Portable mass spectrometry for measurement of anaesthetic agents and methane in respiratory gases

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Abstract

Monitoring the composition of gases breathed by anaesthetised patients requires measurement methods with fast responses, high accuracy and good reliability. There is also an increasing demand for systems to be able to monitor more than one target analyte simultaneously, but some gas analysers can be sensitive to the presence of methane gas in exhaled breath, consequently leading to inaccurate measurements of the anaesthetic agent. This study investigated the feasibility of employing portable quadrupole mass spectrometry to monitor volatile anaesthetic agents (halothane, isoflurane and sevoflurane), methane accumulation in anaesthetic rebreathing systems, and inspired and exhaled carbon dioxide and oxygen concentrations during equine anaesthesia in a clinical setting. The volatile anaesthetic agents were easily measurable and methane was detectable. The instrument had an advantage over short wavelength infrared absorption spectrometry analysers because it could monitor anaesthetic agents and other respiratory gases simultaneously and at extremely low concentrations, although further optimisation is required.
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Introduction

The ability to monitor the physiological or pathological condition of a patient is a constant challenge faced by scientists and clinicians. On-line patient monitoring is essential in many clinical scenarios for example, during anaesthesia or in high-dependency care. The exceptional sensitivity and wide dynamic range of mass spectrometry (MS) make it an ideal technology for analyte monitoring where high accuracy and sensitivity are required. However, high cost and large size have previously confined its use to specialist laboratories.

Recently, miniature and microengineered mass spectrometers have been developed capable of operating at higher pressures than was previously possible (Badman and Cooks, 2000; Taylor et al., 2001). The small size and lower cost of these instruments opens up the opportunity of several diverse stand-alone applications. It is now possible to provide individual, real-time breath analysis in clinics and hospitals. In addition, the flexibility of MS means that one set of apparatus can be used to measure multiple unrelated analytes with little modification or reprogramming.

Exhaled gas analysis during anaesthesia is an obvious and familiar example of patient monitoring requiring immediate, accurate measurement of several analytes simultaneously. Many anaesthetic gas analysers employ differential infrared (IR) absorption techniques to measure carbon dioxide and the volatile anaesthetic agents. These analysers are relatively low cost, simple to use and can provide breath-by-breath waveform displays and numerical

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values for inspired and end-tidal gas concentrations. However, they are often limited in sensitivity, i.e. around 0.1–0.2% for CO_2 and other volatile agents at best (Technical specification for Datex Ohmeda Capnomac Ultima; McPeak et al., 1988).

These machines typically utilise either IR absorption spectrometry (using monochromatic or polychromatic IR wavelengths). Monochromatic and polychromatic IR spectrometry analysers generally use IR wavelengths centred around 3.3 μm; whereas photoacoustic analysers use IR wavelengths in the band 10.3–13.0 μm (Walder et al., 1993). Gaseous species, notably methane, which have absorption spectra overlapping those of the anaesthetic agents, may accumulate during low-flow or closed system anaesthesia. This is a particular problem in IR gas analysers employing the shorter wavelengths, yielding erroneous measurements of anaesthetic agent concentrations. This is a well known difficulty with halothane and, to a lesser extent, isoflurane and has been documented in human anaesthesia (Morita et al., 1985; Rolly et al., 1990, 1994; Versichelen et al., 1996).

It is far more of a problem in animals with an intestinal fermentation compartment producing large amounts of methane such as cattle, sheep and horses (Taylor, 1990; Moens et al., 1991; Moens and Gootjes, 1993; Dujardin et al., 2005), which is then excreted via the lungs (Dougherty et al., 1962, 1964; Sasaki et al., 1999). Falsely high end-tidal anaesthetic agent readings are likely to cause the anaesthetist to reduce the concentration of inspired anaesthetic agent resulting in the patient becoming light under anaesthesia. Besides being detrimental for the patient, this can also be dangerous for the surgical/anaesthetic team in the case of veterinary anaesthesia where large animal such as horses may be involved.

Exhaled pentane or ethane: biomarkers of inflammation/oxidative stress (de Jongste and Alving, 2000; Wyse et al., 2004), products of volatile anaesthetic agent interaction with soda lime or products of hepatic metabolism such as isobutene (Sharp et al., 1979; Hempel et al., 1980) may also accumulate during low-flow or closed system anaesthesia. These can also interfere with measurement of anaesthetic gases but methane appears to be the major culprit during equine anaesthesia (Moens et al., 1991).

Methane is excreted in the exhaled gases in proportion to its rate of production by methanogenic bacteria in the gastrointestinal tract and its absorption from there (Bond et al., 1971; Sasaki et al., 1999). While all ruminants are methane producers (methanogenic bacteria are present mainly in the rumen), the case is not so clear cut with humans. Humans (monogastrics) can be divided into 'non-producers' or 'producers', which have methanogenic bacteria present in the distal colon; but the quantity of methane exhaled by producers varies widely (Bond et al., 1971), probably due to differences in bowel flora (Morita et al., 1985). Morita et al. (1985) monitored the accumulation of methane, acetone and nitrogen in inspired gas dur-

ing closed circuit anaesthesia using gas chromatography–mass spectrometry (GC–MS). Extraneous sources of methane were also considered, e.g., methane is present in urban atmosphere but at low levels (typically <4 ppm). One patient showed an increase to 229 ppm after 72 min, which was attributed to bowel production. It has been suggested that this rate of accumulation of methane over a 14 h period would result in flammable concentration levels, although other workers have disagreed (Baumgarten and Reynolds, 1985).

Horses, which are monogastric but also hindgut fermentors, tend to exhale methane. Like human producers, the amount exhaled varies widely. This is thought to depend upon age, diet and environmental factors (Bond et al., 1971; Sasaki et al., 1999). The variable, unpredictable, nature of methane production greatly complicates anaesthetic agent monitoring, because standard corrections cannot be applied.

While mass spectrometry has been used for monitoring respiratory and anaesthetic gases in experimental rats (Larach et al., 1988) and using large, central multiplexed systems in both human and equine hospitals (Ozanne et al., 1981; A. Dugdale, unpublished data) patient-side, multi-gas mass spectrometry has not previously been described during routine equine anaesthesia.

Mass spectrometers work by ionising different molecular species present in the gas sample and then accelerating and deflecting the ionised species in an electromagnetic or radiofrequency field so that their impingement on a detector is determined by their mass-to-charge (m/z) ratio. Several types of mass spectrometer are available, varying in method of ionisation and types of mass analyser and detector; e.g. magnetic sector, time-of-flight and quadrupole.

Quadrupole mass spectrometers (QMS) have a simple four rod electrode structure, to which a combination of radio frequency AC (V) and DC (U) voltages are applied. For a particular voltage combination (U/V) only ions of a specific mass-to-charge ratio (m/z) value form stable trajectories and are able to successfully pass down the length of the analyser to the detector: the QMS electrode system therefore acts as a mass filter. As such the QMS is a relatively simple and low cost mass spectrometer. Mass spectrometry has developed significantly (Svec, 1985), since the first published description of a quadrupole instrument in 1953 (Paul and Steinwedel, 1953). A substantial amount of work has gone into design improvements including electrode geometry, detector technology and more recently, miniaturisation (Taylor et al., 2001). Design improvements have been aided by computer simulation of ion paths and interactions with the analyser (Gibson and Taylor, 2003). Larger molecular species in a gas sample can fragment in the QMS producing smaller ionised species with characteristic fragmentation patterns. The degree of fragmentation is dependent upon the ionisation energy used.

On fragmentation, different species can yield ions with the same m/z ratio, which are indistinguishable at low-mass resolutions. For example, nitrous oxide, carbon dioxide

and nitrogen can yield different singly charged ions all with nominal mass 28 (N_2^+; CO^+, N_2^+, respectively). This is one possible source of error when employing mass spectrometry for anaesthesia monitoring (Beatty, 1984). The problem can be circumvented by choosing a characteristic high mass/non-overlapping fragment peak or operating at high resolution ($m/\Delta m > 1000$), or overcome by the application of chemometrics (multivariate analysis). Similarly water vapour and background gases present in high concentration need to be resolved from the sample gases (Beatty, 1984). The fact that QMS in not limited to monitoring a pre-selected molecule or reagent provides the flexibility to redesign monitoring protocols 'on-the-fly' accounting for these complicating factors.

QMS traditionally operates at low pressures (e.g., 10^{-4}–10^{-6} mbar) since non-linearity of signal is observed at higher operating pressures. The pressure of the sample (at the inlet) is inevitably much higher than this, so the inlet system must bridge this pressure gap and allow the sample into the vacuum system at a controlled rate such that the integrity of the vacuum is maintained. On-line methods require continued sampling and the particular inlet type depends on the application. Miniature and micro versions of the QMS are capable of operating at much higher pressures than traditional QMS, reducing the need for highly sophisticated (large and expensive) vacuum pumps, increasing the flexibility of the system and its utility in stand-alone applications such as bedside (or operating theatre) on-line patient monitoring.

This study set out to determine the efficacy of patient-side use of miniature, portable QMS for monitoring equine anaesthesia using typical anaesthetic agents: halothane, isoflurane and sevoflurane, delivered in oxygen via a semi-closed breathing system. Horses undergoing elective and emergency surgery (including colic) were included. The aim was to use the portable QMS to: (1) establish suitable mass spectral peaks generated by commonly used anaesthetic gases; (2) establish the extent of methane interference with anaesthetic measurement by short wavelength IR; (3) optimise the portable QMS instrument for on-line anaesthetic monitoring applications and demonstrate quantitative monitoring capability; (4) analyse the composition of inhaled and exhaled respiratory gases, and (5) identify and quantify methane production.

Materials and methods

Instrumentation

Instruments used were a Pfeiffer QS422 QMS enclosed in a portable vacuum system (Fig. 1a) and a larger laboratory-based QMS (MKS Spectra Minilab). For the portable instrument, sampling was via a 2 m long × 0.5 mm diameter unheated capillary inlet to the vacuum system operating at a pressure of $\sim 1 \times 10^{-6}$ mbar. The sampling rate was approximately 20 mL/min. When sampling in this manner there is a two stage drop in pressure, firstly from atmosphere to 10^{-2} mbar. This first vacuum was provided by a rotary vacuum (roughing) pump. High vacuum ($\sim 10^{-6}$ mbar) was achieved using a turbo-molecular pump, a frit or
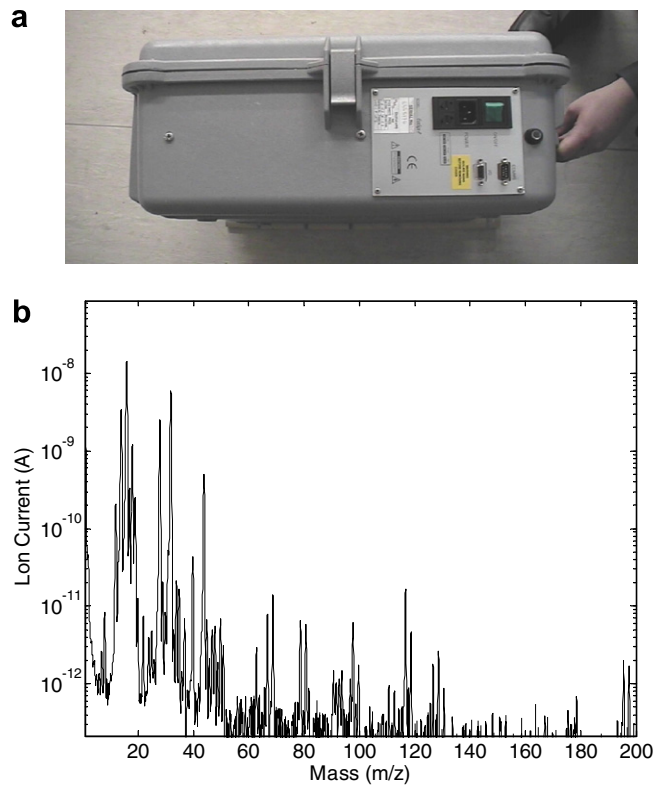

Fig. 1. (a) Field portable miniature quadrupole mass spectrometry (QMS) and vacuum system (b) Analogue mass spectra (ion current versus m/z) recorded during anaesthesia contaminated with the typical air constituents N_2, O_2, Ar, CO_2.

molecular leak separated the 10^{-2} and 10^{-6} mbar pressure regions and maintained vacuum integrity.

For breath-by-breath monitoring the scans were collected as MID (multiple ion detection) spectra. In this mode of operation the instrument monitors the maximum values of selected mass spectral peaks with time, jumping from one peak maximum to the next. The time interval over which the instrument monitors each peak can be varied at discrete points and was defined by the operating software from 10 ms to 10 s. This determines the maximum number of data points recorded per minute.

Sampling from the patient

Anaesthetic gases were sampled from a site in the endotracheal tube (near the incisor arcade) of 23 horses undergoing anaesthesia for a mixture of elective and emergency procedures (see Table 1).

Anaesthetic system

Maintenance of anaesthesia was with halothane, isoflurane or sevoflurane in oxygen, delivered through the endotracheal tube from a semi-closed circle system (oxygen flow of ca. 10 mL/kg/min). The QMS was initially switched on about 10 min in advance of each anaesthetic in order for the vacuum level to stabilise. By sampling room air and monitoring the m/z 28 (N_2) and m/z 32 (O_2) ratio the QMS was thereby approximately 'calibrated' before each patient test.

Calibration of IR and QMS

The outputs of the QMS and a monochromatic (3.3 μm) IR absorption analyser (Datex Ohmeda Capnomac Ultima), were both tested in the laboratory during stepwise increases in volatile anaesthetic agent delivery

Table 1
Details of equine patients monitored

Age (years)	Breed	Sex	Weight (kg)	Surgery	Emergency? (Y/N)	Starved? (Y/N)	Volatile agent
13	New Forest	M/N	352	Cataract phacoemulsification	N	Y	I
14	TB × ID	M/N	601	Sarcoid treatment	N	Y	S
8	Cob	M/N	~520	Foot canker debridement	N	Y	H
1		M/N	350	Septic joint arthroscopy	Y	N	H
13	New Forest	M/N	352	Enucleate eye	N	Y	S
9	Holstein	F	~500	Colic	Y	N	I
2	TB	M/N	~300	Tenoscopy	N	Y	H
9	Irish Sports	M/N	527	Conjunctival pedicle graft	Y	N	H
9	TBX	M/N	570	Sarcoid treatment	N	Y	H
9	TBXID	M/N	580	Sarcoid treatment	N	Y	H
25	TBX	M/N	480	Penile amputation	N	Y	S + IVA
9	TB	F	520	Colic	Y	N	I
29	TBX	F	464	Enucleation	N	Y	S
7	TBX	M/N	568	Colic	Y	N	S
12	Cob	M/N	516	Ocular squamous cell carcinoma treatment	N	Y	H
13	TBX	M/N	512	Third eyelid removal	N	Y	I
15		M/N	575	Colic	Y	N	H
2	TBX	F	557	Limb wound debridement	Y	Y	H
9	Warmblood	M/N	620	Colic	Y	N	I
2	TBX	M/N	466	Spermatic cord resection	N	Y	TIVA
2	TBX	M/N	435	Arthroscopy	N	Y	H
18	TBX	M/N	547	Colic	Y	N	I
4	TBX	M/N	571	Colic	Y	N	I

Key: TB, Thoroughbred; ID, Irish Draught; H, halothane; I, isoflurane; S, sevoflurane; IVA, intravenous anaesthesia; TIVA, total intravenous anaesthesia.

into a stream of oxygen and carbon dioxide. A Boyle International II anaesthetic machine with calibrated rotameter-type flow meters was used to deliver the measured flow of oxygen and carbon dioxide to a Mapleson D non-rebreathing system connected to an endotracheal tube with a sealed distal tip. Gases were sampled from needles placed through the endotracheal tube wall so that their points lay within the tube lumen near the connection with the breathing system, but spaced 6.5 cm apart to reduce the likelihood of sampling errors. Tec 3 halothane and isoflurane vaporisers and a Penlon Sigma Delta sevoflurane vaporiser were used to deliver their respective anaesthetic vapours into the carrier stream of oxygen and/or carbon dioxide. All vaporisers were serviced annually and checked for accuracy of output. Only calibrated positions on each vaporiser dial were used for the analyses. With this in vitro set-up, using a 'fresh gas flow' of 5 L/min, 2–3 min were found to be sufficient for stabilisation between stepwise changes. This was judged by the time taken for IR machine displayed values to stabilise and QMS readings to plateau; and was also checked against time constant calculations.

A commercially prepared gas mixture containing 1% methane, 25% carbon dioxide, 25% oxygen with 49% nitrogen, was used in later laboratory-based calibration experiments, to compare the Datex Ohmeda Capnomac Ultima (Datex Ohmeda, GE Healthcare) with the Datex Ohmeda S/5 compact anaesthesia monitor (Datex Ohmeda), for possible interference with volatile anaesthetic agent measurement by methane.

Results

Establish suitable mass spectral peaks generated by the anaesthetic gases

Table 2 shows the anaesthetic agents monitored and the corresponding m/z values for their molecular ions and their fragments. Analogue mass spectra were collected to identify suitable fragment peaks for on-line monitoring and to ensure correct tuning of the instrument at low and high mass ranges over prolonged periods. Fig. 1b shows the ana-

Table 2
Anaesthetic agents and corresponding peak intensities of their molecular ions and their fragments

	Halothane	Isoflurane	Sevoflurane
	F H | | F – C – C – Br | | F Cl	F H F | | | F – C – C – O – C – H | | | F Cl F	F CF₃ H | | | F – C – C – O – C – F | | | F H H
M_r	197.38	184.49	200.5
Other fragment peaks	CF_3^+ (69)	CHF_2^+ (51)	$C_3H_3F_4O^+$ (131)
	CF_3CCl^+ (117)	CF_3CCl^+ (117)	CF_3^+ (69)
	CF_3CClBr^+ (196)		

logue mass recorded during anaesthesia. For the portable QMS (Pfeiffer QS422) the output (from the detector) is displayed as a 'raw' ion current: this can be converted to a partial pressure using an appropriate scaling factor (e.g., as is the case for the MKS Spectra Minilab QMS) or after calibration against a known gas reference standard. The samples were continuously taken from the endotracheal tube, so the spectra contained the air constituents: H_2O, N_2, O_2, Ar and CO_2 which may be seen in the lower mass regions. Levels of CO_2 in ambient air are subject to variations during the course of a day and in a busy operating theatre elevated levels of CO_2 are normally present.

Establish the extent of methane interference with anaesthetic measurement by IR

Traditional IR monitors that use short IR wavelengths (e.g., Datex Ohmeda Capnomac Ultima) can be affected by the presence of methane in respiratory gases. This was verified using a standard gas mixture containing atmospheric species and 1% methane gas with this monitor. Fig. 2 shows halothane measurements in the presence and absence of methane. Clearly when methane was present even with no anaesthetic agent present (vaporiser setting of zero), the IR instrument indicated a high percentage value (false positive) for halothane. A lower false positive was noted when isoflurane was used, and lower still when sevoflurane was the agent being monitored.

Optimise the portable QMS instrument for on-line anaesthetic monitoring applications and demonstrate quantitative monitoring capability

Fig. 3 shows the calibration of the portable QMS against vaporiser output. Each of the anaesthetic agents (halothane, isoflurane and sevoflurane), was monitored as its respective vaporiser setting was incremented and decre-
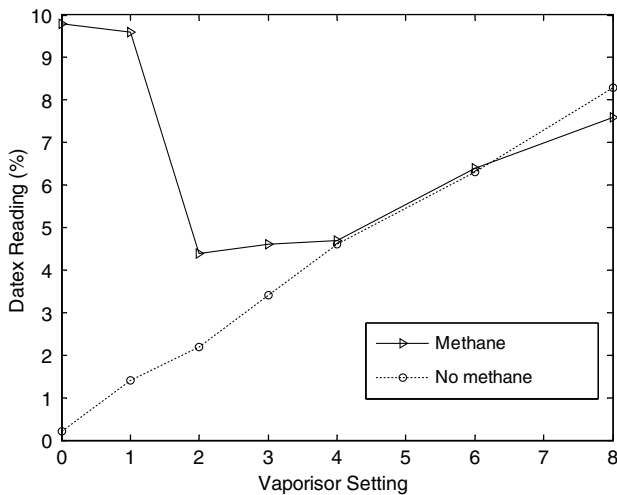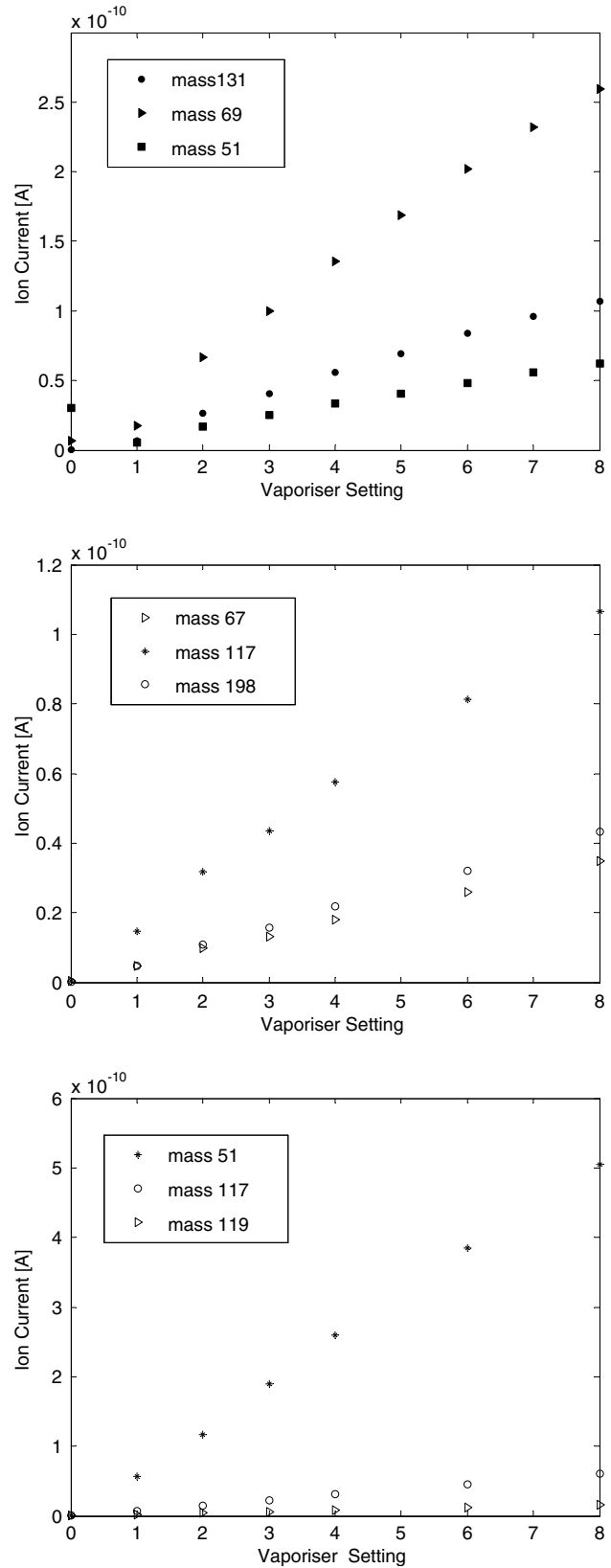

Fig. 3. Measured concentration of halothane (middle), isoflurane (bottom) and sevoflurane (top) using the QMS versus vaporiser setting.

mented from 0% to 8% to 0% in an oxygen flow of 5 L/min. Three mass spectral peaks for each anaesthetic agent were monitored. Some fragment mass spectral peaks were more

Fig. 2. Measured concentration of halothane using a short wavelength IR monitor in the presence and absence of methane.

intense (indicative of greater fragment concentration) than others but in each case there was a linear relationship between vaporiser setting and anaesthetic agent concentration as determined by the portable QMS.

The portable QMS was used in the operating theatre for 23 operations upon horse patients. The levels of anaesthetic peaks of halothane, isoflurane and sevoflurane were well within the detection limits of the mass spectrometer in every case and it was unnecessary to optimise the instrument for maximum sensitivity to particular analytes.

Fig. 4 shows continuous mass spectral peak monitoring of respiratory and anaesthetic gases for a horse patient under anaesthetic undergoing surgery. The QMS was used in MID mode and was also automated to collect a single analogue spectrum in the mass range 10–150 m/z after every 5 min of MID monitoring.

Analysis of the composition of inhaled and exhaled respiratory gases

From Fig. 4 the changes in inspired and expired CO_2 and O_2 can be clearly seen with each breath, however, when the unheated capillary tube sampling at ca. 20 mL/min was used with the Pfeiffer QS422 QMS, there was incomplete resolution of each breath. This was identified, most clearly, by a failure of the carbon dioxide signal to return to a near zero baseline during inspiration.

Identify and quantify methane production

There is normally an overlapping signal at m/z 16 of methane (CH_4^+) with oxygen ($^{16}O^+$) when oxygen is administered at high percentage levels (>80%), for example during anaesthesia. To distinguish between CH_4^+ (m/z 16.0313) and $^{16}O^+$ (m/z 15.9949) at m/z 16 requires a MS instrument with a resolution in excess of 500 and this is not usually possible with miniature (portable) instruments. Methane was therefore identified in this study by using m/z 15 corresponding to a CH_3^+ fragment. Although this overlaps with the $^{15}N^+$ signal, the isotope abundance of $^{15}N^+$ is only 0.02% during anaesthesia after denitrogenation of the circle system and the patient's body, allowing the nitrogen contribution to m/z 15 to be neglected. During anaesthesia, low-mass side 'tails' on the mass spectral peak for m/z 16 were observed using the portable instrument (Fig. 5). This was a consequence of the high intensity of the O^+ (m/z 16) peak and the resolution of the QMS.

Fig. 6 shows the methane region for a sample of air, a 1% methane standard and a horse breath captured in a sample bag, analysed by the laboratory-based MKS Minilab instrument. The resolution is sufficient to give good separation of the peaks at m/z 15 and m/z 16. The equine respiratory sample shows a clear increase in m/z 15, in excess of the m/z 15 air spectra ($^{15}N^+$) and which we attributed to methane production by the patient. The amount of methane produced by the patient may be estimated by comparing the spectral heights of the m/z 15 signal from the 1% methane standard and the m/z 15 signal from the equine respiratory sample (attributed to $^{15}CH_3^+$) subtracting the m/z 15 lab air component ($^{15}N^+$). The quantity of methane produced by the horse in this case was estimated to be between 0.1% and 0.2%. The results of breath analysis on five further bag samples showed that methane was produced in four adult horses but not in a 7 day old foal.
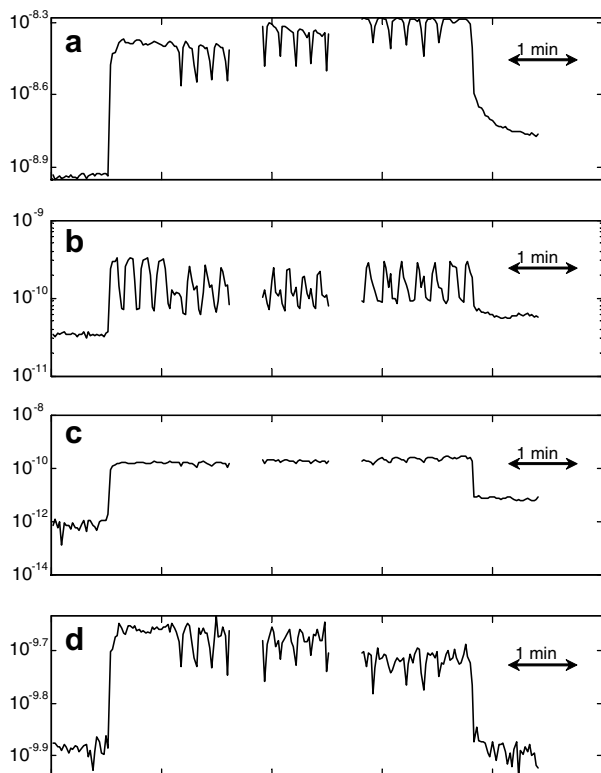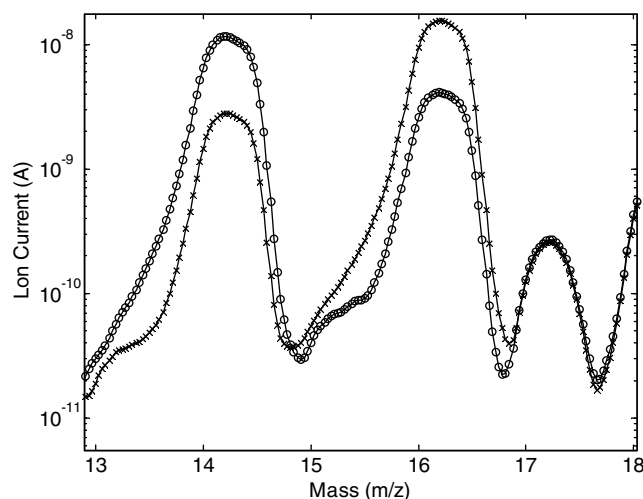

Fig. 4. Operation of QMS in multiple ion detection (MID) mode (ion current versus time) to allow continuous peak monitoring of respiratory and anaesthetic reagent during an operation: (a) CO_2 ($m/z = 44$) (b) O_2 ($m/z = 32$) (c) Methane ($m/z = 15$), and (d) halothane ($m/z = 117$).

Fig. 5. Two mass spectra from the portable instrument (Pfeiffer QS422 QMS) in the mass range m/z 10–20 showing overlap of methane (m/z 15) signal and oxygen (m/z 16).
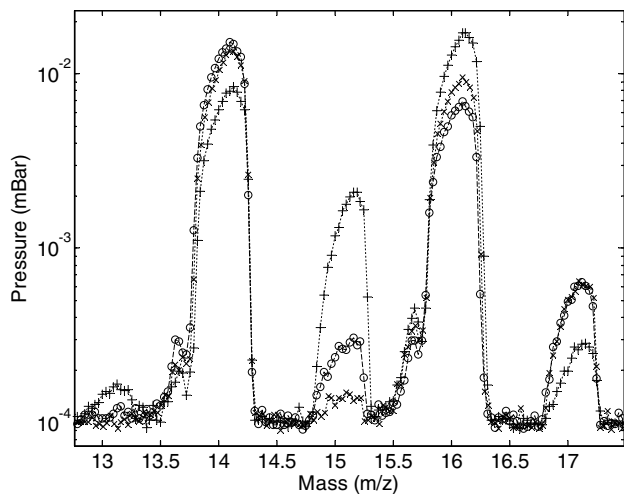
Fig. 6. Mass spectra from the laboratory-based instrument (MKS Minilab) in the mass range m/z 10–20 for a sample of laboratory air (x), 1% methane (standard) (+) and a respiratory sample (o).

Discussion

The anaesthetic agents were found to have individual and isolated peaks on which univariate calibration is possible (e.g., m/z 196 for halothane, m/z 51 for isoflurane and m/z 131 for sevoflurane). However multiple peaks for the same reagent were also identifiable and can be used for multivariate calibration (e.g., m/z 69 and 117 for halothane, m/z 51 and 117 for isoflurane and m/z 69 and 131 for sevoflurane). Since different anaesthetic gases produce different fragmentation patterns, the software in any QMS monitoring system can be configured to auto-detect which reagent is being administered to the patient.

Our study confirmed that the traditional short IR wavelength monitors are unreliable for quantifying the percentage of volatile anaesthetic agents, especially halothane, in the presence of even low levels of methane. High IR wavelength monitors, e.g., Bruer and Kjael MGM 1302 (Moens et al., 1991), and the newer Datex monitors (e.g., Datex Ohmeda S/5 compact anaesthesia monitor), appear to be unaffected by this interference (preliminary laboratory studies), however cross sensitivity effects still require full evaluation.

The linear relationship between QMS signal and delivered anaesthetic agent concentration establishes the suitability of the QMS for quantitative monitoring of anaesthetic gases. Further useful information is obtainable from analogue scans since all mass spectral peaks in the mass range are identified. Sufficiently fast analogue scanning may allow use of multivariate calibration methods on-line which have been shown to have many advantages, but this was not possible with the system used here.

To monitor individual breaths of the horse a rise and fall in the level of an analyte should be characterised by a suitable number of data points. On the Pfieffer QMS422 instrument 11 mass spectral peaks in MID mode were monitored

with a timing of 10 ms between samples giving a total number of 545 data points per analyte per minute ($100 \times 60/11$). QMS sampling rates were therefore not a limiting factor in determining the overall response time of the system. However, as Fig. 4 shows, the measured analyte signals do not return to their original levels between individual breaths. The capillary inlet used limited the response of the QMS system to the order of 1 s. This prolonged recovery time was due to residence times of the gaseous species in the measurement system and was improved in later studies through more effective sampling using a heated capillary tube.

Accurate measurement of the relative concentrations of O_2 and CO_2 in inspired and expired gases allow evaluation of the metabolic status of an individual. The respiratory quotient (RQ) is defined as: $RQ = CO_{2produced}/O_{2consumed}$. Measurements of RQ can be used to monitor the extent of disease states such as the extent of cachexia, the progress of recovery and the nutritional status of critically ill or starved patients (Flancbaum, 1999; Tisdale, 2000).

IR spirometry, as a means of measuring RQ, is limited in sensitivity. It is possible to resolve CO_2 concentrations of <0.001% against the atmospheric background of about 0.036%, however measurement (usually by paramagnetic techniques) of small changes in O_2 concentration are more difficult against a background concentration of about 20.9%. Nevertheless, indirect calorimetry has tended to rely on measurements of O_2 consumption because estimation of metabolic turnover from CO_2 production introduces its own large errors. This is because the thermal equivalent of CO_2 production varies much more with substrate type than that of O_2. Mass spectrometry (MS) permits highly sensitive measurements (parts per billion) which are more than two orders more sensitive than optical methods such as IR. MS therefore offers the means to make fast, accurate determination of RQ. Fig. 3 demonstrates the feasibility of this system for measuring RQ on-line, although breath-by-breath resolution needs improvement.

The low-mass side 'tail' on the O^+ (m/z 16) peaks in Fig. 5 may have occurred because of several factors. It may have been due to the high percentage of oxygen used as the carrier gas for the anaesthetic agent (and hence a high intensity O^+ (m/z 16) peak), or the relatively low resolution of the QMS, but may also have been affected by the sampling procedure. Such tails are commonly observed at lower resolutions and have been modelled theoretically (Gibson and Taylor, 2003). Monitoring the m/z 15 signal in MID mode can give a false positive for methane (CH_3^+) due to the overlap with the long $^{16}O^+$ tail. This was reduced using a higher resolution instrument (MKS Minilab QMS), as demonstrated in Fig. 6. The MKS instrument had a uniformly heated capillary inlet, avoiding excessive absorption of volatile species by the sampling capillary, which otherwise prolongs the response time (see earlier).

The calculated methane concentration of 0.1–0.2% present in the horse breath of Fig. 6 is in agreement with those

measured by Moens et al. (1991) and Sasaki et al. (1999). The lack of apparent methane production in the 7 day old foal breath sample was probably due to incomplete establishment of its gastrointestinal tract flora at this early age (Bond et al., 1971; Perman et al., 1981; Sasaki et al., 1999).

Advances in genomics, proteomics and metabolomics have revealed numerous biomarkers that indicate patient condition. In many cases however these biomarkers can only be measured after time consuming or expensive analysis. Many diagnostic tests also require preparation of the patient such as enforcing overnight fasting, administration of a substrate (e.g., glucose loading) or the use of drugs. Most molecular techniques seek to identify gene products/proteins in a given sample (Weckwerth, 2003) in a non-targeted manner. They represent a 'snap-shot' of a subject's condition at a specific point in time making it impossible to observe changes in a dynamic system. A more physiologically relevant approach is to monitor the dynamic response of an organism to the demands and stresses imposed upon it (e.g., disease, stress, exercise and therapeutic intervention). This is the strategy adopted by metabolomic analyses. Blood, plasma or urine samples have been used extensively, however, these can only be sampled intermittently and the sampling process is inconvenient and may cause discomfort. The challenge faced by metabolomics is to gain continuous access to the changing milieu.

Large numbers of volatile organic compounds, by-products of endogenous biological processes, are present in exhaled breath (Miekisch et al., 2004). Breath testing has largely been neglected as a means of monitoring metabolic state, however, some breath-borne biomarkers are very familiar. Acetone, produced in the oxidation of free fatty acids, is easily detectable on the breath and can be diagnostic of diabetes. Galassetti et al. (2005) studied the concentration of acetone in the breath of patients during episodes of diabetic ketoacidosis, discovering that breath acetone correlates positively with plasma levels. The possible application of breath analysis includes screening for conditions such as disorders of cholesterol synthesis, or the presence of oxidative stress and ketoacidosis. The technology also has potential applications in diagnosis and monitoring of tumours (Deng et al., 2004; McWilliams and Lam, 2005), pulmonary disease including asthma and heart disease. Human breath, and no doubt animal breath, contains thousands of chemicals, therefore, the potential for developing new applications is high.

Within this preliminary work some important operation considerations were noted. Firstly the importance of correct sampling: a moisture trap and a heated capillary line should be used: this prevents condensation and avoids blockages in the capillary inlet. Additionally it was found difficult to monitor m/z 15 in a mainly oxygen environment due to the size of the m/z 16 peak. This highlights the importance of using an instrument with a suitable resolution.

Breath-by-breath analysis, for monitoring of respired oxygen and carbon dioxide, the latter capability being akin to that obtainable with the traditional low IR wavelength machines, is also possible given adequate sampling time and signal resolution. Mass spectrometry offers an advantage, however, in that the same technology is capable of monitoring oxygen in the inspired and expired gases, whereas the IR absorption principle cannot be used to measure any gas molecules composed of identical atoms, necessitating the addition of another type of measurement technique for oxygen, such as a paramagnetic analyser.

Additionally the QMS could also be used as a routine diagnostic tool. For example volatile organic compounds produced as by-products of endogenous biological processes can be biomarkers for disease, for example ketones are produced during oxidation of free fatty acids (ketosis) (Elliott-Martin et al., 1997). Other compounds including dimethyl amine, acetone, hydrogen sulphide, and pentane can be indicative, in man, of diabetes, uraemia/kidney failure, lung carcinoma, liver disease and schizophrenia, (Gardner et al., 2000; Miekisch et al., 2004; Wyse et al., 2004). It remains to be demonstrated whether similar such compounds will prove to be markers of disease in animals.

Conclusions

In this study portable quadrupole mass spectrometry has been shown to be a feasible replacement for traditional IR monitoring when simultaneously monitoring anaesthetic and respiratory gases. Quadrupole mass spectrometry (QMS) provided both qualitative and quantitative information. All the volatile anaesthetic agents were found to be easily measurable. The instrument had an advantage over fixed wavelength analysers as it could be used to monitor anaesthetic analytes and other respiratory gases at extremely low concentrations. Reducing the size of the QMS (and its associated vacuum system), increases portability such that applications for routine patient-side clinical monitoring and assessment can be considered.

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