



# A selective reagent ion-time-of-flight-mass spectrometric study of the reactions of $O_2^+$ with several volatile halogenated inhalation anaesthetics: potential for breath analysis

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**Abstract.** As a part of an ongoing study to determine the concentrations of inhalation anaesthetics in the exhaled breath of patients following surgery, separate investigations are being undertaken to determine which soft chemical ionisation mass spectrometric techniques are most suitable for real-time breath measurements. Towards that goal, we present here details of a selective reagent ion-time-of-flight-mass spectrometer study investigating the reactions of  $O_2^+$  with isoflurane, enflurane, desflurane, and sevoflurane. Information on the product ions as a function of reduced electric field and the influence of humidity in the drift (reaction) tube is presented. With increasing humidity in the drift tube, secondary product ion-water reactions lead to significant decreases in the intensities of many of the primary product ions, resulting here in a reduced analytical sensitivity for the four fluranes. However, for breath analysis this is found not to be a major issue owing to the high concentrations of inhalation anaesthetics found in exhaled breath even several days after surgery. This is demonstrated in a clinical measurement involving a patient who had undergone an operational procedure, with sevoflurane being used for maintenance of general anaesthesia.

## 1 Introduction

Using the relatively new analytical technique of proton transfer reaction-time-of-flight-mass spectrometry (PTR-ToF-MS) [1], in 2012 Professor Kurt H. Becker and colleagues reported an investigation of the reactions of protonated water with 2,4,6-trinitrotoluene (TNT) [2]. Of relevance to the work presented here, they reported that the  $TNTH^+$  intensity started low at the initial reduced electric field ( $E/N$ ) of approximately 100 Td and gradually rose with increasing  $E/N$  by over an order of magnitude at about 180 Td. After reaching this maximum value, the  $TNTH^+$  signal intensity declined with further increases in  $E/N$ . This was unlike the behaviour of any other molecule investigated with PTR-ToF-MS to that date, for which the protonated

parent intensity starts high at the lowest  $E/N$  value and then gradually decreases with increasing  $E/N$ . This results from the decrease in reaction time, an increase in collisional induced dissociation processes, and possibly changes in ion transmission from the drift tube into the detection region. It was hypothesised in [2] that the decreasing  $TNTH^+$  signal from its maximum with decreasing  $E/N$  resulted from reactions with residual neutral water present in the drift tube. Electronic structure calculations suggested that once a  $TNTH^+\cdot H_2O$  adduct is formed it will efficiently react with water to produce  $H_3O^+\cdot H_2O + TNT$ , i.e. a terminal product ion that does not contain any signature of TNT. This was confirmed experimentally.

The study with  $TNTH^+$  was one of the first detailed investigations that highlighted the potential effects of secondary ion-water chemistry on the analytical detection sensitivity of PTR-ToF-MS for a given compound. In a more recent PTR-ToF-MS study, Malásková et al. provided further illustrations of the effects of product ion-water reactions, resulting in a reduced analytical sensitivity for the

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reactions of  $\text{H}_3\text{O}^+$  with four fluorinated inhalation anaesthetics; isoflurane ( $\text{CHF}_2\text{-O-CHCl-CF}_3$ ), enflurane ( $\text{CHF}_2\text{-O-CF}_2\text{-CHClF}$ ), desflurane ( $\text{CHF}_2\text{-O-CHF-CF}_3$ ), and sevoflurane ( $(\text{CF}_3)_2\text{CH-O-CH}_2\text{F}$ ) [3], in agreement with the observations reported in a selected ion flow tube-mass spectrometer (SIFT-MS) investigation of isoflurane and sevoflurane by Wang et al. [4]. In particular, Wang et al. suggested that  $\text{H}_3\text{O}^+$ /sevoflurane ion chemistry catalyses the production of  $\text{H}_3\text{O}^+$ , i.e. just like TNT, a secondary reaction process results in a terminal product ion not containing a signature of the original neutral reactant.

In connection to ion/flurane chemistry, Weiss et al. have recently provided details on the reactions of  $\text{O}_2^+$  with isoflurane, sevoflurane, enflurane and desflurane within the reaction region of a High Kinetic Energy-Ion Mobility Spectrometer-Mass Spectrometer (HiKE-IMS-MS) [5]. Owing to its operating pressure range of  $\sim 10\text{--}40$  mbar, the ion-chemistry occurring there interestingly bridges that taking place in the reaction tubes of PTR-ToF-MS and SIFT-MS instruments (operating pressure  $\sim 1\text{--}3$  mbar) and atmospheric pressure IMS systems. To provide new insights, it is useful to compare the ion-molecule chemistry occurring in the various soft chemical ionisation analytical instruments.

Just as differences in the product ions are observed between the PTR-ToF-MS and SIFT-MS studies dealing with the reactions of  $\text{H}_3\text{O}^+$  with isoflurane and sevoflurane [3, 4], notable differences in the product ions resulting from the reactions of  $\text{O}_2^+$  with isoflurane and sevoflurane are also discerned between the HiKE-IMS-MS and SIFT-MS studies [4, 5]. These differences relate to the operational conditions of the instruments, the complexity of the ion-molecule processes, and the differing effects of secondary reactions of the product ions with water present in the reaction regions. Furthermore, whereas collisions of reagent and product ions with neutrals in the flow tube of a SIFT-MS proceed at thermal translational energies, the presence of the electric field in the drift tube of the HiKE-IMS-MS instrument results in the ions gaining additional (non-thermal) translational energy, as described by the Wannier equation [6]. This gain in kinetic energy results in non-thermal and non-elastic collisional processes, which may be sufficient to drive reaction pathways not possible for SIFT-MS. However, even considering the effects of collisional-induced dissociation, the differences in the observed product ions between the SIFT-MS and HiKE-IMS-MS  $\text{O}_2^+$  investigations are rather extensive. Thus, further investigations of the reactions of  $\text{O}_2^+$  with the fluranes are warranted using other types of soft chemical ionisation mass spectrometers to help try to reconcile these differences.

Here we extend the Malásková et al.  $\text{H}_3\text{O}^+$  study of four inhalation anaesthetics by using  $\text{O}_2^+$  as the reagent ion. We investigate the  $\text{O}_2^+$ /flurane chemistry as a function of  $E/N$  and its dependence on the humidity of the buffer gas in the reaction region. Important objectives of this study are to (i) provide new data that could help to shed some light on the observed

disparities in the product ions between the SIFT-MS and HiKE-IMS-MS studies, (ii) provide an improved understanding of  $\text{O}_2^+$ /flurane processes, (iii) determine suitable product ion(s) and operational parameters needed to detect inhalation anaesthetics using Selected Reagent Ion (SRI)-ToF-MS techniques, and (iv) assess the potential use of  $\text{O}_2^+$  as a reagent ion to monitor inhalation anaesthetics for real-time analytical purposes, e.g. breath analysis.

Given that the work presented in this paper is primarily based on obtaining fundamental knowledge to aid in the development of SRI-ToF-MS to analyse inhalation anaesthetics in exhaled breath, we illustrate its potential to undertake pharmacokinetic studies in order to determine the washout of an inhalation anaesthetic from the human body following surgery. This has implications for post-operative recovery of patients and for workplace exposure of hospital staff in the post-operative observation units.

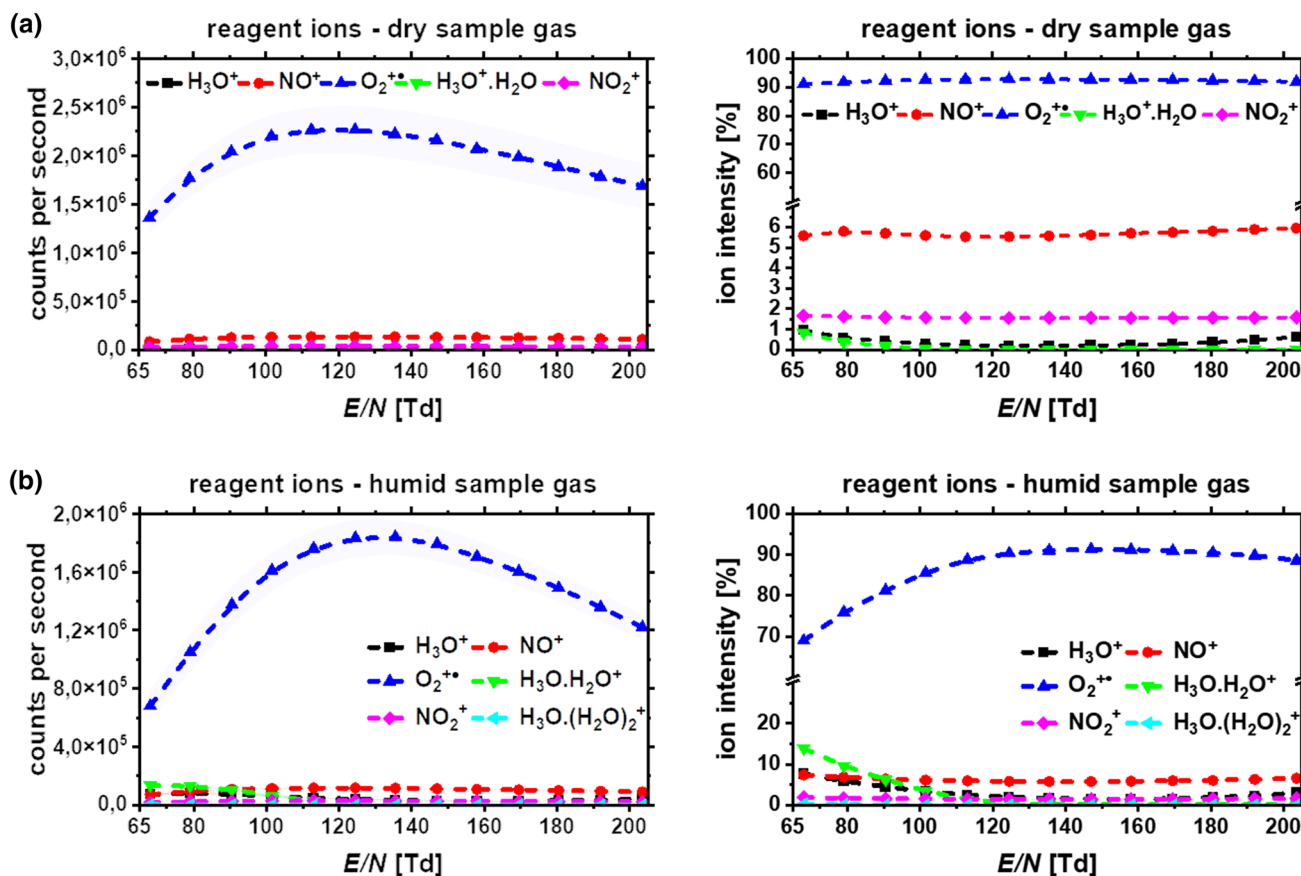
## 2 Methods

### 2.1 SRI-ToF-MS

The operational procedures of SRI-ToF-MS have been discussed in detail for PTR-ToF-MS [1, 7]. Here a compact high-performance Extended PTR-TOF 6000 X2 (Ionicon Analytik GmbH, Austria) was used [7–11], the reaction region of which consists of two sections: a standard drift tube and an ion funnel. The funnel enhances instrumental sensitivity. No RF field was applied in this work, because sensitivity is not an issue, and because the application of the RF funnel may complicate the ion-collisional processes. This also allows us to make semi-quantitative comparisons with the HiKE-IMS-MS results [5], because of having defined  $E/N$  values. The drift tube was maintained at a constant pressure of 2.3 mbar and a temperature of 80 °C. High-purity dry nitrogen (99.9999%, < 3 ppm<sub>v</sub> water) was used. Its flow rate was constrained by a peek capillary. We will refer to this as *dry* sample gas flow. High humidity (i.e. relative humidity (rH) of  $\sim 100\%$ ) gas flow was achieved by bubbling nitrogen through distilled water kept at room temperature. This will be referred to as *humid* sample gas flow.

#### 2.1.1 Reagent ion production

Medical grade oxygen (not less than 99.5% v/v of  $\text{O}_2$ , SOL Technische Gase GmbH) was introduced at six standard cubic centimetres per minute into the ion source.  $\text{O}_2^+$  was produced in a hollow cathode discharge, either through electron impact with  $\text{O}_2$ , or through charge transfer involving other ionic species created in the discharge.  $\text{NO}_2^+$  is also produced in the ion source through the reactions of the nitrogen containing ions with oxygen. In the discharge, excited oxygen atoms ( $\text{O}^*$ ) are also created, which can react



**Fig. 1** Reagent ion distributions as a function of the reduced electric field ( $E/N$ ) in Td, whilst operating the PTR-TOF 6000 X2 in SRI mode with  $O_2$  as the reagent gas being introduced directly into the ion source. The reagent ion intensities are given in counts per second (cps) and percentages for (a) *dry* and (b) *humid* nitrogen sample gas flows into the drift tube of the PTR X2 6000. Only raw output data were used, i.e. no correction for ion transmission has been used. The shaded regions provide a measure of the uncertainties in the values

with  $N_2^+$  leading to another impurity reagent ion  $NO^+$  [12]. Whilst operating in SRI mode, any residual water already present in the ion source results in  $H_3O^+$  and  $H_3O^+ \cdot H_2O$  being formed. All terminal reagent ions present in the ion source were extracted into the drift tube, for which  $O_2^+$  reagent ions dominate, contributing approximately either 90% or 70%–90% (depending on the  $E/N$  value) under *dry* or *humid* sample gas flows, respectively, to the total reagent ion signal (see Fig. 1).

Ions migrate towards the end of the drift tube under the influence of the applied electric field, which was adjusted to provide an  $E/N$  ranging from about 65 Td up to approximately 205 Td. A percentage of these ions exited the drift tube through a 1-mm orifice and were guided and focused into the detection region. The ToF-MS region was maintained at a pressure of  $3 \times 10^{-7}$  mbar using a HiPace 80 turbo pump (Pfeiffer), which is backed by a MD 1 VARIO-SP diaphragm pump (VACUUBRAND). The ToF-MS has a mass resolution ( $m/\Delta m$ ) of approximately 6000 at  $m/z$  147, and a sensitivity  $> 2000$  cps/ppb<sub>v</sub> at  $m/z$  181. Using a ten-second integration time, mass spectra were recorded in

steps of 10 Td. Three  $E/N$  sweeps were performed, and the mass spectra for any given  $E/N$  were averaged.

## 2.2 Halogenated chemicals

Sevoflurane (CAS number: 28523-86-6) and isoflurane (CAS number: 26675-46-7) were purchased from the biopharmaceutical company AbbVie, enflurane (CAS number: 13838-16-9) was supplied by Abbott Products Operations AG, and desflurane (CAS number: 57041-67-5) was sourced from Baxter International.

## 2.3 Sampling procedure for use in identifying the product ions

A preliminary  $O_2^+$ /flurane SRI-ToF-MS investigation was already undertaken by us, which was briefly presented by Chellayah in her PhD thesis [13]. However, for those measurements, a less sensitive and lower mass resolution SRI-ToF-MS was used. Furthermore, direct headspace sampling above the volatile liquid was used resulting in extremely high concentrations of the fluranes entering the drift tube. Therefore, possible secondary processes cannot be ruled out. For this study, all

direct measurements ensured the use of low flurane concentrations ( $\sim 100$  ppb<sub>v</sub>) in the drift tube. To achieve these low concentrations, the following procedure was used. A 1000 mL gas bulb was sealed by two valves and a septum. The bulb was then evacuated at 60 °C in a drying cabinet (Memmert). To reduce evaporation, the flurane was cooled to -18 °C. A 5- $\mu$ L glass syringe (Hamilton®) withdrew 0.5  $\mu$ L of the liquid and injected it into the glass bulb through the septum. The gas bulb was then brought to atmospheric pressure using dry high-purity nitrogen. A 3-L Tedlar® bag (SKR Inc. USA) was flushed five times with high-purity nitrogen and then filled with 1000 mL of dry nitrogen. Using a 5 mL glass syringe (Hamilton®), a known volume (8.5 mL up to 20 mL) of the gas in the bulb was sampled and injected into the bag to give volatile volume-mixing ratios in the range of 1.5 ppm<sub>v</sub> to 3.5 ppm<sub>v</sub>. The bag maintained at 40 °C in an oven was then connected to the inlet of the PTR 6000 X2 via a heated capillary. Using a sample inlet flow of 70 mL/min, a flurane concentration of the order of a hundred ppb<sub>v</sub> was achieved, which is sufficiently high to produce good product ion intensities for identification purposes, but low enough to ensure that no secondary product ion-flurane reactions occur.

#### 2.4 Time-of-flight mass spectrometric analysis

Data were processed using PTR-MS Viewer 3.4.3.12. The  $^{16}\text{O}^{18}\text{O}^+$  ( $m/z$  33.9941) isotopologue was used to determine the  $\text{O}_2^+$  intensity. The peak positions of  $^{16}\text{O}^{18}\text{O}^+$  and an ion at  $m/z$  329.8403, resulting from the reaction of  $\text{O}_2^+$  with 1,3-diiodobenzene, provided the two-point  $m/z$  calibration of the ToF-MS. The reagent and product ion mass spectral peaks were fitted to pseudo-Voigt profiles using Ionicon Analytik GmbH software, from which the peaks' positions and their areas were determined. An average of the three independent measurements were used to obtain the intensities (in counts-per-second) of each product ion resulting from a given flurane.

#### 2.5 Clinical breath sampling and PTR 6000 X2 analysis protocols

The PTR 6000 X2 instrument was not allowed in the university hospital of Innsbruck. Therefore, we had to undertake breath analysis off-line. To achieve this, end-tidal breath and room air samples were collected into 250 mL glass syringes (Socorex®) at the point-of-care, which was the recovery room for the first breath sample following surgery, and a patient's private room for all subsequent breath samples. End-tidal breath samples were also collected from the volunteer some hours prior to surgery. The end-tidal phase of the exhaled breath was determined using a  $\text{CO}_2$  sensor (CAPNOSTAT®, PHASEIN Medical Technologies). Approximately, 4–5 breaths were needed to fill each syringe. Once full, the syringe was then closed using a 3-way Luer-lock stopcock (Braun Medical Limited). Three syringes were

used in total for each measurement. Two were used to obtain two replicates of the exhaled breath. The third syringe was used to obtain a room air sample taken at approximately the same time as the two breath samples, to take into account any background room contamination. All samples were analysed with the PTR 6000 X2 within 1 h of collection.

To avoid saturation of the product ion signals and to use concentrations in the dynamic range used for the calibration to convert counts per second (cps) of a product ion to sevoflurane concentration, breath samples had to be diluted to achieve concentrations levels of  $\sim 40$ – $60$  ppb<sub>v</sub> in the drift tube. For the dilution, a small quantity of the breath sample was drawn into a 2.5 mL glass syringe (Hamilton®). Typically 0.1–0.5 mL of this was injected into another 250 mL glass syringe that contained 250 mL of dry nitrogen. Owing to such high dilution used, breath humidity plays no role in these off-line measurements. The 250 mL syringe was then placed in an oven maintained at a constant temperature of 40 °C and coupled to a heated inlet capillary (80 °C) using a 20-cm peek tube. As the syringes are gas tight and have minimal friction, atmospheric pressure smoothly pushed the plunger so that the breath sample was continuously drawn into the instrument at a constant flow until the syringe was empty.

To convert cps to sevoflurane concentration in ppb<sub>v</sub>, standards at five different concentrations (8.5, 17.0, 25.5, 34.0 and 42.5 ppb<sub>v</sub>) in nitrogen were created. A linear fit ( $R^2 = 0.989$ ) of cps for a selected product ion versus known sevoflurane concentrations at a given  $E/N$  gave the sensitivity in units of cps/ppb<sub>v</sub>.

#### 2.6 Patient's details and ethical approval

A 66 year-old male patient, with a body mass index of 30.9 kg/m<sup>2</sup>, volunteered for this study. Using the American Society of Anaesthesiologists physical status classification (ASA), the patient's pre-anaesthesia medical co-morbidities were categorised with mild systemic disease (ASA 2). General anaesthesia was induced with 200 mg of propofol and 0.5 mg of fentanyl. Anaesthesia was maintained with continuous infusion of 1.37 mg of remifentanyl and sevoflurane as the inhalation anaesthetic. The surgery without intervals for induction and emergence of anaesthesia and time from patient positioning, skin preparation, draping and post-operative dressing lasted for 1.5 h. In total, the patient was exposed to sevoflurane for 2.7 h. Breath samples were taken at approximately 5 h before the start of the surgical procedure and then at 1.5 h, 4.1 h, 19.1 h, 26.8 h, 43.9 h, 51.1 h and 66.8 h after surgery. (We have defined 0 h as the time the surgery finished as the reference point.)

This clinical study was approved by the independent Ethics Committee of the Medical University of Innsbruck (protocol number 1180/2017) on the 26 April 2018 and was renewed on the 12 April 2022. All procedures performed were in accordance with the ethical standards of the institutional and national research

committees and with the 1964 Helsinki Declaration. The clinical investigation undertaken in this study is explorative and, being non-invasive, it did not influence routine patient care.

### 3 Results and discussion

Note that only those product ions that contribute at least 5% to the total product ion signal at any of the  $E/N$  values are discussed in any detail. Below 5%, there is little use for such product ions in terms of analytical applications.

#### 3.1 Product ion distributions for the inhalation anaesthetics

Figure 2 displays the product ion distributions resulting from the  $O_2^+$ /flurane reactions as a function of  $E/N$  for (a) *dry* and (b) *humid* nitrogen sample gas. (Note that the signal intensities, given in cps, have not been corrected for ion transmission dependencies.) For *dry sample* gas flow drift tube conditions, Table 1 presents a summary of all of the identified product ions (> 5% contribution at any  $E/N$ ) and their percentages calculated at three  $E/N$  values, thereby covering the  $E/N$  range used. The product ion intensity values presented (cps in the figures and percentages in the table) have taken into the  $^{37}\text{Cl}$  contributions for the chlorine containing product ions, which result from the reactions involving isoflurane and enflurane. From the product ion distributions, we can determine the best operating reduced electric field value to achieve maximum cps (and hence sensitivity) for any given compound and what is the best product ion to monitor.

##### 3.1.1 Dry sample nitrogen gas flow

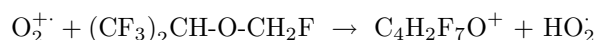
For isoflurane, and its isomer (enflurane), two product ions dominate the product ion distributions over the whole (isoflurane) or the majority (enflurane) of the  $E/N$  values investigated. These are  $\text{CHF}_2^+$  ( $m/z$  51.005) and  $\text{CHFCl}^+$  ( $m/z$  66.975/67.978). This is in good agreement with the results we obtained in our recent, but much lower mass resolution, HiKE-IMS-MS study using reaction region conditions under which  $O_2^+$  was the dominant reagent ion [5]. For enflurane, these two product ions result from a single-bond breakage. Single-bond breakage also accounts for  $\text{CHF}_2^+$  from isoflurane, but there is no obvious pathway of getting to  $\text{CHFCl}^+$  from isoflurane, and hence, considerable rearrangement must be occurring following charge transfer. Two additional product ions were observed from the reaction of  $O_2^+$  with enflurane, namely  $\text{C}_2\text{HF}_3^+$  ( $m/z$  82.003) and  $\text{C}_2\text{HF}_2\text{Cl}^+$  ( $m/z$  97.974/99.971), that either were not detected or only weakly, respectively, in our HiKE-IMS-MS study.  $\text{C}_2\text{HF}_3^+$  makes a negligible contribution to the total product ion signal at any  $E/N$  value. However,

$\text{C}_2\text{HF}_2\text{Cl}^+$  becomes the dominant ion species below about 80 Td.

For isoflurane, the only other product ion we detected is  $\text{C}_2\text{H}_2\text{F}_2\text{ClO}^+$  (at  $m/z$  114.976/116.973), resulting from a single-bond breakage, whose maximum intensity occurs at the lowest  $E/N$  value investigated, with a product ion branching percentage of about 20%. In agreement with the observed increasing branching percentage for  $\text{C}_2\text{H}_2\text{F}_2\text{ClO}^+$  with decreasing  $E/N$ , under thermal conditions Wang et al.[4] report this ion to be the most dominant species (with a branching percentage of 45%). Other product ions resulting from isoflurane and reported by Wang et al. are  $\text{CF}_3^+$  ( $m/z$  69) (10%),  $\text{CF}_3\text{CH}_2\text{O}^+$  ( $m/z$  99) (20%) and  $\text{CF}_2\text{HOCHCF}_3^+$  ( $m/z$  137) (25%). None of these have been observed above 5% in this present study. However, Wang et al. state that the intensity of these three ions is seriously reduced in the presence of humid air. Hence, under our more humid drift tube conditions, even when using *dry* nitrogen as the drift gas, and compared to the extremely dry (helium gas) flow tube conditions of a SIFT-MS, it is possible that they are lost in our study through secondary reactions with water. This may also be the reason why these ions were not observed in our HiKE-IMS-MS study.

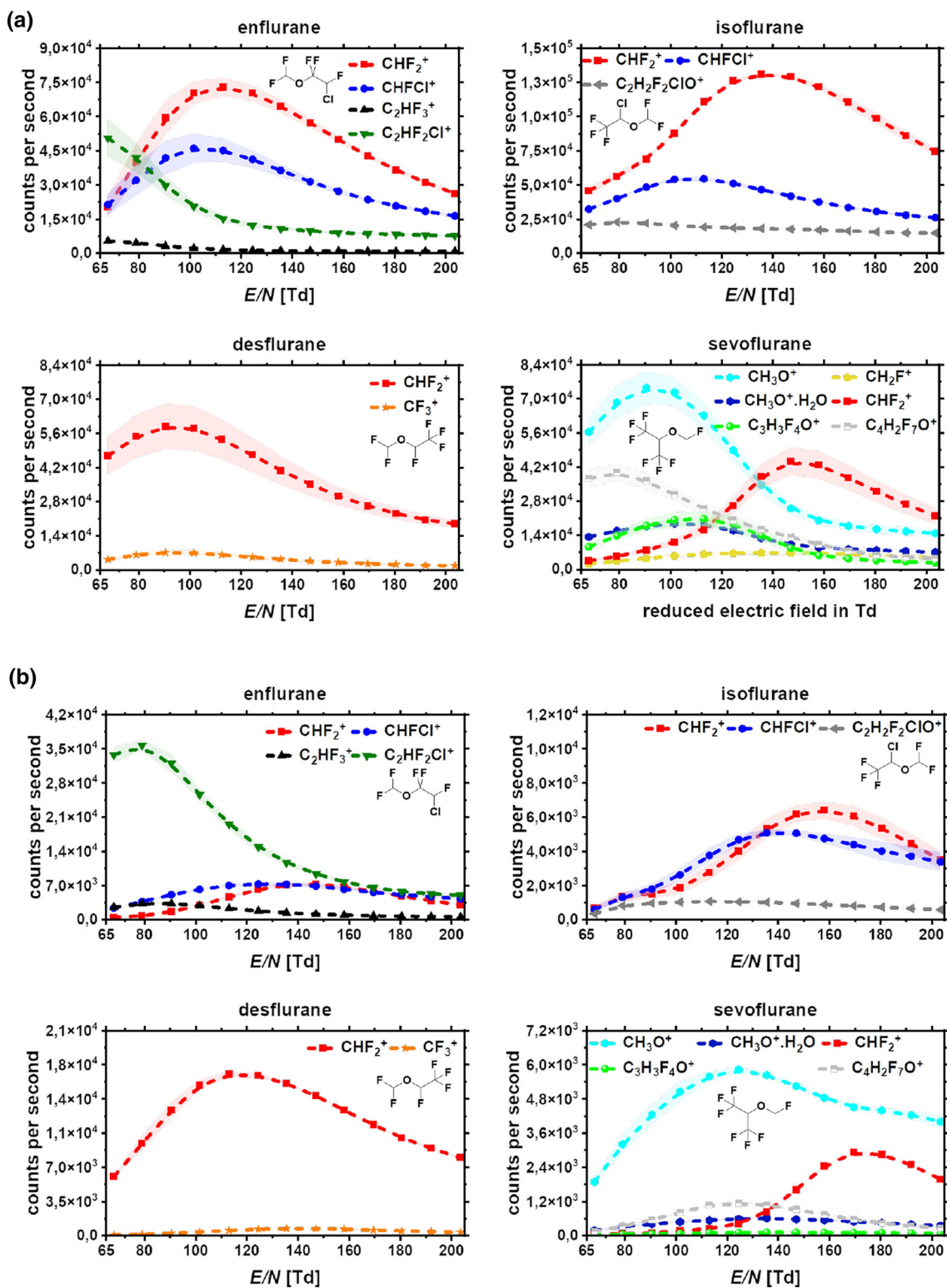
In comparison with enflurane and isoflurane, the product ion distributions for desflurane and sevoflurane are, respectively, simpler and more complicated. For desflurane only two product ions are observed,  $\text{CHF}_2^+$  and  $\text{CF}_3^+$  ( $m/z$  68.995), resulting from simple bond breakage, with  $\text{CHF}_2^+$  being by far the most intense.  $\text{C}_3\text{H}_2\text{F}_5\text{O}^+$  is also observed from desflurane, but with an intensity always below 5%. These results are in reasonable agreement with our less sensitive HiKE-IMS-MS results [5]. However, in the HiKE-IMS-MS study  $\text{C}_3\text{H}_2\text{F}_5\text{O}^+$  ( $m/z$  149) is reported as a product ion with a higher intensity. The cause for this discrepancy is unknown.

Five product ions are directly assigned to sevoflurane:  $\text{CH}_3\text{O}^+$  ( $m/z$  31.018),  $\text{CH}_2\text{F}^+$  ( $m/z$  33.014),  $\text{CHF}_2^+$  ( $m/z$  51.005),  $\text{C}_3\text{H}_3\text{F}_4\text{O}^+$  ( $m/z$  131.012) and  $\text{C}_4\text{H}_2\text{F}_7\text{O}^+$  ( $m/z$  198.999). Rather than a dissociative charge transfer process leading to  $\text{C}_4\text{H}_2\text{F}_7\text{O}^+$ , this product ion could also result from a hydride abstraction mechanism:



In the HiKE-IMS study, a product ion with a nominal  $m/z$  199 was assigned to result from the product ion  $\text{C}_4\text{H}_3\text{F}_6\text{O}^+$  associating with water. However, this is not found to be the case in this study, given that the  $m/z$  value for  $\text{C}_4\text{H}_3\text{F}_6\text{O}^+$  associated with water is 199.019. Although  $\text{C}_4\text{H}_3\text{F}_6\text{O}^+$  ( $m/z$  181.009), was observed in this current study, its intensity was always below 5% at any  $E/N$  value. The two product ions  $\text{CH}_2\text{F}^+$  and  $\text{C}_3\text{H}_3\text{F}_4\text{O}^+$  result from a single-bond breakage. In agreement with the results from the HiKE-IMS-MS study,  $\text{CH}_3\text{O}^+$  is found to be the dominant product ion up to about 130 Td (115 Td in HiKE-IMS-MS study), but there is no obvious reaction





**Fig. 2** Product ion distributions in cps for the four inhalation anaesthetics enflurane, isoflurane, desflurane and sevoflurane using (a) a *dry* and (b) *humid* nitrogen sample gas flows into the drift tube of the PTR X2 6000. Note that the raw output was used, i.e. no correction for transmission has been used. The significance of the secondary reaction leading  $\text{CH}_3\text{O}^+\cdot\text{H}_2\text{O}$  is shown in the figure for sevoflurane. The shaded regions provide a measure of the uncertainties in the values

**Table 1** Product ions and their percentages for enflurane, isoflurane, desflurane, and sevoflurane, resulting from their reactions with  $O_2^+$ 

Compound, formula, $m/z$ (lightest-isotopomer)	Product ions	% ion intensity			Measured $m/z$ [Th]	Actual $m/z$ [Th]	$\Delta m/z$ [Th]
		70 Td	140 Td	200 Td			
Enflurane	$CHF_2^+$	21	57	51	51.008	51.005	0.003
$C_3H_2ClF_5O$ 183.971	$CHFCl^+$	22	32	32	66.978	66.975	0.003
	$C_2HF_3^+$	5	1	2	82.005	82.003	0.002
	$C_2HF_2Cl^+$	52	10	15	97.976	97.974	0.002
Isoflurane	$CHF_2^+$	46	67	64	51.009	51.005	0.004
$C_3H_2ClF_5O$ 183.971	$CHFCl^+$	33	24	23	66.978	66.975	0.003
	$C_2H_2F_2ClO^+$	21	9	13	114.98	114.98	0.003
Desflurane	$CHF_2^+$	92	90	91	51.007	51.005	0.002
$C_3H_2F_6O$ 168.001	$CF_3^+$	8	10	9	68.997	68.995	0.002
Sevoflurane	$CH_3O^+$	46	28	26	31.021	31.018	0.003
$C_4H_3F_7O$ 200.007	$CH_2F^+$	2	6	9	33.014	33.014	0
	$CH_3O^+ \cdot H_2O$	11	10	13	49.031	49.029	0.002
	$CHF_2^+$	3	31	39	51.007	51.005	0.002
	$C_3H_3F_4O^+$	8	12	5	131.02	131.01	0.004
	$C_4H_2F_7O^+$	30	13	8	199	199	0.005

The percentages of the product ions are given at three  $E/N$  values of 70, 140 and 200 Td with *dry* nitrogen sample gas flow as sample matrix. Note that the percentage shown for the product ion  $CH_3O^+$  from sevoflurane can be added to the secondary  $CH_3O^+ \cdot H_2O$  percentage to appreciate the full extent of the primary reaction. Also listed are the measured and expected  $m/z$  values along with their deviation from each other. The  $m/z$  values given are for the lightest isotopomer, but the percentages provided have taken into account isotopic contributions from chlorine in isoflurane and enflurane

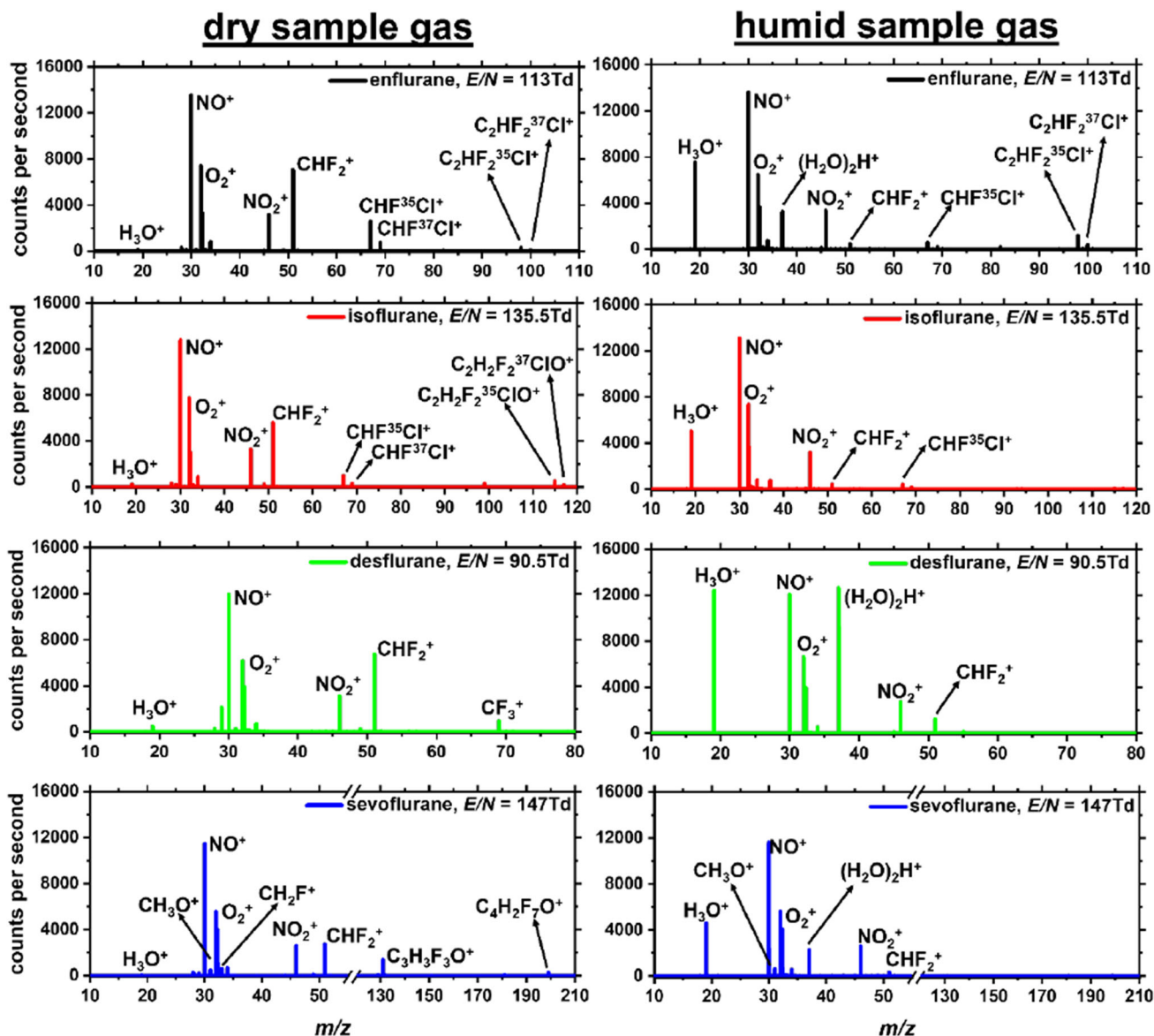
pathway that can lead to its formation and considerable rearrangement is required. After 130 Td,  $CHF_2^+$  is the dominant species for sevoflurane, for which again there is no obvious pathway to its formation, other than major rearrangement following the electron transfer.

A recent soft electron impact study of sevoflurane using a TOF-MS with a resolution of 1500 at  $m/z$  106 [14] reports ions with nominal  $m/z$  values at 33 ( $CH_2F^+$ ), 51 ( $CHF_2^+$ ), 69 ( $CF_3^+$ ), 79 ( $C_3H_5F_2^+$ ), 131 (assigned as  $C_3F_5^+$ , but most probably  $C_3H_3F_4O^+$ ), 151 ( $CH(CF_3)_2^+$ ), 181 ( $C_4H_3F_6O^+$ ), and 200 ( $C_4H_3F_7O^+$ ). Four of the assigned product ions agree with our measurements within the 5% threshold that we set. Only two product ions reported in the paper by Wang et al. are in agreement with our measurements for sevoflurane:  $C_3H_3F_4O^+$  and  $C_4H_2F_7O^+$ . Other product ions mentioned in the Wang et al. paper, at nominal  $m/z$  values of 49 and 129, assigned to be  $CH_2FO^+$  and  $C_3F_4OH^+$ , respectively, were not found in our study.  $C_4H_3F_6O^+$  was also observed in the SIFT-MS study, but its intensity was significantly reduced in the presence of humid air in the flow tube.

As mentioned above, only nominal  $m/z$  values of the product ions are given in the SIFT-MS and HiKE-IMS-MS studies, owing to the low mass resolving power of the mass spectrometers used (a quadrupole mass spectrometer for the SIFT-MS and a home-built ToF-MS

for the HiKE-IMS-MS), which makes the identification of a product ion uncertain. This is particularly true for the product ion coming from the reaction involving sevoflurane observed in both studies with a nominal  $m/z$  49. This product ion is assigned to be  $CH_2FO^+$  in the SIFT-MS study, contributing a significant percentage to the total product ion signal, namely 20–80%, depending on the reaction length used.

In this SRI-ToF-MS, we did observe an ion at a nominal  $m/z$  49 when sevoflurane was introduced into the drift tube, and with a reasonable ion intensity, contributing approximately 10% to the total product ion signal over the full  $E/N$  range investigated. However, by taking advantage of the high mass resolution power of the PTR 6000 X2, we determined a more accurate value for its peak position, namely  $m/z$  49.030. This cannot be  $CH_2FO^+$ , because that would be at  $m/z$  49.009. We therefore propose that the ion observed by us at  $m/z$  49.030, and possibly the ion identified in the SIFT-MS study with a nominal value of  $m/z$  49, results from a secondary process involving an association reaction of  $CH_3O^+$  with residual  $H_2O$ . This leads to a product ion at an actual  $m/z$  49.029, which is within the experimental uncertainty of our measurements. This also explains the observation made by Wang et al. in that the product ion at  $m/z$  49 becomes the most important in the presence of water. The effects of humidity on the product



**Fig. 3** Mass spectra under *dry* and *humid* sample nitrogen gas flow conditions for the four inhalation anaesthetics enflurane, isoflurane, desflurane and sevoflurane. Concentrations for each flurane sample used were the same for both *dry* and *humid* drift tube conditions

ion distributions for the four fluranes are now discussed in more detail.

### 3.1.2 Effects of humidity on detecting the inhalation anaesthetics

Given that we are providing information for the potential use of SRI-ToF-MS to detect the inhalation anaesthetics in exhaled breath (high humidity gas sample) in real time, we describe here the changes in the product ion distributions occurring in the drift tube in going from the *dry* to the *humid* nitrogen gas sample flows. The results for *humid* nitrogen drift tube gas flow are summarised in Fig. 2b, which for each type of flurane can be directly compared to those shown in Fig. 2a,

because approximately the same concentrations of the fluranes were used in both cases.

Figure 3 provides a more visual representation of the influences of humidity on the product ion distributions for the four-inhalation anaesthetics, by comparing mass spectra obtained using *dry* (rH ~ 0%) and *humid* (rH ~ 100%) nitrogen gas. The majority of the primary product ions resulting from the reactions  $O_2^{+}$  with the four fluranes are significantly reduced when using the *humid* nitrogen. This is especially noticeable for  $CHF_2^+$ . The only exceptions are  $CH_3O^+$  coming from the reaction with sevoflurane, and the  $C_2HF_2Cl^+$  coming from the reaction with enflurane. For the latter, Fig. 3 shows a small increase in intensity of  $C_2HF_2Cl^+$  when using the *humid* sample nitrogen gas flow, which



agrees with the observations from the HiKE-IMS-MS study [5].

The observed decrease in many of the product ion intensities with the *humid* nitrogen gas flow for all  $E/N$  values is in part caused by the reduction in the  $O_2^+$  signal (compare Fig. 1a, b). However, this alone cannot account for the loss in the product ions' signals. This is most obvious for enflurane, for which the dramatic reduction in the signal intensity of  $CHF_2^+$  and  $CHFCl^+$  results in  $C_2HF_2Cl^+$  becoming the dominant product ion over the full range of  $E/N$  values investigated. These observations can only be explained by secondary reactions involving water.

### 3.2 An applied research application: a longitudinal study of sevoflurane concentrations in breath samples post-surgery

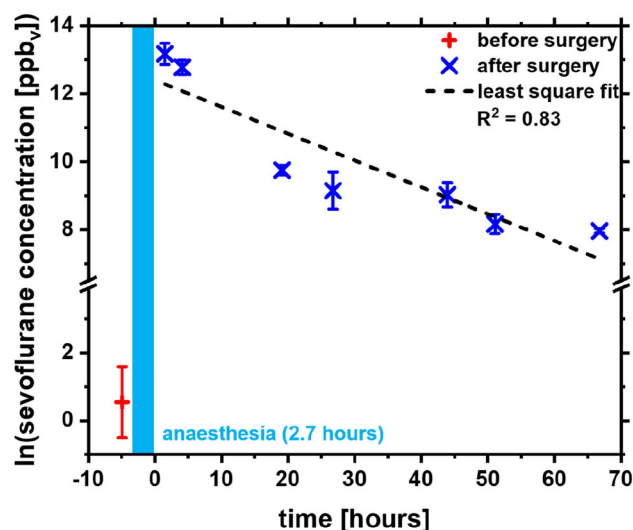
Although Fig. 2a shows that  $CH_3O^+$  is the most intense product ion coming from sevoflurane at an  $E/N$  of approximately 90 Td, we did not select to use it for determining the washout of sevoflurane from the body. The reason for this is due to a large and variable breath background signal associated with methanol in breath, which can have endogenous and exogenous origins. This resulted in significant uncertainties in determining the concentrations of sevoflurane via  $CH_3O^+$ . In comparison, only sevoflurane in the breath samples could give rise to an ion signal at  $m/z$  51.005. Hence, we selected the product ion  $CHF_2^+$  intensity, measured in cps at an  $E/N$  of 147 Td, to determine the concentration of sevoflurane in exhaled breath in units of  $ppb_v$ .

Figure 4 provides a plot of the natural logarithm of the concentration of sevoflurane versus time. The first data point shown in Fig. 4 corresponds to breath samples taken in the patient's hospital room approximately five hours prior to the start of his surgery. The low concentration level determined (approximately 2.5  $ppb_v$  on average) was similar to that found in the room air sample. After surgery, the room air sevoflurane concentrations were always found to be insignificant compared to that found in the exhaled breath samples.

Using an exponential fit, a washout half-life of 4.4 h has been determined for this patient. A meaning to this half-life will only be obtained once we have completed a full clinical study involving many other patients. However, it is of note that this half-life is sufficiently long to ensure that even more than sixty hours after surgery the patient still had high levels of sevoflurane in his exhaled breath ( $\sim 2900$   $ppb_v$ ) and hence would have had substantial concentrations in his systemic circulation upon discharge.

## 4 Concluding remarks

Key aims of this study have been to identify the product ions resulting from the reactions of  $O_2^+$  with isoflurane, enflurane, desflurane and sevoflurane and



**Fig. 4** Graph of the natural logarithm of the concentration of sevoflurane in exhaled breath versus time to illustrate the washout characteristics of sevoflurane. The data points arise from an analysis of breath samples taken from a male patient prior to and following his surgery. The intensities of the product ion  $CHF_2^+$  in cps were used to determine the concentrations of sevoflurane in the exhaled breath samples. All measurements were taken using an  $E/N$  of 147 Td

to determine their relative intensities as a function of  $E/N$ .  $O_2^+$  reacts with the fluranes predominantly via dissociative charge transfer [15, 16], with the exothermicity of the initial charge transfer reaction being generally transferred to the internal energy of the product ion [17]. This internal energy gained may be sufficient to break bonds (dissociative charge transfer), and, if not, collisional processes in the drift tube can increase the internal energy leading to various product ions. For all of the four fluranes, it is found that only one product ion needs to be used for analytical purposes, namely  $CHF_2^+$ . In our earlier PTR-ToF-MS study involving the four inhalation anaesthetics with  $H_3O^+$  as the reagent ion, concerns were raised with regards to the dramatic decrease in product ions signal intensities under the '*humid*' compared to the '*normal*' drift tube operating conditions [3]. A similar behaviour is reported in this paper for the product ions resulting from the reactions of  $O_2^+$  with the fluranes. Therefore, and independent of the reagent ion used, direct (i.e. no dilution) measurements of exhaled breath will reduce the analytical sensitivities of soft chemical ionisation mass spectrometric techniques to monitor these halogenated compounds. However, even with the loss of product ions, this study, and an earlier one (investigating the decrease of isoflurane in the breath of patients following liver transplant surgery [18]), shows that this is not a significant issue. This is because of the extremely high concentrations of inhalation anaesthetics that remain in the human body for a considerable time following the completion of a general anaesthesia using fluranes. Thus, the product ion(s)

used to detect an anaesthetic will still have more than sufficient intensities even for much less sensitive (and hence cheaper) on-line soft chemical ionisation mass spectrometers than the one used in this study, even for those measurements that take place days or weeks after surgery.

The washout study of anaesthetics from the body form a part of major clinical study we have been undertaking for several years, and involving various soft chemical ionisation spectrometric techniques. These studies provide useful information that can be used to determine what influences the rate of elimination of the inhalation anaesthetics from the human body. This will be the subject of a later and more detailed clinical paper.

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## Author contributions

FW developed the methods, performed all the experiments, collected all of the data, analysed the measurements, produced all of the figures and was involved in the writing of all drafts of the paper. TDM, VR, and WL contributed to the scientific discussions and were involved in drafting the final paper. CAM proposed the study and, together with VR, supervised FW. CAM took the lead in writing all of the drafts of the paper. All authors have read, corrected, edited and approved the final manuscript.

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## Declarations

**Conflict of interest** The authors declare no competing interests.

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## Dedication to Professor Kurt Becker

Professor Kurt Becker has had a long and very fruitful affiliation with the University of Innsbruck, Austria, and in particular with colleagues at the Institute for Ion Physics and Applied Physics. This includes collaborative research projects reaching from basic atomic and plasma physics all the way to applications such as Proton Transfer Reaction-Time-of-Flight-Mass Spectrometry (PTR-ToF-MS). Therefore, we dedicate this paper, which deals with advances in the general field of PTR-ToF-MS instrumentation, to Professor Kurt Becker in recognition of his forthcoming 70th birthday.

The work and results presented in this paper very much reflects Kurt's professional interests. At the heart of a PTR-ToF-MS is a low-pressure electron-driven plasma discharge, leading to the production of terminal reagent ions that are delivered into a drift tube region to react with trace chemical compounds. Furthermore, we are presenting a study that investigates charge transfer. This is a major ion-molecule reaction process of fundamental importance to terrestrial, industrial, and astrophysical plasmas. Thus, this paper covers Kurt's interests in both fundamental and applied plasma researches. However, the use of PTR-ToF-MS will also appeal to Kurt's passion of using fundamental and applied research to drive innovation and entrepreneurship.

Within the Institute for Ion Physics and Applied Physics of the 1990s, Professor Werner Lindinger and associates first began to develop PTR-MS technology by leveraging and developing breakthroughs in fundamental science and engineering concerning the study of ion-molecule reaction kinetics. Their rapid realisation of its considerable analytical potential to identify, quantify and monitor volatile organic compounds in many complex chemical environments led to a spinout company being formed in 1998. This company, Ionicon Analytik GmbH, was established with only one employee (Dr. Alfons Jordan) and working only part-time, with an objective to exploit commercially the novel technology.

Following Werner Lindinger's premature death just three years later in 2001, one of the co-authors of this paper, Tilmann D. Märk, stepped in to take over as

the CEO of the company. His son, Lukas Märk, followed him as the company's CEO in 2011. Under their leadership, the company expanded, and, owing to its university origins, they innovatively combined instrumental development with active internationally leading fundamental and applied research, involving numerous collaborations worldwide, including one with Kurt Becker.

Today, Ionicon Analytik GmbH has more than 50 employees. As the company enters its twenty-fifth anniversary it has sold more than 500 analytical instruments to a wide international customer base that includes many academic, industrial, private and environmental establishments. Ionicon Analytik GmbH is therefore a testament to Kurt's desire of translating fundamental and applied research into successful commercial products, and, in the case of PTR-MS, products that bring considerable benefits to society by addressing many important societal needs. Matching academic excellence with societal impact also lives up to what the University of Innsbruck stands for, which has been recently enhanced by its involvement in the Aurora European University Alliance.

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