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TECHNICAL NOTE

Confirmation of natural gas explosion from methane quantification by headspace gas chromatography–mass spectrometry (HS-GC-MS) in postmortem samples: a case report

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Abstract A new analytical approach for measuring methane in tissues is presented. For the first time, the use of in situ-produced, stably labelled CDH_3 provides a reliable and precise methane quantification. This method was applied to postmortem samples obtained from two victims to help determine the explosion origin. There was evidence of methane in the adipose tissue (82 nmol/g) and cardiac blood (1.3 nmol/g) of one victim, which corresponded to a lethal methane outburst. These results are discussed in the context of the available literature to define an analysis protocol for application in the event of a gas explosion.

Keywords Methane · Explosion · HS-GC-MS

Introduction

Although methane does not have major toxic effects on organisms, the gas has two characteristics that can lead to death. First, significant quantities of methane can compete with and deplete oxygen levels. Thus, oxygen is not sufficiently distributed within an organism, causing hypoxia (dizziness and fainting) and, ultimately, lethal anoxia (suffocation and neuronal death) [1]. This property has been exploited in suicides with natural gas [2] and has played a role in accidental deaths due to sewer shaft falls [3], drainage pit work [4], handling decaying material [5] or working in mines [6, 7]. Secondly, methane is extremely flammable and may form explosive mixtures with air at concentrations between 5 and 15 % by volume [8]. Therefore, methane combustion in mines [9, 10], during tunnel urban works [11] or domestic incidents [12], can lead to lethal explosions through the conversion of methane chemical energy into mechanical and thermal energy [13–15]. Conversely, only one suicide case has been reported that involved methane explosivity as a deliberate lethal agent [16]. The highly flammable and potentially explosive properties of methane make it an extremely noxious agent.

In mining industry accidents, methane determination in postmortem tissues is necessary to identify the cause of death. Indeed, the chemical combustion of methane can be lethal for several reasons, including extremely high temperatures (up to 2,650 °C) [15], mechanical effects (blast and pressure following explosion) or asphyxia due to oxygen depletion [6]. Consequently, it is very difficult to establish a lethal methane concentration. Local concentration (especially in the lungs) can be more important in asphyxia cases, when the victim inhales methane for a long period of time, than in cases of death due to gas explosion (thermal, mechanical effects). Moreover, in cases of asphyxia following an explosion, continued respiration during survival period allows methane to distribute into the organs, and this interval is always unknown, which complicates the interpretation of the implication of various methane concentrations in organs. The initial ambient air composition can provide information regarding methane concentration for estimation of the survival period. Finally, to avoid methane release from the body and to exclude methane generation due to decomposition, minimal delay between death and sample collections is of great importance.

From an analytical point of view, these measurements are performed via injections of gaseous samples into a gas chromatograph (GC) equipped with a flame ionisation detector (FID) [17, 18] or a thermal conductivity detector (TCD). Mass spectrometry (MS) has recently been used as

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a confirmatory tool, but quantification by GC-MS has not been previously performed. The main drawback of GC-FID and GC-TCD methods is the absence of an internal standard. Indeed, the quantifications were performed with external calibration with standard gaseous methane [6], pentane [12] or a mixture of methane/argon [4]. Methanol has also been used as an internal standard [19]. However, taking into account the high reactivity of Grignard reagents such as methylmagnesium chloride (CH_3MgCl) towards water, it becomes possible to generate gaseous methane. By using deuterated water (D_2O) instead of H_2O, an internal standard (CDH_3) can be generated. The control of concentration is performed by the stoichiometry of the reaction and the volume in which the reaction takes place.

The aim of this study was to develop a new method of methane quantification by headspace gas chromatography–mass spectrometry (HS-GC-MS) using a labelled stable isotope of methane generated in situ. The procedure was applied to postmortem samples from victims of a gas explosion.

Materials and methods

Reagents

Methylmagnesium chloride 3.0 M in tetrahydrofuran (THF) was from Sigma-Aldrich (Saint Louis, USA). Deuterated water was obtained from Cambridge Isotope Laboratories, Inc. (Andover, USA). Certified methane was obtained from Carbagas (Lausanne, Switzerland).

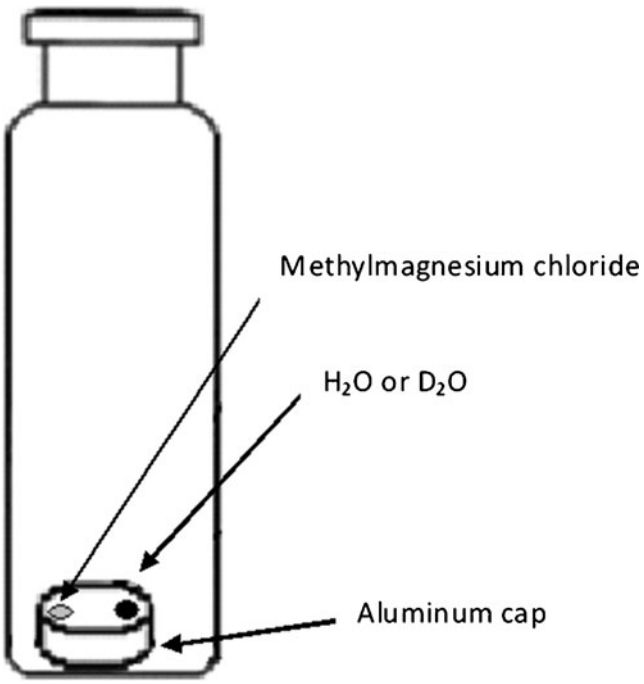

Fig. 1 Design of methane generation in situ

Standard generation

The methodology for generating standards in situ was described previously [20]. Methane (CH_4) and deuterated methane (CDH_3) were generated separately in 20-mL headspace vials. The reactions of Grignard reagent with water and deuterated water are given below:

$$CH_3MgCl + H_2O \rightarrow CH_4 + MgClOH \quad (1)$$

$$CH_3MgCl + D_2O \rightarrow CDH_3 + MgClOD \quad (2)$$

Due to the high reactivity of these reactions, it is important to proceed quickly (methylmagnesium chloride reacts with water in ambient air) and safely (in a fume hood). Grignard reagent and water were added without any contact between them in an aluminium cap with no septa or holes and introduced into a headspace vial (Fig. 1). The vial was rapidly and hermetically closed and then vortexed to allow methane generation. Precise volumes of gas (CH_4 and CDH_3) were sampled (automatically or manually) with a gas syringe through the vial septum and directly introduced into the GC injector.

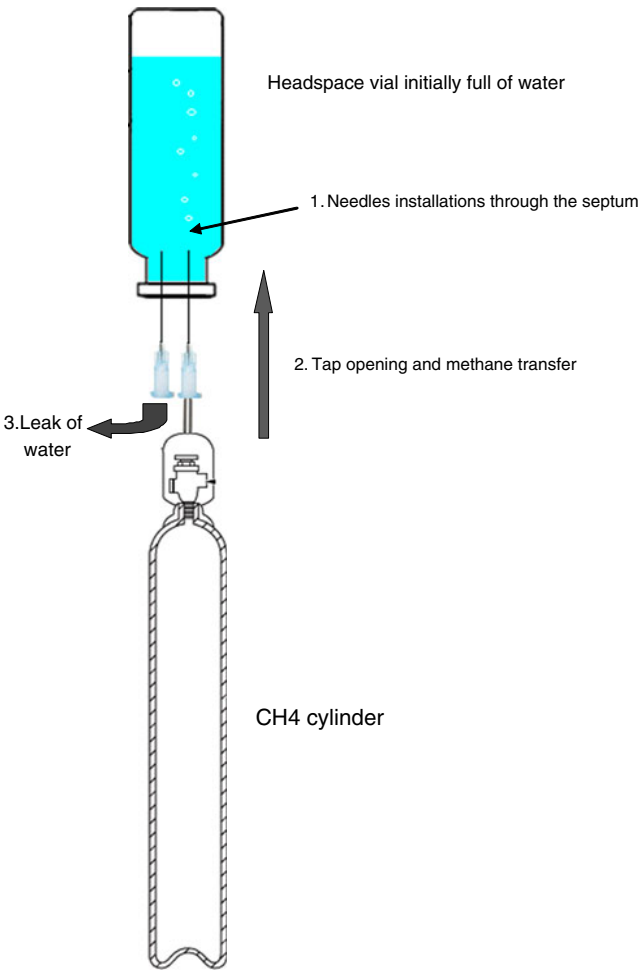

Fig. 2 Design of methane transfer to perform external control samples

Table 1 Concentration of methane in tissues of the two victims and HbCO saturation

	Woman (31 years old)	Girl (5 years old)
Methane concentration (μmol/g)		
Cardiac blood	ND	1.3×10^{-3}[a]
Brain	ND	
Adipose tissue		82×10^{-3}
HbCO (%)		
Peripheral blood	5	19.5

ND not detected

[a] Approximate value

Control samples

Certified methane (99.995 % purity) was used to make control samples. Known volumes of methane were diluted in headspace vials previously saturated with nitrogen: 0.053, 0.105 and 0.210 μmol/mL were used (Fig. 2). The methane yield was compared against these external controls (relative bias <25 % for each concentration, n=3).

Calibration curve

The amounts of Grignard reagent and water were calculated to produce concentrations of 0.5 μmol/mL of headspace in the vial: 3.3 μL CH_3MgCl (3.0 M in THF) and 20 μL water (or deuterated water) were introduced into a 20-mL headspace vial. A calibration curve (R^2 0.9756, n=4 for each point) was built with five concentrations: 0.025, 0.05, 0.1, 0.25 and 0.5 μmol/mL corresponding to 50, 100, 200, 400 and 1,000 μL of injected gaseous sample from the standard vial (obtained by reaction 1). For unknown sample measurement, a 1,000-μL gaseous sample was taken. Before each injection, a volume of 100 μL (corresponding to 0.5 μmol/mL) was taken from the internal standard vial (obtained by reaction 2).

GC-MS analysis

An Agilent 6890N GC (Agilent Technologies, Palo Alto, CA) combined with a headspace gas autosampler and equipped with a HP Molecular sieve 5-Å PLOT capillary column (30 m× 0.32 mm, 30 μm) from Restek (Bellefonte, USA) was used. A CP Porabond Q column was also used. The temperature program was 40 °C held for 5 min. The injector (splitless mode) was set at 100 °C, and the interface MS temperature was 230 °C. Helium was employed as a carrier gas at a flow rate of 1.9 mL/min.

The detection was performed with an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA), operating in an electron ionisation mode at 70 eV. Selected ion monitoring mode was used to acquire the methane signal at m/z 16 and 17 for CDH_3.

Case report

A 31-year-old woman and her 5-year-old daughter were killed when there was an explosion in their apartment. Both victims underwent autopsy in our laboratory a few hours after the explosion. Only brain and cardiac blood were sampled from the woman, and cardiac blood and adipose tissue were collected from the daughter for the purpose of gas analysis. The objective was to assess methane exposure to confirm the hypothesis of a natural gas explosion. The lungs were too damaged to be useful for analysis.

Results and discussion

Only a handful of studies have assessed methane concentrations in postmortem tissue. A minimal methane concentration of 6 μL/g or 0.25 μmol/mL of cardiac blood was reported in a study performed on 22 victims of a gas outburst accident in a coal mine [6]. The methane concentration in cardiac blood was 14.1±5.3 μL/g or 0.59 μmol/mL (n=22). This result is in

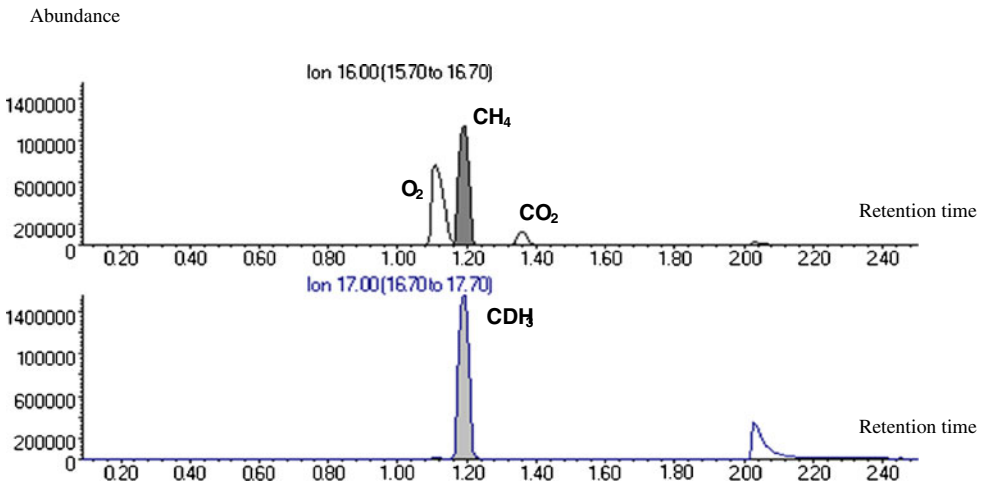

Fig. 3 Chromatograms of methane and deuterated methane in adipose tissue (daughter)

agreement with results obtained from rats subjected to various methane/oxygen concentrations in a closed space [7]. A lethal methane concentration of 19 μL/g (0.79 μmol/mL) was found in rat blood (n=5) after 2 min under 100 % CH_4. A lethal methane concentration of 25 μL/g (1.04 μmol/mL) was found in rat blood (n=5) after 20–25 min of exposure to a CH_4 atmosphere increasing from 0 to 100 % at 1 L/min. Finally, a lethal methane concentration of 29 μL/g (1.21 μmol/mL) was found in rats blood (n=5) after 80–85 min under an oxygen/CH_4 atmosphere mixture (2:8, v/v%) increasing to 100 % of methane after 1 h. The initial composition of ambient air is therefore very important for interpreting blood methane concentration. The lung is the best tissue for diagnosing acute methane exposure. A minimal lung methane concentration above 160 μL/g (6.67 μmol/mL) was measured in coal miners (n=2) [6] and seems consistent with the results obtained in rats [7]: 163±48 μL/g (6.8 μmol/mL) under oxygen/CH_4 atmosphere mixture and 442±107 μL/g (18.4 μmol/mL) under 100 % methane. However, brain or adipose tissues are also useful because methane is soluble in fat.

The methane concentration in the tissue from the two autopsied victims is presented in Table 1. Methane was only detected in samples from the daughter, with the highest levels in the adipose tissue (Fig. 3). It is interesting to note that only the daughter's tissue showed significant HbCO saturation (19.5 % in cardiac blood compared to 5 % in the mother). These results indicate that the daughter received a higher exposure to methane and CO gases than the mother before the explosion or that the mother passed away shortly after the explosion because her methane concentration values were not sufficient for death from anoxia.

The observed methane concentrations were considerably below the 0.25 μmol/g value that is considered indicative of "methane death" caused by asphyxia due to oxygen depletion [6]. However, this cutoff value should be only used for gas outbursts in mines or confined, underground spaces. Moreover, this value was established without information regarding the true role of methane. Methane concentration variations in the miners' tissues show that the causes of death and survival periods were different. Some of the workers could have died due to asphyxia before the explosion, and others may have died after the explosion due to anoxia or explosion-related injuries. Nevertheless, it can be assumed that a methane concentration greater than 0.25 μmol/g, even in the absence of an explosion, is an indicator of "methane-related death".

The difficulty in determining lethal methane concentrations can be illustrated by the different methane concentrations obtained in different tissues according to gas exposure (Tab. 2). It is necessary to identify four interconnected parameters before interpreting methane concentrations:

1. Initial ambient air composition. The results compiled in Table 2 [4, 11] indicate a very low methane exposure. In

Table 2 Methane concentrations in various tissues and organs after several types of accidents (adapted from [4, 6, 7, 11])

Methane exposure						
Methane concentration (μmol/g)	Work in pit [4]	Urban tunnel explosion [11]	Coal mine explosion [6]	Rats under 100 % CH_4 [7]	Rats under 0→100 % CH_4 (1 L/min) [7]	Rats under $CH_4/0_2$ (8:2) (1 h)→100 % CH_4 [7]
Blood	$12\times10^{-3}\pm11\times10^{-3}$ (n=3)		0.59±0.2 (n=22)	$0.80\pm16\times10^{-3}$ (n=5)	$1.04\pm50\times10^{-3}$ (n=5)	$1.21\pm75\times10^{-3}$ (n=5)
Brain	$15\times10^{-3}\pm14\times10^{-3}$ (n=3)	2×10^{-3} (n=1)	0.89 (n=2)	$1.00\pm88\times10^{-3}$ (n=5)	$1.09\pm67\times10^{-3}$ (n=5)	1.00±0.1 (n=5)
Liver	$7.3\times10^{-3}\pm7.0\times10^{-3}$ (n=3)	$12\times10^{-3}\pm18\times10^{-3}$ (n=3)	0.40 (n=2)	$0.59\pm54\times10^{-3}$ (n=5)	$0.67\pm34\times10^{-3}$ (n=5)	0.92±0.14 (n=5)
Kidney	$14\times10^{-3}\pm7.9\times10^{-3}$ (n=3)		0.17 (n=2)	$0.96\pm63\times10^{-3}$ (n=5)	$0.92\pm88\times10^{-3}$ (n=5)	1.00±0.2 (n=5)
Heart	$19\times10^{-3}\pm20\times10^{-3}$ (n=3)		0.48 (n=2)	1.1±0.14 (n=5)	$0.83\pm34\times10^{-3}$ (n=5)	$0.92\pm59\times10^{-3}$ (n=5)
Lung	0.13±0.2 (n=3)	$14\times10^{-3}\pm6.0\times10^{-3}$ (n=3)	9.27 (n=2)	19±4.5 (n=5)	7.96±3.4 (n=5)	6.79±0.2 (n=5)
Fat	$7.4\times10^{-3}\pm6.0\times10^{-3}$ (n=3)	$16\times10^{-3}\pm14\times10^{-3}$ (n=4)	0.14 (n=2)	0.71±0.1 (n=5)	$0.67\pm50\times10^{-3}$ (n=5)	1.00±0.2 (n=5)
Vitreous	$7.4\times10^{-3}\pm4.7\times10^{-3}$ (n=3)					
Muscle	$4.9\times10^{-3}\pm2.3\times10^{-3}$ (n=3)					
Medulla oblongata			0.51 (n=2)			
Spleen			0.20 (n=2)			
Pancreas			0.16 (n=2)			

The last three columns show the results of a study on methane asphyxia on rats

the first case, there was no explosion, and the cause of death was CO_2 intoxication leading to lethal asphyxia of three workers in a draining pit [4]. In the second case, there was a gas outburst following a rapidly increasing methane leak past the explosion limit of 5 % and a simultaneous decrease of O_2 [11]. The main causes of death were wounds caused by the explosion and CO intoxication. However, in both cases, the methane concentrations were too low to cause death due to asphyxia.

2. Room geometry. Results compiled in Table 2 [6, 7] illustrate the importance of room geometry and gas flow rates on methane concentrations. Mixtures of methane with oxygen influence survival time because the methane is diluted. A large methane leak without ventilation can rapidly transform the composition of ambient air until the explosive threshold is reached. As methane is lighter than air, it collects at the top of a room. Depending on the room volume, the time to reach 5 % methane can vary, and this influences the final methane concentrations in victims' organs.
3. Delay of gas exposure before and after explosion (if an explosion occurs). The results listed in Table 2 [7] demonstrate that the survival period is strongly related to ambient air composition, which is itself related to room geometry. Increased oxygen could allow a longer time of methane exposure, which could explain the greater distribution of methane in the body and its accumulation in fat-rich tissues (e.g. adipose tissue and brain). The delay and magnitude of methane exposure (initial ambient air composition) can be deduced from the methane concentrations obtained in the different samples and the room geometry.
4. Analysed sample. As illustrated in Table 2, methane concentration can be influenced by the nature of the sample, the length of methane exposure and the victim's metabolism. Usually, the lungs are the first organs impacted by methane, but if the methane concentration in the ambient air is sufficiently low, the concentration can decrease in the lungs and increase in the heart, blood and especially in lipophilic organs, such as brain and adipose tissue. Conversely, methane is not stored in appreciable quantities in spleen, pancreas or kidney, which are further along in the detoxification pathway. Therefore, a high methane concentration in lipophilic tissue and a low concentration in the lungs could mean that the victim did not die rapidly and could have been asphyxiated. Similarly, a high methane concentration in the lungs and low concentrations in other tissues could indicate a rapid, lethal methane explosion [11].

In our case (Table 1), the values of methane concentrations in cardiac blood and adipose tissue were between those measured in samples from a sudden lethal methane explosion (no asphyxia) [11] and those measured in samples following asphyxia due to a methane explosion [6]. The fact that no methane was measured in the mother seems to indicate a relatively sudden and lethal methane explosion.

From an analytical point of view, employing deuterated methane as an internal standard before assessing the unknown samples allows troubleshooting that can prevent the loss of valuable samples. Leaks or analytical discrepancies should affect methane and deuterated methane equally, which results in more reliable measurements.

Conclusion

The labelled stable isotope of methane allows precise identification and quantification. Further work is necessary to fully validate the analytical method of methane measurement by HS-GC-MS. These findings are important for establishing a sampling protocol following explosions. Cardiac blood and lungs provide the best samples to monitor methane after a gas outburst, but injuries sustained during the explosion may render these tissues unusable. Therefore, because methane is lipophilic, brain and adipose tissue can be analysed instead. In the reported cases, the absence of HbCO saturation and the weak methane concentration in cardiac blood seem to indicate a rapid natural gas explosion. The presence of methane in the adipose tissue of one of the victims confirmed that natural gas was the lethal explosive agent.

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