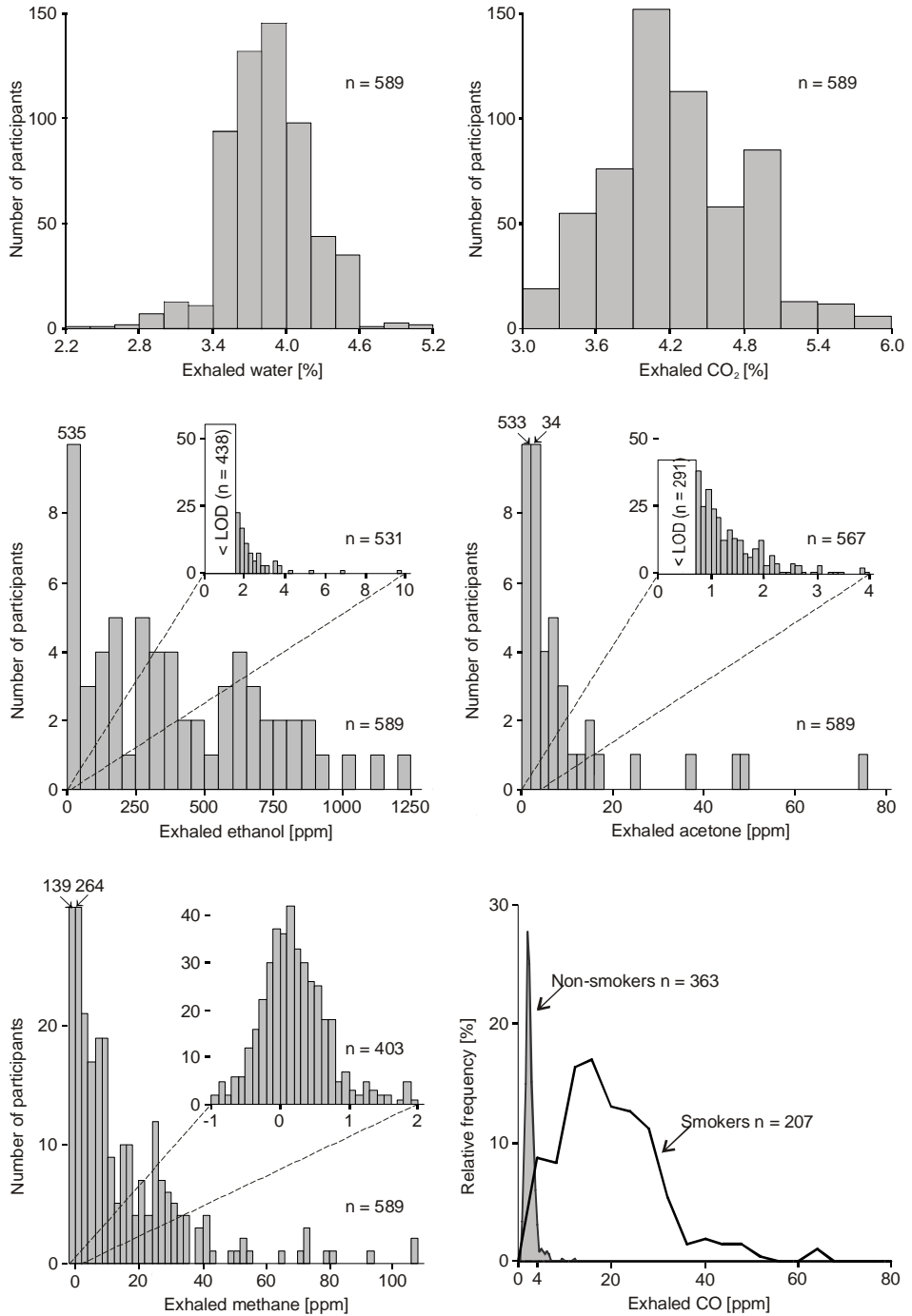
Figure 12. *Concentration distribution of the main breath components among emergency room patients. Open columns represent the results below the detection limit. The methane concentration displayed is the difference between concentrations in exhaled and ambient air, which is the reason for the negative values.*

7.1.2.2. Composition of the breath after ingestion of technical ethanol products

Acetone, 2-propanol, methyl ethyl ketone and methyl isobutyl ketone were the most abundant solvents found in the breath, in addition to ethanol (Table 5). Breath acetone concentrations were high and correlated linearly with 2-propanol concentrations (Figure 13). The acetone concentration even exceeded the toxic limit in four samples. Sub-toxic amounts of exhaled 2-propanol were detected in 33 out of 46 samples. The concentrations of other solvents were toxicologically insignificant.

Twenty-four (69%) of the men were methane producers (methane >10 ppm [6.4 µg/l]). Ambient air methane was 1.5 to 2 ppm. Carbon monoxide was over 4 ppm [4.5 µg/l] in 39 of the samples (85%), indicating a smoking habit.
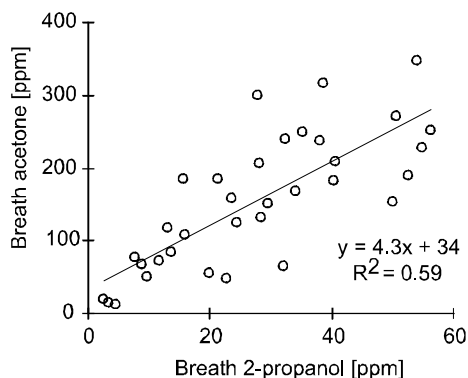
Figure 13. *Correlation of acetone and 2-propanol concentrations in the 34 breath samples with values over the lower limit of quantification. The line represents linear regression.*

Table 5. *Composition of the breath after ingestion of technical ethanol products.*

Component	Concentration			
	median		maximum	
	[vol%]		[vol%]	
Carbon dioxide	4.1		5.3	
Water	2.5		3.4	
	[ppm]	[µg/l]	[ppm]	[µg/l]
Methane	18	11	79	50
Carbon monoxide	12	13	32	36
Acetone	101	233	347	800
Ethanol	427	781	918	1677
Methanol	< LLOQ		9.9	13
1-propanol	< LLOQ		13	31
2-propanol	18	43	56	134
Butane	< LLOQ		6.0	14
MEK	28	80	88	253
MIBK	5.5	22	47	187
MTBE	1.9	6.6	5.3	19
Toluene	< LLOQ		2.2	8.0
Diethyl ether	< LLOQ		1.0	3.0
Ethyl acetate	0.7	2.4	2.6	9.1

< LLOQ = under the lower limit of quantification determined in simulated breath spiked with 500 ppm EtOH;
MEK = methyl ethyl ketone; MIBK = methyl isobutyl ketone; MTBE = methyl tert-butyl ether

7.2. Performance of the Gasmet FT-IR analyzer in solvent breath testing

7.2.1. Calibration model (linearity)

The correlation between concentrations of ethanol (EC, ppm) or methanol (MC, ppm) and the corresponding largest absorbances at 1049 cm^{-1} (9.533 µm) for ethanol (EA, absorbance units) or 1,057 cm^{-1} (9.461 µm) for methanol (MA, absorbance units) were practically linear: EA = $3.53 \times 10^{-4} \times$ EC − 8.30×10^{-4}, r^2 = 0.9990; MA = $4.12 \times 10^{-4} \times$ MC + 6.37×10^{-3}, r^2 = 0.9993 (Figure 14). The relative residuals ranged from -5% to +11% for ethanol and from -33% to +4% for methanol. Observation of the residuals revealed one outlier among the ethanol measurements (verified by Single Grubb's test, α = 0.01) and a slight non-linearity (convexity) in the methanol measurements. When the outlier was excluded from the ethanol regression calculations, the correlation was: EA = $3.56 \times 10^{-4} \times$ EC + 6.12×10^{-5}, r^2 = 1.0000, and the relative residuals for ethanol were ±2% (the outlier was -6.5%).

The correlation between the calculated (EC, ppm) and measured (EM, ppm) ethanol concentration in the samples of simulated breath was quite linear: EM = $1.02 \times$ EC − 6.3, r^2 = 0.9992. Observation of the residuals revealed quite extensive variation in the highest level of ethanol, but no deviation from the linear relationship. The mean relative residuals varied from -3.3% to 1.0%.
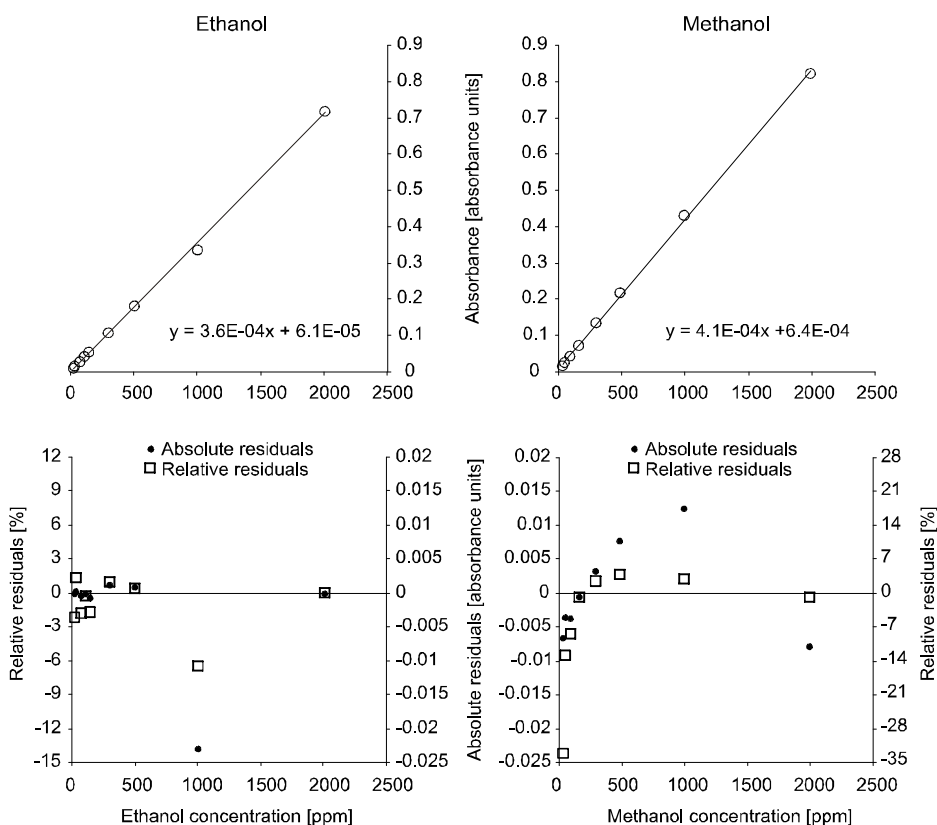
Figure 14. *Linear calibration model. The lines in upper diagrams represent the linear regression lines for correlation between liquid concentration and the IR-absorbance. The lower diagrams show the absolute and relative residuals.*

7.2.2. Accuracy

The literature values for Henry's law constants (corrected to 21 °C, 1 atm) ranged from 216 to 311 M/atm (median 257) for ethanol and from 266 to 292 M/atm (median 285) for methanol (Sander 1999). According to our bubbling tests, the Henry's law constant was 235 M/atm for ethanol and 243 M/atm for methanol. Our values were 9% and 15% smaller than the median values, respectively.

The bias in the preliminary accuracy testing (the bubbling tests) ranged from 10.9% to 16.9% for ethanol and from 10.6% to 24.6% for methanol. The within-day coefficient of variation for the ten measurements at each level ranged from 0.1% to 1.1% for ethanol and from 0.4% to 1.0% for methanol.

The bias of the analysis results was ± 2% for ethanol and -11% for methanol (Figure 15), when simulated breath samples generated with the calibrator system were analyzed. Within-day and between-day coefficients of variation were < 1% for ethanol and < 4% for methanol (Table 6).

7.2.3. Sensitivity

According to the bubbling tests, the limits of detection were 0.9 ppm and 1.2 ppm [1.7 and 2.3 µg/l] for ethanol and 0.9 ppm and 1.3 ppm [1.2 and 1.7 µg/l] for methanol in N_2 matrix and N_2 + 3.0 vol% water matrix, respectively.

The detailed results of the systematic
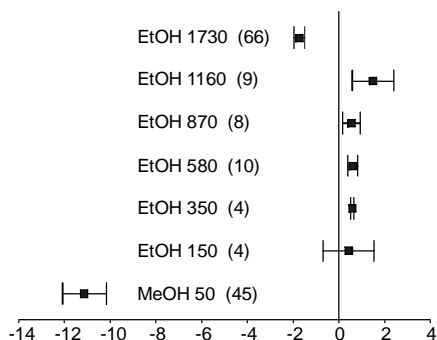
Figure 15. *Bias [%] of analysis results in simulated breath. Vertical axis: ethanol or methanol concentration in ppm. Boxes represent the means and lines the 95% confidence intervals of means. The number of measurements is displayed in brackets.*

sensitivity tests with the calibrator system are shown in Table 7. When compared to plain N_2 matrix, the addition of water and carbon dioxide to the matrix caused marked elevation in the LODs and LLOQs. The LOD and LLOQ for tested components in plain simulated breath ranged from 0.2 to 2.4 ppm [0.5 to 6.8 µg/l] and from 0.5 to 6.5 ppm [1.7 to 12 µg/l], respectively.

7.2.4. Selectivity

In the preliminary bubbling tests, the calculated bias of the methanol analyses due to the presence of ethanol ranged from -2.7% to +1.4%, the average absolute bias

Table 6. *Summary of the analytical precision studies.*

Component	Nominal conc. (ppm)	Number of days	Analyses per day	Measured conc. (ppm)	Within-day		Between-day		Total	
					SD^2	CV (%)	SD^2	CV (%)	SD^2	CV (%)
Ethanol	150	5	9	148	1.4	0.8	1.6	0.9	3.0	1.2
Methanol	50	3	12	44	2.4	3.5	0.5	1.6	2.9	3.8

SD^2 = variation

CV = coefficient of variation

was 1.7%. The high ethanol concentration in the sample did not markedly affect the linear correlation between liquid and measured vaporous methanol concentrations. The effect to the correlation coefficient was only -1.4%, when compared to the value without ethanol.

The effects of various solvents on ethanol and methanol analyses in systematic tests with the calibrator system are displayed in Table 8 (page 50). The absolute effect on ethanol and methanol readings ranged from -1.2 to +3.3 ppm and from -2.4 to +1.3 ppm, respectively. The bias of ethanol analysis due to interfering compounds ranged from -0.8% to +2.2%, the average absolute bias being 0.8%. Similarly, the bias of methanol analysis ranged from -5.6% to +3.2%, the average absolute bias being 1.7%.

7.2.5. Stability

The concentration of breath components decreased if the sample was stored in the measuring cell (Table 9). The relative decrease in the component was approximately the same regardless of the original concentration.

Table 9. Sample stability.

Storage time	Sample concentration compared to original (%)		
(min)	EtOH	CO_2	H_2O
15	98	98	96
30	96	97	92
60	92	95	87
120	86	91	79
180	81	86	72

Table 7. Lower limits of quantification for components in five different matrixes.

| Component | in N_2 | | in simulated breath spiked with * | | | | | | | |
| | | | none | | ethanol† | | methanol‡ | | Marinol§ | |
	ppm	[µg/l]	ppm	[µg/l]	ppm	[µg/l]	ppm	[µg/l]	ppm	[µg/l]
Ethanol	4.4	[8.0]	6.5	[11.9]			6.9	[12.7]		
Methanol	1.8	[2.2]	5.2	[6.6]	2.4	[3.0]			2.6	[3.3]
1-propanol	4.0	[9.6]	4.3	[10.3]	4.3	[10.2]	7.3	[17.5]	3.4	[8.2]
2-propanol	2.9	[7.0]	4.7	[11.3]	2.5	[6.0]	3.9	[9.3]		
Acetone	1.2	[2.8]	2.2	[5.0]	1.3	[2.9]	1.7	[3.8]		
Methane	0.8	[0.5]	2.6	[1.7]	0.8	[0.5]	1.8	[1.2]	0.7	[0.4]
Butane	1.1	[2.6]	2.3	[5.2]	2.1	[4.9]	2.6	[5.9]	2.6	[6.1]
MEK	0.7	[2.1]	3.6	[10.3]	3.6	[10.3]	3.1	[8.8]		
MIBK	1.3	[5.0]	2.3	[9.1]	1.5	[6.1]	4.1	[16.3]		
MTBE	0.4	[1.5]	1.0	[3.4]	0.8	[2.6]	1.6	[5.5]	2.5	[8.7]
Toluene	0.7	[2.5]	1.3	[4.8]	1.2	[4.3]	1.6	[5.8]	1.1	[4.2]
Diethyl ether	0.4	[1.2]	0.6	[1.8]	0.5	[1.3]	0.7	[2.0]	1.3	[3.7]
Ethyl acetate	0.2	[0.7]	0.5	[1.9]	0.3	[0.9]	0.3	[1.0]	0.4	[1.3]
Carbon monoxide	1.2	[1.4]	1.5	[1.7]	1.7	[1.9]	2.8	[3.1]	1.6	[1.8]

* Simulated breath: carbon monoxide 5 vol% and water 2.5 vol% in N_2

† Sample ethanol concentration 500 ppm

‡ Sample methanol concentration 500 ppm

§ Sample contains ethanol 440 ppm, 2-propanol 41 ppm, acetone 250 ppm, MEK 53 ppm, MIBK 24 ppm

MEK = methyl ethyl ketone; MIBK = methyl isobutyl ketone; MTBE = methyl tert-butyl ether
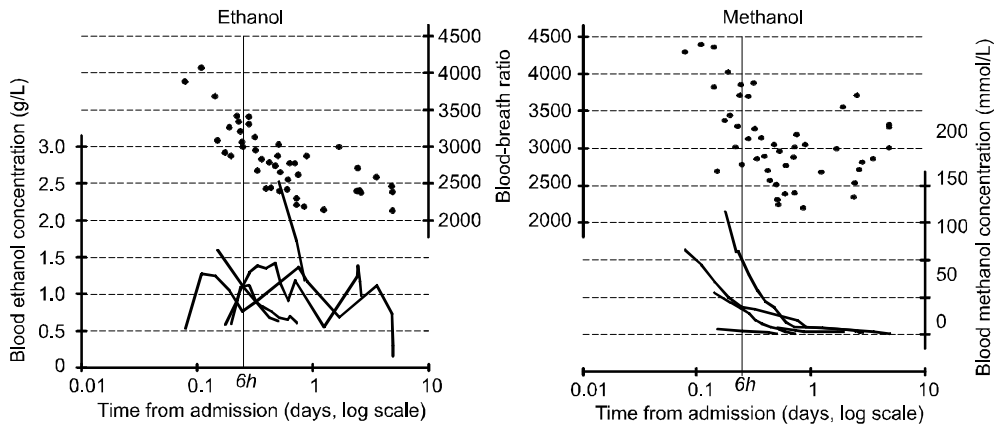
Figure 16. *Blood-breath ratio of ethanol and methanol (dots) during the treatment of methanol intoxication (n = 5). The lines represent the individual participants' ethanol and methanol blood concentration.*

Table 8. *The effect of solvents on the breath ethanol and methanol readings.*

Interferent	Concentration	Effect of solvent on analysis results of ethanol (150 ppm)		Effect of solvent on analysis results of methanol (50 ppm)	
	(ppm)	mean (%)	95% confidence interval for mean	mean (%)	95% confidence interval for mean
Ethanol	250			-0.1	-2.1 – 2.0
	500			-1.8	-4.6 – 1.0
Methanol	150	0.4	0.1 – 0.7		
	300	1.0	0.3 – 1.7		
1-propanol	150	-0.3	-1.0 – 0.5		
	300	0.1	-1.0 – 1.1		
2-propanol	150	0.4	0.2 – 0.5	-1.8	-3.4 – -0.3
	300	0.4	-0.1 – 0.9	-1.1	-2.8 – 0.7
Acetone	150	0.8	0.2 – 1.3		
	300	0.3	-0.4 – 1.0		
	400	1.1	0.8 – 1.5	-2.1	-3.1 – -1.0
	800	1.5	0.5 – 2.5	-4.0	-5.9 – -2.1
MEK	150	0.6	0.2 – 0.9	0.0	-2.5 – 2.6
	300	0.9	0.3 – 1.6	-0.6	-1.3 – 0.1
MIBK	150	0.8	0.4 – 1.2	2.0	0.1 – 3.8
	300	1.9	1.6 – 2.2	1.2	-1.0 – 3.4
Diethyl ether	400	-0.7	-0.9 – -0.4	-1.3	-2.8 – 0.3
	800	0.0	-0.6 – 0.5	-2.1	-3.1 – -1.1
Ethyl acetate	150	1.4	0.8 – 2.1	-0.6	-2.0 – 0.8
	300	0.5	-0.5 – 1.4	-2.9	-3.3 – -2.4

MEK = methyl ethyl ketone; MIBK = methyl isobutyl ketone

7.3. Feasibility of the Gasmet FT-IR analyzer in clinical settings

7.3.1. Correlation of blood and breath concentrations

During the postabsorptive phase, the ethanol concentrations measured in the exhaled breath of volunteers (BrEC, ppm) correlated linearly with the blood concentrations (BEC, g/l): BrEC = 203×BEC, r^2 = 0.81 (Study I). The results of the analysis of one person's samples seemed to differ from those of the others noticeably. When the measurements of this subject were ignored, the equation became BrEC = 199×BEC, r^2 = 0.95. The mean [SD] calculated blood-breath ratios were 1,616 [276], 2,216 [304] and 2,466 [216] at the absorption, plateau and postabsorption phases, respectively.

In the study on methanol intoxication, the calculated blood-breath ratios were high and varied inter-individually during the first hours of treatment (Figure 16). At that time, most of the patients required extensive support of their vital functions. They were treated with vaso-active medication and received large
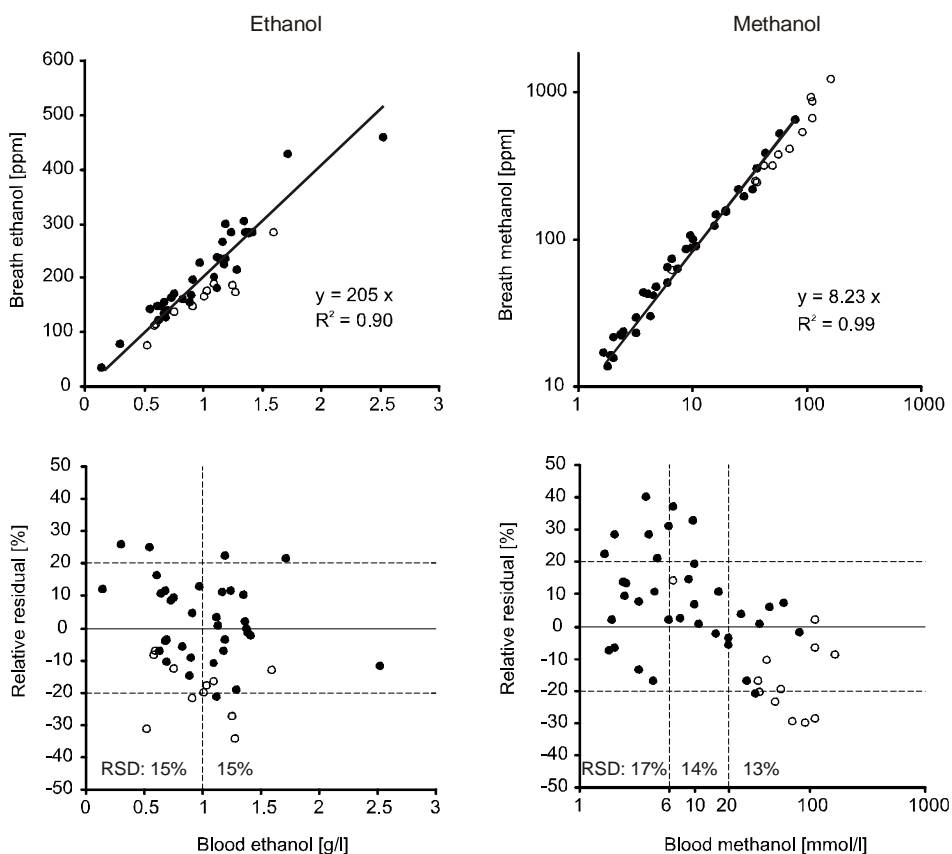
Figure 17. Linear regression lines express the correlation between ethanol and methanol blood and breath concentrations. Relative residuals express the deviation of breath analysis results from the regression line. The regression line was calculated from the data after six hours of patient arrival. The relative standard deviation (RSD) of the analysis results was calculated from all data points. Outline and solid symbols represent, respectively, the data collected before and six hours after patient arrival.

amounts of intravenous fluids, among other therapies. Four patients were initially connected to a respirator. After the first six hours of treatment, the measured breath ethanol (BrEC, ppm) and methanol (BrMC, ppm) concentrations correlated fairly well with the respective blood ethanol (BEC, g/l) and methanol (BMC, g/l) concentrations: $BrEC = 205 \times BMC$, $r^2 = 0.90$, $n = 35$; $BrMC = 257 \times BMC$, $r^2 = 0.99$, $n = 35$ (Figure 17, page 51).

The relative residuals were high. The relative standard deviation was 15% for ethanol and 13%–17% for methanol. The methanol relative standard deviation was highest in blood concentrations below 6 mmol/l.

The blood-breath ratios calculated from the linear regression coefficients were 2,668 for ethanol and 3,063 for methanol. When compared to the *in vivo* literature values, the ethanol blood-breath ratio exceeded even the range (Table 2, page 20). It was 16% higher than the literature median. The methanol blood-breath ratio had not been determined before. The *in vitro* blood-air partition coefficients have been determined mainly at 37 ℃. According to Jones, the coefficient for methanol is 19% higher in 34 ℃ than in 37 ℃ (Jones *et al.* 1990). The methanol blood-breath ratio was 3% smaller than the median (3,092) of the temperature-corrected water-air partition coefficients in the literature.

7.3.2. Sampling and analyzing

The delivery or collection of the samples was successful in five out of six methanol intoxication cases (four comatose, one cooperative). The failed case was a non-cooperative man. He had not been intubated, and the samples had to be taken by means of a face mask. The sampling was laborious and the CO_2 concentration remained low, thus rendering the samples unsuitable for quantitative analysis.

In the emergency room population, the sampling and analysis was successful in 589 out of 609 cases (96.7%). Samples were considered failed in 16 cases because of low exhaled carbon dioxide (< 3 vol%, median exhaled CO_2 2.7 vol%). Nurses considered the sampling difficult in only two of these cases. Technical problems in computing caused the loss of data on four additional participants. Difficulties in sampling appeared in some cases due to a lack of patient cooperation (15 confused and 4 comatose patients). An acceptable sample was finally obtained from all but two of these patients.

The exhaled CO_2 concentration varied quite a lot between the subsequent samples of the individuals among the men intoxicated with technical ethanol products. This was mainly due to the reduced coordination of the participants. Nevertheless, even very inebriated men were able to give an acceptable sample in a few attempts.

7.4. Response of the Dräger 7110 evidential breath analyzer to intoxicating solvents

Most of the possible interfering compounds studied either caused the "interference detected" message in very low concentrations (methanol, ethyl acetate and diethyl ether) or had a negligible effect on ethanol analysis (small coefficient (C_x); acetone, methyl ethyl ketone and methyl isobutyl ketone). When combined with ethanol, even possibly toxic concentrations of acetone and propanols did not activate the "interference detected" message. Additionally, 1-propanol and 2-propanol had a significant impact on ethanol readings. 1-propanol had a more pronounced effect in comparison with 2-propanol, the coefficients being 0.60 and 0.26, respectively. The effects of potentially interfering compounds on the Dräger 7110 evidential breath analyzer's ethanol recordings are summarized in Table 10.

Table 10. *The effect of interferents on the Dräger 7110 evidential breath analyzer readings. The values in bold correspond with potentially toxic blood concentrations.*

EtOH conc. mg/l	Interferent	Coefficient * (C_x)	Max. interf. conc. † mg/l	[ppm]	Reading ‡ mg/l	Error § (%)
0	1-Propanol	0.58	0.17	[70]	0.09	
0.24	Methanol	1.35	0.019	[15]	0.26	+8
	Acetone	0.017	**1.8**	**[800] ¶**	0.27	+13
	Ethyl acetate	0.54	0.052	[15]	0.27	+13
	Diethyl ether	((	0.044	[15]	0.25	+4
	MEK	0.025	0.86	[300] ¶	0.26	+8
	MIBK	0.018	1.2	[300] ¶	0.26	+8
	2-Propanol	0.26	0.17	[70]	0.28	+17
	1-Propanol	0.60	**0.48**	**[200]**	0.52	+117
0.60	Ethyl acetate	0.65	0.10	[30]	0.67	+12
	2-Propanol	0.25	**0.60**	**[250]**	0.74	+23
	1-Propanol	0.59	**1.4**	**[600] ¶**	1.40	+133

* Biasing power of an interferent on the ethanol reading (see text for details)

† Highest observed concentration of a possible interfering compound not to trigger the "interfering compound" sign

‡ Analysis result at the maximum interference level

§ Relative difference between the true ethanol concentration and the reading

¶ Higher concentrations were not tested

((Regression not relevant due to a small number of observations

MEK = methyl ethyl ketone; MIBK = methyl isobutyl ketone

8. Discussion

8.1. Configuration of the Gasmet FT-IR analyzer for breath testing

8.1.1. Impact of sampling system components on analysis results

According to our studies, 0.5 l of the sample gas was required to achieve 95% sample concentration into the 200 ml gas cell. The gas mixing was tested with 0.5–1.0 l/min flow. Nevertheless, even the 1.0 l/min flow is over tenfold lower than the flow in a normal exhalation. The higher gas flow was not tested, because the mixing would have occurred during a short time, allowing only few FT-IR measurements to be made.

According to the actual sampling experiment, a 1.5 l exhalation was needed to achieve 70% of the final CO_2 concentration. In contrast to the literature (Hlastala 1998), no plateau for CO_2 was ever reached. 1.5 l is the point where the CO_2 concentration curve bends to a more horizontal direction. From this point on, the changes in the exhaled volume would lead to a smaller difference in the CO_2 concentration. Hence, it was rational to require over 3 vol% CO_2 in an exhaled sample for FT-IR measuremets. The low end-exhaled CO_2 and the missing of an "alveolar plateau" might have been, at least partially, due to the sampling protocol. Deep inhalation immediately before the exhalation might have caused dilution of the CO_2 in the lungs, especially in the case of a test subject with a large lung volume. Further investigation is required to verify this assumption.

The total dead space due to the bacterial filter (35 ml) and hosing (25 ml) was 60 ml. The volume of the analyzer's hosing and measuring cell should be smaller in order to reduce the required sample volume. In the beginning of the study, the measuring cell size was minimized to be able to catch an alveolar sample at the end of the exhalation. Still, the cell makes up 70% of the volume of the sampling system. However, if the cell volume were reduced even further, the analyzer's performance would be impaired. A shorter sample line would be impractical, and smaller sample line diameter would lead to higher delivery pressure. The bacterial filter could be replaced with a smaller one, perhaps without significantly reducing the protection.

A very low resistance to blow has a few advantages. The effort required to give a sample is minimal, and even fragile elderly individuals or very inebriated persons are thus able to produce a satisfactory sample. Furthermore, the ventilation of a comatose patient is possible through the low-resistance measuring cell. A high delivery pressure has been supposed to prevent the attaining of an exhalation plateau and low pressures to generate a non-uniform exhalation profile (Gullberg 1990). In our studies, a higher resistance seemed to lead to a higher expired volume and higher end tidal CO_2. The measured ethanol concentration was independent of the delivery pressure.

The effect of condensation was significant, especially in the water analysis. Only a small part of the effect might have been due to the dead space effect, because the test subject was a 192 cm, 90 kg man with a large lung volume. The dead space effect would be more significant with a smaller sample volume. The effect of condensation on low-concentration ethanol was clearly smaller than on saturated water vapour. In any case, the problem could be easily solved by developing a heated filter and sampling hose.

8.1.2. Analysis software configuration in relation to breath composition

The objective was to configure analysis settings so as to minimize the effect of interfering components and maximize the analytical accuracy. Due to a considerable number of components in the reference library, negative component concentrations were mostly inhibited by the software settings. This is acceptable, because the background sample should be free of the library components (Saarinen *et al.* 1991).

Negative analysis results had to be allowed for methane in the screening study with ER attendees (Study III), because medical oxygen gas was used for zero calibration. Unexpectedly, the medical oxygen contained five to ten ppm of methane, which made it impossible to express the absolute exhaled methane concentration. Because the ambient air was measured before each patient, it was possible to calculate and show the exhaled methane concentration over the ambient air value. This points out the importance of analyzing the ambient air before breath testing.

Despite the ability of the analyzer to detect even subtoxic levels of common solvents other than ethylene glycol, the screening of ER-attendees' exhaled breath revealed only one case of methyl ethyl ketone, in addition to ethanol and acetone. A considerable exhaled concentration of ethanol was a common finding, especially among male trauma patients at night. Elevated acetone in ten patients was related to an apparently strong alcohol dependence or undernourishment.

Most of the men tested in Study V had drunk technical ethanol products and had a variety of solvents in their breath. The breath ethanol concentrations measured were quite high. These concentrations correspond to blood concentrations of 1.3 to 2.7 g/l, if a blood-breath equilibration ratio of 1,616 is used for calculations in the absorption phase. The most commonly misused product was Marinol (cooker fuel). In addition to ethanol (over 80% w/w), it contains MEK (2% w/w), MIBK (2% w/w) and 2-propanol (1%–5% w/w) (Berner 2005). All of these components were expectedly detected in the exhaled breath of alcoholics.

Ketoacidosis and high acetone concentrations have been found in alcoholics with unknown causes of death (Brinkmann *et al.* 1998). In addition to ketoacidosis, intoxication with 2-propanol or the use of technical ethanol products containing 2-propanol leads to very high blood acetone levels (Kelner *et al.* 1983). In Study V, breath screening revealed unexpectedly high concentrations of acetone. The high acetone concentrations measured in our study were most probably due to metabolism of 2-propanol.

8.2. Performance of the Gasmet FT-IR analyzer in solvent breath testing

8.2.1. Calibration model (linearity)

The linearity was checked by analyzing samples generated with the Temet calibrator. The variation due to the calibrator was estimated to be less than ± 2.5%. In spite of that, one of the analyzed ethanol samples was statistically an outlier. Because only one reference sample was prepared and analyzed at each concentration level, random error could not be excluded. Moreover, no other analysis method was used to confirm the sample concentration.

According to the validation protocol (Peters *et al.* 2002), the outlier should be excluded. This was not done in the original publication. When the outlier was later excluded, the measured ethanol absorbance of the remaining samples correlated linearly with the calculated concentration. The intercept of the regression equation was also very small; the calibration line went almost through the origo. The relative residual of the

outlier was -6.5%; not very high but more than would have been anticipated.

Methanol absorbance behaved somewhat non-linearly. This may be partially due to the fact that at high concentrations, the growth of the peak in the IR-spectrum decelerates. The sharp shape of the methanol peak at 1,057 cm^{-1} augments this phenomenon. When the two points over 0.4 absorbance units were deleted, the remaining six points remained significantly closer to the straight line passing the origo very closely.

The non-linear behaviour of absorbance as a function of concentration, if not corrected, can produce significant quantitative errors, because the analysis algorithm assumes a linear relationship between absorbance and concentration. The extent of the non-linearity will vary depending on the natural line width of the absorption band and the resolution of the spectrometer. The correction can be accomplished by determining the non-linearity factor from the reference spectra at different concentrations (Bak *et al.* 1995). The effect of non-linearity can also be minimized by measuring a sufficient amount of reference spectra within the non-linear concentration range.

The curving could have been mostly avoided by calculating the linearity from a blunter type peak, for example, at 2,972 cm^{-1} for ethanol and 2,979 cm^{-1} for methanol. On the other hand, the non-linearity can be an advantage in increasing the dynamic range of quantitative analysis. It is possible to measure high concentrations with a low-resolution instrument, because at low resolution, the absorbances measured at high concentrations are lower than at high resolution. This is a significant advantage in the case of a low concentration component required to be measured in the presence of strongly absorbing components with a high degree of spectral overlap (Saarinen *et al.* 1991).

8.2.2. Accuracy
As we did not have any secondary analysis method, we had to compare the results of the bubbling test analyses with previously published values. According to a comprehensive review, the literature values for Henry's law constants vary widely (Sander 1999). The reference values used in this study originate from publications of experimental determinations of Henry's law constants. The values calculated based on our study results were slightly lower than those found in the literature. This was clearly shown in the bias calculations.

The tests with the calibrator system proved a very good accuracy of the analyzer, except for the bias in the methanol measurements.

8.2.3. Sensitivity
The Gasmet FT-IR analyzer was adequately sensitive in ethanol and methanol breath analysis, even in the presence of high concentrations of other solvents. The calculated LLOQ for methanol was one tenth of the toxic concentration. The analyzer was most sensitive when a single component was analyzed in plain N_2. Even though the overlapping compounds were taken into account in the analysis method, the strong absorptions of the matrix components in the simulated human breath increased the LLOQ. When ethanol, methanol or even more components were added to the matrix, the LLOQ did not increase further.

8.2.4. Selectivity
Dadgar and co-workers (1995) proposed to determine the selectivity by analyzing up to 20 blank samples spiked with an analyte at the lower limit of quantification and, if possible, with interferents at their highest likely concentrations. In our studies, high concentrations of interferents were used, but the analyte concentration was not at LLOQ level. Instead, the selectivity measurements were made at clinically critical levels.

The Gasmet FT-IR analyzer is designed to analyze several components simultaneously. Due to this feature, it was also able to cope with several possible interfering compounds. All of the interfering effects remained well below acceptable limits.

8.2.5. Stability

The decrease in the sample components during storage was more probably due to leakage through the sampling hose than any chemical reaction inside the measuring cell. New components were not detected during the storage test. The significant decrease during the three-hour storage test is practically inconsequential, because the sample is blown directly into the sampling cell and usually analyzed immediately thereafter. A few minutes' delay in analysis would cause practically no error in results.

8.2.6. Procedural aspects

Preparing an exact gaseous sample is a challenge, especially in low analyte concentrations. The bubbling method was used in the early study period. It is easy to set up and use, but it has some disadvantages. The vaporization depends strongly on temperature. According to their thermodynamic properties, the components in the solution vaporize at varying rates. The composition of the sample gradually changes, if the sample gas is not led back to the bubbling system and the temperature controlled strictly. Interactions between the components in the liquid phase are also possible, although unlikely in dilute solutions.

Due to these factors, a breath simulator based on Temet calibrators was developed. The main advantage of this system was the possibility to easily calculate and adjust the composition of the sample gas, simply by regulating the speed of the syringe pumps. The main problem was a sway in the injection speed of the syringe pumps. The sway was clearly detected by the analyzer, because of the small measuring cell volume. Obviously, the sway was most disturbing at low syringe injection speeds. The problem was solved by adding a heated 1.0 l reservoir to the system.

The sample stabilization after changes was always verified by non-stop 5 s measurements. Even though, in theory, the calibrator system accurately vaporized the components into the stream of the carrier gas, the sample was sometimes unacceptable. The problems mostly occurred with methanol. Typically, the stabile state was not achieved or it was significantly delayed. The problem was usually solved by resetting the methanol syringe once or twice. In spite of this, the measured methanol concentration remained, for the most part, lower than could have been theoretically anticipated. Even though no explanation was found for the malfunction of the calibrator system, it probably explains part of the methanol bias.

The performance testing in a laboratory was carried out without a secondary analysis method. The nominal concentration in the bubbling tests was estimated according to the literature values for Henry's law constants, the range of which was quite wide. The nominal values for the calibrator system were calculated on the basis of the carrier gas flow and injection rates. If results of a secondary analysis method had been available, the error component due to the calibrator system could have been estimated. Even though the FT-IR method is traceable to the principal laws of physical chemistry, further investigation is needed in order to exactly validate the breath test method—e.g., by comparing it with another analysis method.

8.3. Feasibility of the Gasmet FT-IR analyzer in clinical settings

The most challenging part of solvent breath testing is estimating the correspond-

ing blood concentration. The criteria for precision and bias for a bioanalytical method were summarized in a recent review (Peters *et al.* 2002). The precision should stay within 15% relative standard deviation, with the exception of the lower limit of quantification (LLOQ) where 20% relative standard deviation is accepted. Bias was required to be within ±15% of the accepted true value, with the exception of the LLOQ where ±20% is accepted. If blood concentration is to be determined with a breath test, the human body becomes, so to speak, a part of the bioanalytical method. The abnormal vital functions of a seriously ill patient add many factors of uncertainty to the method.

Fortunately, on many clinical occasions—such as diagnosing a solvent intoxication—an exact quantification is not necessary. It is enough to know the name of the intoxicant and an approximated concentration. In the following, the feasibility of the Gasmet FT-IR analyzer is discussed from the clinical point of view.

8.3.1. Correlation of blood and breath concentrations

The blood-breath ratios from the experiments with volunteers agreed with previous investigations and showed higher and more constant values in the postabsorptive phase. The number of observations in this preliminary study was very limited.

It is important from the clinical perspective that the lower limit of quantification is low enough to enable any toxic amount of solvent to be found. Methanol is considered toxic in blood concentrations over 6 mmol/l. Even though the linear regression parameters of blood-breath pairs derived from methanol intoxications showed a quite good linear fit, the variation in the standardized residuals remained extensive even after omitting the data from the first six hours. The high and widely variable ethanol and methanol blood-breath ratios in the

methanol-intoxicated patients during the first hours of treatment could be explained by an unstable cardiorespiratory state and a marked ventilation-perfusion mismatch in the lungs.

Due to a limited number of blood and breath measurement pairs, the calculation of relative standard deviation for *in vivo* data was not statistically convincing. Nevertheless, these figures support the observational impression that the method was only just accurate enough to detect the mildest methanol intoxication.

Collecting the breath sampling was difficult with one of the methanol-intoxicated patients. The CO_2 concentration in a typical breath sample of this patient was 2.3 vol%. At the same time, the arterial pCO_2 was 5.0 kPa and the methanol blood concentration 12.1 mmol/l. In spite of the low-quality breath sample, the measured exhaled methanol concentration was 68 ppm. This case affirms the above assumption of methanol detection in subtoxic levels.

As was pointed out previously, there is no single true value for a blood-breath ratio of a solvent. In fact, only the blood-breath ratio for ethanol has been reasonably estimated. The methanol blood-breath ratio in toxic concentrations was determined for the first time in Study II. This blood-breath ratio was compared to *in vitro* blood-air partition coefficients in the literature. The *in vitro* coefficients do not account for the complex excretion of the solvents in the human respiratory system. The difference (bias) to the literature values was surprisingly small, if one considers the critical illness of the intoxicated patients. As we did not have any secondary analysis method for breath samples, the observed difference may derive from factors related both to the analyzer and the patients. During the treatment, the bias could be individually compensated for by comparing the breath analysis results with values derived from blood tests.

To discover the possible interfering compounds, hundreds of breath samples were screened in Studies III and V. The effect of these interfering compounds on the results of ethanol and methanol analysis was determined by laboratory tests with simulated breath samples, as described above. Because the composition of the simulated breath is very close to genuine human breath, the results of the *in vitro* interference studies are most probably valid *in vivo*, as well.

8.3.2. Sampling and analyzing

The Gasmet FT-IR analyzer was easy to use, even for non-laboratory personnel. As a battery-operated device, it was easy to carry to the bedside in the emergency room study. Even fragile elderly patients were able to give a satisfactory breath sample. Quickly trained nurses succeeded in obtaining samples, even from unconscious patients. Adding a no-return valve into the sampling hose would be a simple improvement to assist patients in correctly blowing into the analyzer. It would also facilitate the timing of the manual valve closure.

The nearly on-line breath solvent measurement turned out to be a valuable aid in controlling the haemodialysis and ethanol treatments (Figure 18). For example, it was clearly seen from the breath test trends how slowly methanol would have been eliminated without the third haemodialysis.

8.3.3. Procedural aspects

The method of sampling exhaled breath varies between individual studies in the literature. One common method is to inhale deeply and hold the breath for 15 to 25 seconds prior to exhaling. The purpose is to get a more "alveolar" sample, as the volatile compounds have time to equilibrate between the blood and breath. The non-breath-holding method was chosen for this study to get appropriate
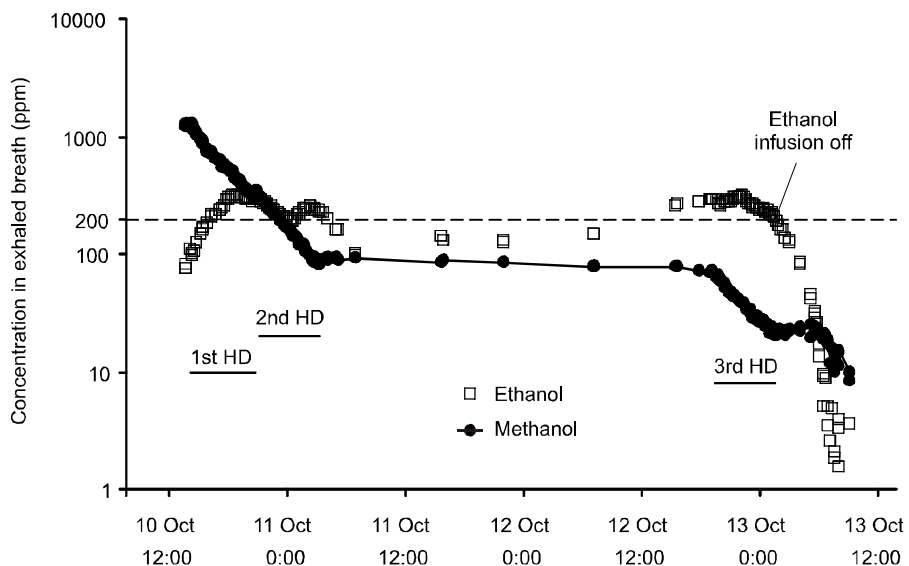
Figure 18. *Elimination of methanol during treatment of intoxication. Three six-hour haemo-dialysis treatments (HD) were performed. Between the second and third HD, the exhaled methanol concentration declined only from 90 to 70 ppm. An exhaled ethanol concentration of 200 ppm corresponds with the target blood concentration of 1 g/l.*

and equivalent samples even from non-cooperative patients. This choice was supported by Kirkham and co-workers (1988). They achieved an equally good correlation without breath-holding, even for gases changing predominantly in the alveoli, such as carbon monoxide.

Two subsequent breath samples were averaged to decrease random variation. An adequately high and roughly similar carbon dioxide level was required for the two samples. This method was used in all studies, except in Study V. Due to poor cooperation, the blowing technique of the inebriated men varied to a great extent between subsequent samples. In these circumstances, one good sample was considered adequate.

The large measuring cell size (0.2 l) is a clear disadvantage of the FT-IR method. The gas mixing in the measuring cell requires 0.4–0.7 l of extra sample volume, and dilutes the end-expiratory sample in all cases. Even though the sample was diluted and not truly alveolar, the solvent blood-breath correlation stayed linear and the variation in analysis result was acceptable for rough clinical purposes.

8.4. Value of the Dräger 7110 evidential breath analyzer in revealing the presence of intoxicating solvents

The Dräger 7110 evidential breath analyzer has been designed to analyze breath ethanol accurately, in spite of the other breath components. In order to be detected, a possible interfering compound must have a sufficient vapour pressure to pass from the blood to the breath, and the resulting breath concentration must be high enough to cause a significant difference between the responses of the EC and IR detectors (Lagois 2000). Neither the electro-chemical detector nor the IR-method is absolutely specific to ethanol.

A detection of volatile compounds other than ethanol might in some rare cases reveal poisoning. With regard to methanol, this could be of vital importance. The Dräger 7110 evidential breath analyzer was tested in order to determine whether it could be utilized in diagnosing a solvent intoxication.

The Dräger 7110 evidential breath analyzer was able to detect most of the potentially interfering common solvents in concentration levels which did not significantly affect ethanol analysis. Methanol had a very strong relative effect on the ethanol reading (coefficient 1.35), but the interference was detected in a very low concentration, before it began to influence the results of the ethanol analysis. The threshold concentration in breath was 15 ppm [19 µg/l]. It corresponds with a sub-toxic blood concentration (< 2 mmol/l).

On the other hand, acetone and propanols were not detected even in toxic concentrations, when combined with ethanol. Co-existence of 1- or 2-propanol significantly interfered with the ethanol analysis without activating the "interfering compound" message. The effect of 1-propanol on ethanol readings was more than two times stronger than that of 2-propanol. Corresponding interference by 2-propanol has been published earlier (Lagois 2000).

According to our results, it seems that a successful breath ethanol analysis by the Dräger 7110 evidential breath analyzer could exclude any significant methanol intoxication. In contrast to the Gasmet FT-IR analyzer, the Dräger 7110 evidential breath analyzer is not intended or equipped to qualify the interfering component. Therefore, verifying a suspected intoxication by methanol (or other volatile compound) relies on clinical signs and symptoms, if the "interfering compound" message is displayed. In hospital settings, the reason for the "interfering compound" message would naturally lead to further examinations (blood tests).

8.5. Ethylene glycol

Most of the solvent analyses are required in order to exclude a toxic solvent intoxication. Ethylene glycol is an important intoxicating solvent in addition to methanol and 2-propanol. Unfortunately, a toxic blood concentration of 8–24 mmol/l at 34 ℃ would possibly lead to approximately 5–16 ppb in air; this is at least a hundred times below the assumable detection limit of the Gasmet FT-IR analyzer (Equation 10.3, estimated temperature correction term 10^4 K). For the same reason, an ethylene glycol intoxication will not activate the "interfering compound" message in the Dräger 7110 evidential breath analyzer. In other words, these analyzers can not be used for ethylene glycol intoxication diagnostics, which leads to a marked weakness in the breath diagnostics of a typical case of severe metabolic acidosis.

9. Conclusions

The following conclusions can be drawn on the basis of the present studies:

1. The Gasmet FT-IR analyzer proved suitable for breath testing after the sample cell fittings were widened and the cell size reduced. The analyzer was also equipped with a battery for bedside diagnostics. In the future, a heated sampling hose and filter as well as a non-return valve are required for the sampling system.
   The analysis method was insensitive to the hundreds of minor endogenous or exogenous breath components reported in the literature. The most abundant detectable breath components in the breath were carbon dioxide, water, methane, carbon monoxide and acetone. Measurable amounts of solvents were also detected in breath samples after ingestion of technical ethanol products. All of the measurable components were included in the reference library and were thus taken into account in the analysis procedure. The settings of the analysis software were adjusted for breath testing.
   (Studies I–III, V)

2. The Gasmet FT-IR analyzer proved suitable for solvent intoxication breath testing where linearity, accuracy, selectivity and sensitivity were concerned. In laboratory settings, the lower limit of detection for methanol was 10 times lower than the lowest toxic concentration, even in the presence of a high concentration of other solvents. Toxic blood concentrations of ethylene glycol could not be detected by this method.
   (Studies I, V)

3. The analyzer was easy to use and also suitable for use in non-laboratory settings by non-laboratory personnel. Even though the blood-breath ratio of methanol varied to a great extent in clinical settings, the method was just accurate enough to detect the mildest methanol intoxication.
   (Studies I–III, V)

4. It seemed that a successful breath ethanol analysis by the Dräger 7110 evidential breath analyzer could exclude any significant methanol intoxication. In contrast, very high levels of acetone, 1-propanol, and 2-propanol were not detected in simulated breath. Due to its physiochemical properties, ethylene glycol will not be detected either. The Dräger 7110 evidential breath ethanol analyzer is not intended for and equipped to recognize the interfering component. In case an interfering compound is detected, the suspicion of intoxication by a toxic solvent relies on clinical signs and symptoms.
   (Study IV)

10. Equations

10.1. Blood-breath ratio

Correlation between blood and breath concentrations is determined by the equation:

$$BBR = \frac{C_{Blood}}{C_{Breath}}$$

where BBR = blood-breath ratio; C_{Blood} = concentration of solvent in the blood; C_{Breath} = concentration of solvent in the breath.

10.2. Liquid-air partition coefficient

Blood-air or water-air partition coefficients were calculated by the following equation:

$$\lambda_{la} = \frac{c_l}{c_a} = \frac{c_l}{p_g / RT} = \frac{c_l RT}{p_g}$$

where λ_{la} = liquid-air partition coefficient; c_l = concentration in liquid phase [mol/l]; c_a = concentration in gas phase [mol/l]; R = gas constant [0.08206 atm l / mol K]; T = temperature [K]; p_g = partial pressure in gas phase [atm].

10.3. Henry's law

Henry's law predicts a linear relationship between the partial vapour pressure of the solute and its concentration in the liquid phase (Sander 1999):

$$k_H = \frac{c_l}{p_g}$$

where k_H = Henry's law constant for the component; c_l = concentration of the component in the liquid phase [mol/l]; p_g = partial pressure of the component in the gas phase [atm]. The Henry's law is valid for ideal dilute solutions—i.e., the mole fraction of the solute should be below 0.01. Examples of the constants are shown in Table 3 (page 21).

The Henry's law constant depends strongly on temperature and slightly on pressure. In constant pressure, the effect of temperature change on the constant value can be calculated:

$$k_H = k_H^\theta \times \exp\left(\frac{-\Delta_{soln} H}{R} \left(\frac{1}{T} - \frac{1}{T^\theta} \right) \right)$$

where k_H = Henry's law constant [M/atm] for the component in the new temperature T [K]; k_H^θ = Henry's law constant [M/atm] for the component at the standard temperature T^θ

[298.15 K]; $\Delta_{soln}H$ = enthalpy of the solution [L atm mol^{-1}]; R = gas constant [8.2057 * 10^{-2} l atm mol^{-1} K^{-1}].

The calculation of the temperature effect is possible, because empirical determinations of the

term $\dfrac{-\Delta_{soln}H}{R}$ have been published in the literature (Sander 1999).

10.4. Ventilation-perfusion ratio

The fraction of the compound excreted (E) to the breath is dependent on alveolar blood flow and pulmonary ventilation according to the equation:

$$E = \frac{\dot{V}/\dot{Q}}{\lambda + \dot{V}/\dot{Q}}$$

where λ = blood-gas partition ratio; $\dot{V}$ = alveolar ventilation; $\dot{Q}$ = alveolar blood flow (Hlastala *et al.* 1988). In a normal subject at rest total $\dot{V}$ is approximately 4.5 l/min and the corresponding total $\dot{Q}$ 5.0 l/min. Overall ventilation-perfusion ratio ($\dot{V}/\dot{Q}$) equals 0.9 (range 0.7 to 1.0). In different regions of the lung $\dot{V}/\dot{Q}$ varies widely, from 0.5 to 3.0.

10.5. Beer's law

According to Beer's law, the absorbance of the IR-radiation is directly proportional to the concentration of the sample (and the path length):

$A(v) = -logT(v) = -log[I(v) / I_0(v)] = \varepsilon(v)cl$

where $A(v)$ = absorbance; $T(v)$ = transmittance; $I_0(v)$ = the original intensity of the radiation; $I(v)$ = the intensity of the radiation passed through the sample; c = concentration of the sample; l = optical path length (the distance the radiation goes through the sample); $\varepsilon(v)$ = molar coefficient of absorption of the sample; v = wave number.

The IR spectrum of the sample is the sum of the spectra of the N components:

$$A(v) = \sum_{i=1}^{N} \varepsilon_i(v)cl$$

10.6. Transmittance and absorbance

A transmittance spectrum is obtained as follows:

$$T(v) = \frac{I(v)}{I_0(v)}$$

where $T(v)$ is transmittance; $I(v)$ is the intensity measured with a sample in the beam (from the sample single beam spectrum); $I_0(v)$ is the intensity measured from the background spectrum.

The absorbance spectrum can be calculated from the transmittance spectrum using the following equation:

$$A(v) = -\log T(v)$$

where $A(v)$ is the absorbance.

10.7. Resolution

The resolution (Δv) of an FT-IR instrument depends on the maximum displacement (δ_{max}) of the moving mirror according to the following equation:

$$\Delta v = \frac{1}{\delta_{max}}$$

10.8. Precision

The precision was calculated by one-way ANOVA and the following equations (Bookbinder *et al.* 1986):

Within-day variance: $SD_{WD}^2 = MS_{WG}$

Between-day variance: $SD_{BD}^2 = \dfrac{MS_{BG} - MS_{WG}}{n}$

Total variance: $SD_{I(CT)}^2 = SD_{BD}^2 + SD_{WD}^2$

where MS_{WG} = within-groups mean squares value from the ANOVA results;
MS_{BG} = between-groups mean squares value from the ANOVA results; n = number of measurements per day.

Coefficient of variance: $CV\ [\%] = \dfrac{\sqrt{SD_{XX}^2}}{\bar{x}} \times 100$

where SD_{XX}^2 = within-day, between-day or total variance.

10.9. Bias

The bias of analysis results was calculated for ethanol and methanol with the equation:

$$Bias\ [\%] = \frac{\overline{x} - \mu}{\mu}$$

where $\overline{x}$ = mean of the measured values; μ = nominal value.

10.10. Limits of detection and quantification

The limits of detection (*LOD*) and the lower limits of quantification (*LLOQ*) were calculated with the equations:

$$LOD = |C_0| + 3 * SD$$

$$LLOQ = |C_0| + 10 * SD$$

where $|C_0|$ = absolute value of the measured component concentration; *SD* = standard deviation of analysis results of the component in question.

10.11. Average absolute bias

$$Average\ absolute\ bias\ [\%] = \frac{\sum_{j=1}^{n} \left| Bias\ [\%]_j \right|}{n}$$

where j = number of different combinations of interfering compounds and their concentrations.

10.12. Coefficient for biasing power

The coefficient C_x describes the biasing power of the interfering compounds on the ethanol readings:

$$EtOH_{app} = C_x \times Interf_x + EtOH_{act}$$

where $EtOH_{app}$ = apparent ethanol concentration displayed by the Dräger 7110 evidential breath analyzer; $Interf_x$ = concentration of the interfering component X in the sample; $EtOH_{act}$ = actual ethanol concentration of the sample.

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