Journal of Chromatography B, 913–914 (2013) 155–160

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

# Accuracy profile validation of a new analytical method for butane measurement using headspace-gas chromatography–mass spectrometry

V. Varlet*, F. Smith, M. Augsburger

*Toxicology and Forensic Chemistry Unit, University Center of Legal Medicine Lausanne – Geneva, CH-1011 Lausanne, Switzerland*

ARTICLE INFO

*Article history:*
Received 7 August 2012
Accepted 27 November 2012
Available online 12 December 2012

*Keywords:*
In situ stable labelled isotope generation
Butane
Deuterated butane
Grignard reagent
Headspace gas extraction

ABSTRACT

The aim of our study was to provide an innovative HS-GC/MS method applicable to the routine determination of butane concentration in forensic toxicology laboratories. The main drawback of the GC/MS methods discussed in literature concerning butane measurement was the absence of a specific butane internal standard necessary to perform quantification. Because no stable isotope of butane is commercially available, it is essential to develop a new approach by an in situ generation of standards. To avoid the manipulation of a stable isotope-labelled gas, we have chosen to generate in situ an internal labelled standard gas ($C_4H_9D$) following the basis of the stoichiometric formation of butane by the reaction of deuterated water ($D_2O$) with Grignard reagent butylmagnesium chloride ($C_4H_9MgCl$). This method allows a precise measurement of butane concentration and therefore, a full validation by accuracy profile was presented.



## 1. Introduction

Butane gas comes from the liquation and distillation of petroleum. The toxicity of butane is not only related to its chemical properties as it is mainly due to the tissue asphyxiation. Effectively, butane will replace air leading to oxygen depletion [1]. Butane is mainly involved in two kinds of fatalities: suicides [2–7] and involuntary accidents [8] as well as deliberate inhalation [5,9–12]. Indeed, butane is easily available because it is commercialized for domestic uses (oven, gas stove, etc.) and it is present in mixtures of different purity in other devices (gas lighters, antiperspirant gas aerosols, etc.). Gas cylinders are particularly used to commit suicide as an important quantity of butane is required. Such victims are often found with a bag over their head to accelerate asphyxia. Smaller quantities of butane are available in gas lighters (Liquid Petroleum Gas – butane content: 30–50%) and aerosols. They are more often used in inhalation. Indeed, in many countries, the deliberate sniffing or inhalation of aerosols has become increasingly popular, especially among young and poor people, with recreational or drug purposes [13–15]. Butane deaths linked to these practices exhibit a lower concentration than in butane suicide. The depletion role of butane could be less important and the cause of death is more often related to butane cardiotoxicity properties. Rhabdomyolysis was also reported [16,17]. Among butane sniffers, a cardiac arrhythmia such as ventricular fibrillation is often put in evidence at autopsy [18–28]. Blood samples are often analyzed in these cases but other samples such as lung, brain, liver, heart muscle and adipose tissue may also be used because of butane lipophily [29].

Butane monitoring in exhaled breath was also used to investigate health disorders and cancers [30]. This monitoring proves that an endogenous source of butane is present in the organism. Several concentrations of butane in exhaled breath were proposed such as 0.7 nmol/kg $h^{-1}$ [31] even though the origin of its synthesis is still discussed [32,33].

The analytical measurement of butane was easily performed by gas chromatography (GC) coupled to Flame Ionization Detector (FID) [10,12,34,35], Thermal Conductivity Detector (TCD) [36] or Mass Spectrometry (MS) [5,34,37,38] and tandem mass spectrometry (MS/MS) [39]. However, the quantification was always carried out with external calibrations coming from butane standards [40]. These procedures could give a satisfactory estimation of butane concentrations but the use of an internal standard would be of a great benefit, as it takes into account possible gas losses during sampling. 1,1,2-Trichlorotrifluoroethane, t-butanol and pentane/isobutanol were already used [34,38,40,41] but until now, no internal standard specific to butane was available. However, a first study led on methane and deuterated methane in situ generated was already performed [42]. The reaction between Grignard reagent methylmagnesium chloride ($CH_3MgCl$) and water (respectively deuterated water) can produce methane (and deuterated methane). Following the same technique, the use of butylmagnesium chloride ($C_4H_9MgCl$) instead of methylmagnesium chloride ($CH_3MgCl$) can lead to butane and deuterated butane.

* Corresponding author. Tel.: +41 795 566 293.
*E-mail address:* vincent.varlet@chuv.ch (V. Varlet).

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2012.11.031

The aim of our study was to validate an innovative HS-GC/MS method applicable to routine forensic work in toxicology laboratories for the determination of butane in biological matrices. The analytical protocol is fully described, which is subsequently applied to interpret butane concentrations found in biological matrices from autopsied cadavers linked to butane intoxication.

## 2. Materials and methods

### 2.1. Materials and reagents

Butylmagnesium chloride ($C_4H_9MgCl$) 2.0 M in tetrahydrofuran (THF) was purchased from Sigma–Aldrich (St. Louis, USA). Deuterated water was obtained from Cambridge Isotope Laboratories CIL Inc. (Andover, USA). All headspace extractions were carried out in headspace vials of 20 mL. Certified butane C106 cylinder from Camping gaz (Givisiez, Switzerland) was used as external control. Technical data sheet of C106 butane cylinder indicates a certified butane concentration of 32%.

### 2.2. Extraction method

Butane ($C_4H_9$) and deuterated butane ($C_4DH_9$) were generated separately in 20 mL headspace vials. Reactions of Grignard reagent with water and deuterated water are given below:

$$C_4H_9MgCl + H_2O \rightarrow C_4H_{10} + MgClOH \quad (1)$$

$$C_4H_9MgCl + D_2O \rightarrow C_4DH_9 + MgClOD \quad (2)$$

Due to the high reactivity of these reactions, it is important to proceed fastly (butylmagnesium chloride reacts with the water of ambient air) and safely (under hood). Grignard reagent and water are added without any contact in an aluminium cap with no septa and no hole, introduced in a headspace vial. The vial is rapidly and hermetically closed, and then vortexed to allow the reaction of butane generation at room temperature [42,57]. Precise volumes of gas ($C_4H_{10}$ and $C_4DH_9$) are sampled (automatically or manually) by a gas syringe through the vial septum and directly introduced in the GC injector.

### 2.3. GC/MS analysis

An Agilent 6890N GC (Agilent Technologies, Palo Alto, CA) combined with a headspace gas autosampler and equipped with an Agilent Select Permanent Gases column was used. This column is specially designed for gas analysis and is constituted of two capillary columns set in parallel: a molecular sieve 5 Å PLOT capillary column (10 m × 0.32 mm) and a Porabond Q (50 m × 0.53 mm). The temperature programme was as follows: 100 °C, held for 2 min, and raised at 10 °C/min to 250 °C; the injector (splitless mode) set to 100 °C and the interface MS temperature to 230 °C. Helium was employed as the carrier gas. The detection was performed with an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA), operating in the electron ionization (EI) mode at 70 eV. The selected ion monitoring (SIM) mode was used to acquire the $C_4H_{10}$ signal at $m/z$ 58 and 59 for $C_4DH_9$.

### 2.4. Calibration standards and controls

Five working calibration standards at concentrations corresponding to 6.30, 12.5, 25.0, 50.0 and 100 nmol of butane/mL of vial HS were prepared daily by reacting butylmagnesium chloride with water and respectively deuterated water.

Intermediate quality control samples were also prepared daily from the same reactions with the following concentrations: 15.0, 25.0 and 75.0 nmol of butane/mL of vial HS. For internal standard sampling, 100 μL of the working internal standard was sampled in a gas syringe resulting in a final concentration of 50.0 nmol of butane/mL of vial HS. For gas sampling, after sampling of internal standard in gas syringe, sampling of calibrators or real sample was performed with the same gas syringe. The different gases were mixed in the gas syringe and the total volume is therefore injected in GC injector. Butylmagnesium chloride was stored at +4 °C and deuterated water at room temperature while not in use.

### 2.5. Validation procedure

The validation procedure was performed according to the guidelines of the "French Society of Pharmaceutical Sciences and Techniques" (SFSTP) based on the following criteria: selectivity, response function (calibration curve), linearity, trueness, precision (repeatability and intermediate precision), accuracy, limit of detection (LOD) and limit of quantification (LOQ). Linearity was achieved with a minimal coefficient of determination that was above 0.998. The validation experiments were performed with calibration standards and control samples over 3 non-consecutive days ($p = 3$) and were not analyzed in the same week. The trueness was assessed by controls repetitions and an external control (certified gas cylinder containing butane at 32%).

## 3. Results and discussion

### 3.1. Selectivity of the method

The selectivity of the method was investigated by analysing butane in alkane and alkenes mixtures (Fig. 1). Several negative blood samples ($n = 10$) were analyzed as well as various samples from one autopsy such as kidney, lung, liver, bile, heart, muscle, urine, peripheral blood and cardiac blood. All these analyses were evaluated for co-eluting chromatographic peaks that might interfere with the detection of butane or deuterated butane. No interference peak was observed at the butane retention time and for the $m/z$ of 58, indicating that the method provides satisfactory selectivity for butane determination (Fig. 2).

### 3.2. Calibration curve for the method

Each point on the calibration curve was defined as the area ratio of butane to deuterated butane within a concentration range. Three assay calibration curves were performed for butane determination, prepared on 3 non-consecutive days ($p = 3$), over two weeks. Calibration standards were prepared at 5 ($k = 5$) concentration levels: 6.30, 12.5, 25.0, 50.0 and 100 nmol/mL of headspace, each in triplicate ($n = 3$). Calculated concentrations of each calibrator were compared to target values and were found to be within ±22%. A linear relationship was established between the butane

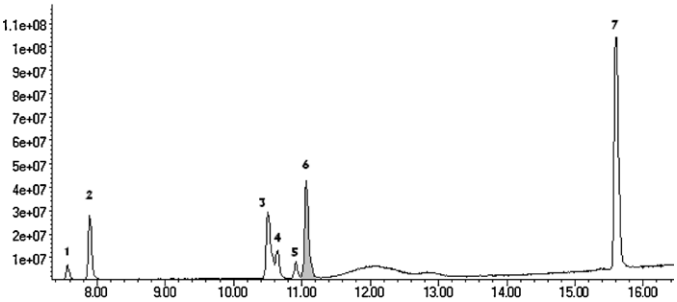


**Fig. 1.** TIC chromatogram of several alkanes and alkenes from external control susceptible to co-elute with butane (1: 1-propene, 2: propane, 3: isobutane, 4: 1-butene, 5: 2-methyl-1-propene, 6: butane and 7: tetrahydrofuran).

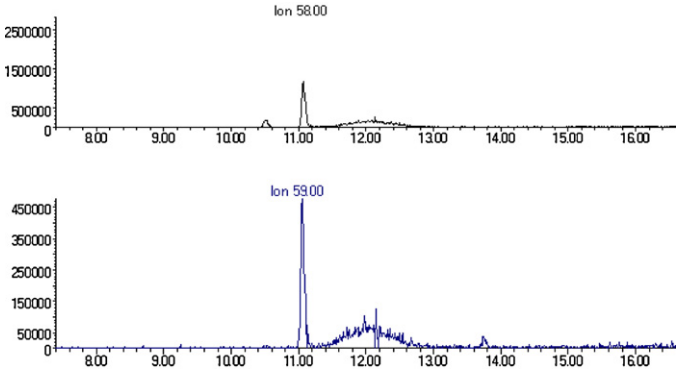


**Fig. 2.** Extracted ion chromatograms of butane ($m/z = 58$) and deuterated butane ($m/z = 59$) obtained from TIC chromatogram (from Fig. 1).

concentrations from butylmagnesium chloride and the measured response in the calibration range. The validation results for the calibration curves are compiled in Table 1.

The validation range was deliberately selected between 6.30 and 100 nmol/mL HS. Indeed, too weak butane concentrations do not give relevant information from a forensic point of view. As presented in Table 2, the lethal butane concentrations are comprised between 1.40 and 130 μg/g blood, 1.05 and 280 μg/g in brain, 0.38 and 310 μg/g in liver, 0.78 and 20.3 μg/g in lungs, 0.73 and 54 μg/g in kidney and around 5 μg/g in heart muscle. Consequently, a risk of "butane death" could occur from 21.0 nmol/g in blood, hence the choice of the validation range. If 1 g of biological matrix is sampled, the calibration range will cover the lowest butane concentrations found in cases related to butane death.

### 3.3. Linearity of the method

The linearity was assessed by fitting back-calculated concentrations of the control samples against the theoretical concentrations. Each non-consecutive day, control samples were measured at 3 concentration levels ($k = 3$) in triplicate ($n = 3$). The control

**Table 1**
Validation parameters of the butane measurement method.

| Calibration curve (6.30–100 nmol/mL HS vial) ($k = 5$, $n = 3$, $p = 3$) | | | |
|---|---|---|---|
| | Day 1 | Day 2 | Day 3 |
| Slope | 24.3 | 25.3 | 25.7 |
| Intercept | 0.35 | 0.39 | 0.40 |
| $r^2$ | 0.98782 | 0.99574 | 0.99551 |

| Linearity (6.30–100 nmol/mL HS vial) ($k = 3$, $n = 3$, $p = 3$) | |
|---|---|
| Slope | 0.9762 |
| Intercept | 0.0005 |
| $r^2$ | 0.9989 |

| Trueness (relative bias %) ($k = 3$, $n = 3$, $p = 3$) | |
|---|---|
| Levels (nmol/mL HS) | Trueness (%) |
| 15.0 | 0.4 |
| 25.0 | 0.4 |
| 75.0 | −1.7 |
| External control | |
| 72.0 | −1.0 |

| Precision (RSD %) ($k = 3$, $n = 3$, $p = 3$) | | |
|---|---|---|
| Levels (nmol/mL HS) | Repeatability | Intermediate precision |
| 15.0 | 1.22 | 2.58 |
| 25.0 | 0.28 | 0.83 |
| 75.0 | 0.08 | 0.08 |

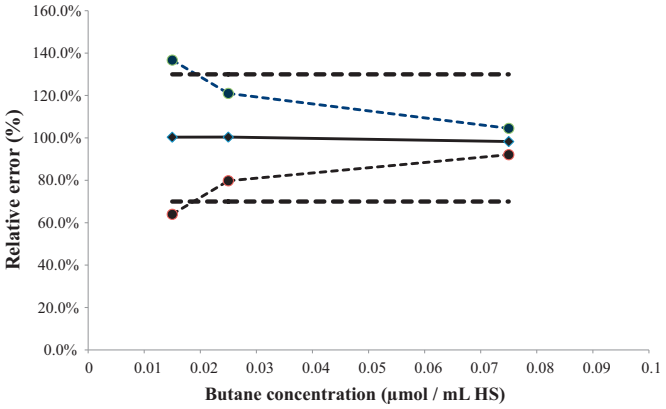


**Fig. 3.** Butane accuracy profile using a simple linear regression model within a range of 6.30–100 nmol/mL HS (continuous line: trueness, bold dashed lines: acceptance limits set at ±30%, dashed lines: lower and upper accuracy limits in relative values).

sample concentrations were calculated using a calibration curve determined for each measurement day. As presented in Table 1, good linearity was obtained with a slope value of 0.9762 and a coefficient of determination above 0.999 in the range of 6.30–100 nmol/mL HS.

### 3.4. Trueness of the method

Also called the bias, the trueness test expresses the closeness between the experimental average value and the accepted reference value. This test detects systematic errors and is expressed as a percent deviation from the accepted reference value. Several daily repetitions of control samples were analyzed over several weeks at their respective concentrations, which were used to establish a true value for each concentration. An additional trueness evaluation was performed using an external quality control of certified gas butane. As shown in Table 1, trueness was found to be lower than the acceptance criteria (within ±15% of the accepted reference value and within 20% at LLOQ, 19.0 nmol of butane/ml HS vial). In fact, trueness was measured within ±3% of the accepted reference value in the considered range (0–100 nmol/mL HS vial) and was consequently satisfactory for butane analysis. The evaluation of trueness with the external quality control of certified butane was performed at a concentration of 72.0 nmol/mL HS vial. Six repetitions were done on two different days and have led to a mean trueness measured at −1% of the target value.

### 3.5. Precision (repeatability and intermediate precision) of the method

Precision was assessed by calculating the repeatability (intra-day precision) and intermediate precision (inter-day precision) for each control sample concentration. The repeatability variance was estimated by calculating the intra-days variance ($S_r^2$) and the intermediate precision variance was estimated by adding the intra- and inter-day variances ($S_{IP}^2$). As shown in Table 1, the relative standard deviation values for repeatability and intermediate precision were between 0.08 and 2.58%.

### 3.6. Accuracy and LOQ of the method

The accuracy expresses the total error defined by the sum of trueness (systematic error) and precision (random error). The accuracy profile given in Fig. 3 shows the ability of the method to provide an analytical result using systematic and random errors

**Table 2**
Concentrations of butane in various samples from lethal cases in which butane was implied in gaseous mixture or alone.

| Butane concentrations (μg/g) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Blood | Brain | Liver | Lung | Kidney | Heart muscle | Spleen | Urine | Gastric content | Fat tissue | Administration | References |
| 1.76 | 2.03 | 3.46 | | 1.16 | | | | | | Lighter refill inhalation | [43, 44] |
| 0.65 | | | | | | | | | | Butane inhalation (antiperspirant aerosol) | [43, 45] |
| 0.85 | | | | | | | | | | Charcoal lighter fluid inhalation (butane 75-89 %) | [40] |
| 4.3 | 13 | 4.5 | 4.4 | 2.1 | | 1.71 | 0.16 | 0.77 | | Propane bottle inhalation | [46] |
| 0.3 | 0.57 | 1.03 | 0.35 | 0.38 | | | | | | Cigarette lighter oil inhalation | [47] |
| | 0.3 | 0.51 | | | | | | | | Liquefied petroleum gas inhalation | [47] |
| 0.12 (PB) / 0.10 (CB) | 0.08 | 0.16 | 0.06 | 0.09 | | | | | 8.34 | Isobutane inhalation | [48] |
| 0.09 (PB) / 3.56 (CB) | 7.28 | | 6.55 | | | | | | 9.56 | Butane inhalation (antiperspirant aerosol) | [48] |
| | 0.31 | | 0.19 | | | | | | | Butane/gasoline explosion | [48] |
| 0.15 | 0.44 | 0.48 | 0.03 | | | | | | 1.76 | Gas cartridges inhalation (50 % propane, 50 % butane) | [38] |
| 0.75 (CB) | | | | | | | | | | Gas cartridges inhalation (40 % propane, 60 % butane) | [49] |
| 18.36 (PB) | | | | | | | | | | Butane gas inhalation | [50] |
| 18.9 | 25.9 | 27.5 | | | | | | | | Lighter refill inhalation | [51] |
| 2.54 | 10.56 | 5.4 | 1.65 | 5.67 | | | | | | Lighter refill inhalation | [52] |
| 41.3 | 35.9 | 71.8 | 20.3 | 36.7 | | | | | | Butane inhalation (antiperspirant aerosol) | [43] |
| 129 | 282 | 310 | | 54 | | | | | | Butane inhalation | [46] |
| 1.4 | 1.05 | 0.38 | 0.78 | 0.73 | | | | | | Butane inhalation | [47] |
| 2.02 (PB) / 1.94 (CB) | 17.29 | | 0.93 | | 4.95 | | | | | Butane inhalation | [48] |
| 6.48 | 8.1 | | 2.97 | | | | | | | Butane inhalation | [53] |
| 7.83 | | | | | | | | | | Butane inhalation | [54] |
| 24.84 | 56.7 | | 8.1 | | | | | | | Butane inhalation | [55] |
| | 65.07 | | | | | | | | | Butane inhalation | [56] |
| | 10.8 | | 2.7 | | | | | | | Butane inhalation | [56] |

Butane concentration ranges in lethal cases (μg/g) (butane identified as the unique compound in the gaseous mixture and responsible of the death)

| Blood | Brain | Liver | Lung | Kidney | Heart muscle | Spleen | Urine | Gastric content | Fat tissue |
|---|---|---|---|---|---|---|---|---|---|
| 1.4–129 | 1.05–282 | 0.38–310 | 0.78–20.3 | 0.73–54 | 4.95 | | | | |

PB: peripheral blood, CB: cardiac blood.
In grey: cases where butane was identified as a compound present in the gaseous mixture.
In white: cases where butane was identified as the unique compound in the gaseous mixture and responsible of the death.

with a risk of $\alpha = 5\%$ at each concentration level. The mean bias (%) confidence interval limits for the control samples were within the ±30% acceptability limits typically allowed by Swiss forensic laboratories.

With a threshold of 30% as the acceptability limit, the lower limit of quantification (LLOQ) of butane was set to 19.0 nmol/mL HS vial.

### 3.7. Limit of detection (LOD) of the method

The LOD was determined by headspace extraction of blank samples containing water and butylmagnesium chloride in order to reach a butane concentration of 500 nmol/mL HS. Several dilutions of the headspace in air were performed and the LOD was assessed

using a signal-to-noise ratio of $S/N > 3$. The noise was estimated by measuring more than 10 blank samples. As a result, the LOD for butane quantification was estimated to be 3.5 nmol/mL of vial HS.

### 3.8. Real cases from literature

The nature of sample (blood or tissue) and its state (solid, liquid, putrefied) are not really important because butane can easily be extracted from the sample with a temperature above butane boiling point (close to 0 °C). As previously mentioned, the critical point is to avoid loss of butane during sampling. The initial matrix amount is not a crucial parameter but must be sufficient to generate a butane signal above LOD, this being the reason why butane concentration is expressed in μmol/mL HS, but could eventually be then expressed in μg/g.

It is also necessary to combine the different concentrations obtained in several samples to interpret the butane exposure. Indeed, due to its lipophily, butane will be preferentially stored in fat tissue in case of long butane exposure and death by anoxia. But in case of butane exposure consecutively to a butane outburst, it is reasonable to think that butane concentration in lungs should be higher than in fat tissue or brain, because of a very short gas exposure. Moreover, due to its volatility, butane evaporation and post mortem redistribution can cause important variations (increase or decrease).

According to the initial health state of the victim, the agonal/survival period, possible reanimation, the context of butane exposure (gas outburst, anoxia, use of sniffing bag), butane concentration can vary in the different organs. The role played by butane in a lethal intoxication must be ponderated by all these parameters, hence the necessity to combine all different results obtained in several matrices for the same case when they are available.

It is very difficult to assess lethal butane concentrations. The main lethal cases with butane measurement available in the literature have been listed in Table 2 and have been expressed in μg/g of sample. Butane concentration (alone or in gaseous mixtures) ranges from 0.09 to 129 μg/g in blood ($n = 22$), from 0.08 to 282 μg/g in brain ($n = 18$), from 0.16 to 310 μg/g in liver ($n = 11$), from 0.03 to 20.3 μg/g in lungs ($n = 13$), from 0.09 to 54.0 μg/g in kidney ($n = 8$), close to 5 μg/g in heart muscle ($n = 1$), close to 1.71 μg/g in spleen ($n = 1$), close to 0.16 μg/g in urine ($n = 1$), close to 0.77 μg/g in gastric content ($n = 1$) and from 1.76 to 9.56 μg/g in fat tissue ($n = 3$). These thresholds were established in lethal cases where butane was involved in the gaseous mixture inhaled. However, in cases directly linked to solely butane inhalation, the minimal butane concentration is higher whatever the matrix (Table 2).

## 4. Conclusion

A selective and sensitive method for the identification and quantification of butane in postmortem samples was presented. This method offers a new opportunity of butane measurement in forensic sciences, particularly for postmortem cases when the samples are often of low quality. The principle of stable labelled isotope generation from Grignard reagent can be extended to all the alkanes, using the respective alkylmagnesium chloride. The technique was validated according to the guidelines of the French Society of Pharmaceutical Sciences and Techniques (SFSTP). This method allows an accurate and reliable measurement (±30%) of butane concentrations in a range of 19–100 nmol/mL HS. The method is not time-consuming and is safe because the generation of butane takes place in a hermetically closed headspace vial. The method also provides a precise quantification because deuterated butane is used as internal standard from butylmagnesium chloride. This is especially useful in cases where only a small amount of tissue is available.

The method described herein was evaluated satisfying to provide reliable, accurate and repeatable butane results in a short time period and from various samples (blood, tissue, etc.) whatever their state (putrefied, solid, and liquid). This is the first time that the high reactivity of Grignard reagents towards water is used to generate gaseous alkanes.

## Acknowledgments

The authors would like to thank the entire team at the Forensic Toxicology and Chemistry Unit, especially Dr Christèle Widmer from Geneva for discussion about butane related autopsy cases.

## References

[1] B.L. Zhu, T. Ishikawa, S. Oritani, L. Quan, D.R. Li, D. Zhao, T. Michiue, M. Ogawa, H. Maeda, Chudoku Kenkyu 18 (1) (2005) 77.
[2] V. Živković, F. Juković, S. Nikolić, Srp. Arh. Celok Lek. 138 (5–6) (2010) 376.
[3] M.J. De Luis, Y. Garcia, V. Uroz, O. Fernandez, J.A. Sanchez, Toxicol. Lett. 196S (2010) S292.
[4] S. Perotti, M. Bernini, Fifth Cross Channel Conference on Forensic Medicine, 1995, p. 2.
[5] F. Musshoff, S.A. Padosch, L.A. Kroener, B. Madea, Am. J. Forensic Med. Pathol. 27 (2) (2006) 188.
[6] A. Gross, M. Kłys, Arch. Med. Sadowej Kryminol. 52 (1) (2002) 37.
[7] R. Rossi, F. Suadoni, L. Pieroni, F. De Giorgio, M. Lancia, J. Forensic Sci. (2011), http://dx.doi.org/10.1111/j.1556-4029.2011.02003.x.
[8] T. Fukunaga, H. Yamamoto, A. Tanegashima, Y. Yamamoto, K. Nishi, Forensic Sci. Int. 82 (1996) 193.
[9] J. Nowicka, J. Kulikowska, M. Korczyńska, R. Celiński, C. Chowaniec, Arch. Med. Sad. Kryminol. LXI (2011) 43.
[10] H. Pfeiffer, M. Al Khaddam, B. Brinkmann, H. Köhler, J. Beike, Int. J. Legal Med. 120 (2006) 168.
[11] E. Siegel, S. Wason, N. Engl. J. Med. 323 (23) (1990) 1638.
[12] T.P. Rohrig, Am. J. Forensic Med. Pathol. 18 (3) (1997) 299.
[13] B. Mathew, E. Kapp, T.R. Jones, Br. J. Addic. 84 (5) (1989) 563.
[14] M. Hirt, A. Brzobohatá, I. Dvořáček, M. Stańková, Soud. Lék. 49 (4) (2004) 54.
[15] A.A. El-Menyar, M. El-Tawil, J. Al Suwaidi, Eur. J. Emerg. Med. 12 (2005) 137.
[16] M. Khatouf, B. Ifkharen, M. Drissi, B. Housni, M. Harandou, N. Kanjaa, Ann. Fr. Anesth. Reanim. 23 (11) (2004) 1080.
[17] S. Prasad, R. Singh, R. Manocha, M. Narang, B.D. Sharma, P. Rajwanshi, B. Gupta, J. Assoc. Physicians India 57 (2009) 472.
[18] R.C. Kamm, Forensic Sci. 5 (1) (1975) 91.
[19] F. Girard, S. Le Tacon, M. Maria, O. Perriard, P. Monin, Ann. Fr. Anesth. Réanim 27 (2008) 83.
[20] K. Godlewski, B. Werner, M. Sterliński, M. Pytkowski, H. Szwed, M. Domagała, L. Koc, Kardiol. Pol. 64 (3) (2006) 305.
[21] M.J. Roberts, R.A. McIvor, A.A. Adgey, Br. J. Hosp. Med. 44 (4) (1990) 294.
[22] A.H. Douglas, L. Marienberg, N.Y. State J. Med. 49 (23) (1949) 2845.
[23] A.A.J. Adgey, P.W. Johnston, S. McMechan, Resuscitation 29 (1995) 219.
[24] D.R. Williams, S.J. Cole, Resuscitation 37 (1998) 43.
[25] A.H. De Naeyer, S.W. De Kort, M.C. Portegies, D.J. Deraedt, C.M. Buysse, Ned. Tijdschr. Geneeskd 155 (34) (2011) A3443.
[26] J. O'Neill, C. McCarthy, Ir. Med. J. 92 (4) (1999) 344.
[27] J. Gunn, J. Wilson, A.F. Mackintosh, Lancet 1 (8638) (1989) 617.
[28] J. Rieder-Scharinger, R. Peer, W. Rabl, W. Hasibeder, W. Schobersberger, Wien Klin. Wochenschr. 112 (24) (2000) 1049.
[29] C.L. Winek, W.W. Wahba, R.M. Huston, J. Anal. Toxicol. 21 (1997) 323.
[30] S. Kischkel, W. Miekisch, P. Fuchs, J.K. Schubert, Eur. Respir. J. (2012), http://dx.doi.org/10.1183/09031936.00125411.
[31] F. Hartmut, T. Hintze, D. Bimboes, H. Remmer, Toxicol. Appl. Pharmacol. 56 (3) (1980) 337.
[32] J. King, H. Koc, K. Unterkofler, et al., J. Theoret. Biol. 267 (2010) 626.
[33] B.M. Ross, S. Shah, M. Peet, O. J. Psych. 1 (2011) 1.
[34] J. Park, J.S. Min, S. Heo, M.A. Lim, S.W. Park, Forensic Sci. Int. 151 (2005) 165.
[35] R. Gagliano Candela, B.M. Altamura, M. Colonna, Boll. Soc. Ital. Biol. Sper. 55 (1979) 38.
[36] G.C. Rhoderick, J. Chromatogr. A 1017 (2003) 131.
[37] A.V. Kirichek, L.A. Rassinskaya, L.V. Shirokova, E.A. Simonov, Sud Med. Ekspert. 52 (3) (2009) 21.
[38] C. Jackowski, W. Römhild, B. Aebi, W. Bernhard, D. Krause, R. Dirnhofer, Am. J. Forensic Med. Pathol. 26 (4) (2005) 355.
[39] M. Jungheim, H. Kijewski, Arch. Kriminol. 215 (3–4) (2005) 103.
[40] M.P.L.A. Bouche, W.E. Lambert, J.F.P. Van Bocxlaer, M.H. Piette, A.P. De Leenheer, J. Anal. Toxicol. 26 (2002) 35.
[41] I. Novosel, Z. Kovačić, S. Gusić, L. Batelja, M. Nestić, S. Seiwerth, J. Škavić, J. Forensic Legal Med. 18 (2011) 125.
[42] V. Varlet, M. Augsburger, Int. J. Legal Med. (2012), http://dx.doi.org/10.1007/s00414-012-0726-2.
[43] M. Ago, K. Ago, M. Ogata, Legal Med. 4 (2002) 113.

[44] T. Aoki, H. Namiki, N. Fukuie, Act. Crim. Japon. 48 (1982) 202.
[45] L.M. Al-Alousi, Med. Sci. Law 29 (1989) 189.
[46] A. Graefe, R.K. Müller, R. Vock, H. Trauer, H.J. Wehran, Arch. Kriminol. 203 (1999) 27.
[47] H. Sugie, C. Sasaki, C. Hashimoto, H. Takeshita, T. Nagai, S. Nakamura, M. Furukawa, T. Nishikawa, K. Kurihara, Forensic Sci. Int. 143 (2004) 211.
[48] W. Römhild, E. Logemann, G. Schmitt, A. Alt, K. Krebs, H. Bartels, I. Pedal, U. Schmidt, N. Beck, D. Krause, Beitraege zum XIII symposium der gesellschaft für toxikologie und forensische chemie, Mosbach, 2003, p. 109.
[49] F. Wehner, D. Benz, H.D. Wehner, Arch. Kriminol. 209 (5–6) (2002) 164.
[50] N. Tanaka, H. Kinishita, R. Haba, M. Jamal, E. Ohkubo, K. Ameno, Soud. Lék. 55 (4) (2010) 44.
[51] M. Terada, T. Watanabe, H. Kashiwade, S. Yoshimura, Jpn. J. Legal Med. 37 (1983) 696.
[52] K. Nishi, N. Ito, J. Mizumoto, K. Wada, T. Yamada, Y. Mitsukuni, S. Kamimura, Jpn. J. Legal Med. 39 (1985) 214.
[53] C. Fuke, T. Miyazaki, T. Arao, Y. Morinaga, H. Takaesu, T. Takeda, T. Iwamasa, Legal Med. 4 (2002) 134.
[54] T. Nagata, M. Kageura, K. Hara, K. Totoki, S. Komura, S. Oshiro, Jpn. J. Legal Med. 31 (1977) 161.
[55] F. Kawata, A. Ito, T. Takeshita, M. Ito, Jpn. J. Forensic Toxicol. 18 (1990) 68.
[56] S. Oritani, B.L. Zhu, K. Ishida, L. Quan, M. Taniguchi, M. Fujuta, K. Fukita, H. Maeda, Jpn. Forensic Toxicol. 19 (2001) 257.
[57] V. Varlet, E. Lagroy De Croute, M. Augsburger, P. Mangin, J. Chromatogr. B 880 (2012) 125.