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Selected ion flow tube mass spectrometry of 3-hydroxybutyric acid, acetone and other ketones in the headspace of aqueous solution and urine

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Abstract

A study has been carried out of the reactions of three isomers of hydroxybutyric acid, giving special attention to 3-hydroxybutyric acid, 3-HBA, with H_3O^+ and NO^+ ions to acquire the required kinetic data for a selected ion flow tube mass spectrometry, SIFT-MS, search for 3-HBA in the headspace of urine since it is known to be one of the "ketone bodies" important in the diagnosis of ketoacidosis. Thus, the product ions formed in the reactions of the H_3O^+ and NO^+ precursor ions with the three hydroxy acids were established by sampling the headspace above the pure compounds over a range of absolute humidities from 1.5% (ambient air) to 6% (liquid headspace at 37 C and exhaled breath). Then these data, together with the rate coefficients for the reactions estimated by calculation, were used to detect and quantify 3-HBA in the headspace of an aqueous solution of this compound of known concentration and above urine donated by two volunteers. The level of 3-HBA above the urine samples after they were acidified with hydrochloric acid was seen to be typically 40 parts-per-billion, ppb, which is much lower than that for acetone seen to be typically 800 ppb. Exploiting the aqueous solution data as a reference, the 3-HBA concentration in the urine samples was estimated to be about 1–2 mmol/L, which is typical of the urine from healthy individuals.

Keywords: Ketone bodies; SIFT-MS; Urine; Breath; Ion-molecule reactions

1. Introduction

Acetone is present in the blood stream of all humans and is generally considered to be linked to dextrose metabolism, to be a product of lipolysis and to be elevated by ketoacidosis [1,2]. Elevated breath acetone has been commonly associated with diabetes [3,4], but this is now being questioned and requires further investigation. It also appears in exhaled breath, in healthy people typically at a relative concentration of a few hundreds of parts-per-billion, ppb [5,6], and also in urine and its headspace vapour [7,8]. The details of the biochemical routes to acetone production are well understood; the precursors are considered to be the so-called ketone bodies, acetoacetic acid and 3-hydroxybutyric acid, 3-HBA (or β-hydroxybutyric acid), which in blood and urine predominantly exist as ions in solution and are assayed as acetoacetate and β-hydroxybutyrate, the decarboxylation of which gives acetone [9,10].

A question often asked of us is "can these ketone bodies be detected in urine headspace and in exhaled breath"? We have developed selected ion flow tube mass spectrometry, SIFT-MS, for trace gas analysis of air with special attention given to exhaled (very humid) breath [11,12]. Using SIFT-MS we can readily measure the concentrations of the major breath metabolites, including acetone, in single exhalations on-line and in real time [11–15]. It is also straightforward to quantify trace gases in urine headspace using SIFT-MS [8,16]. Similarly, some carboxylic acids can also be quantified by SIFT-MS above liquid headspace, but in common with all SIFT-MS analyses the rate coefficients and product ions of the reactions of the compounds to be analysed with the precursor ion available for SIFT-MS analyses, viz. H_3O^+, NO^+ and O_2^+, should ideally be estab-

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lished by experiment. However, it is often difficult to measure the rate coefficients for the reactions of compounds with very low vapour pressures and then calculated values have to be relied upon, as is the case for the hydroxy acids involved in this study. So a major objective of the study reported in this paper was to determine the required kinetic data for the reactions of the ketone bodies 3-HBA and acetoacetic acid with the three SIFT-MS precursor ions. Unfortunately, this was not feasible for acetoacetic acid (see Section 2), but was achieved for 3-HBA and two other structural isomers of hydroxybutyric acid. Having obtained the kinetic data for 3-HBA, a successful search was made for gaseous 3-HBA in the headspace of an aqueous solution of this compound and in the headspace of urine obtained from two healthy volunteers. As a breakdown product of the ketone bodies, acetone was also measured in parallel. During these studies, several other ketones were detected in the urine headspace and the identity of these was confirmed by analysing the headspace of a reference mixture of several ketones in aqueous solution.

2. Experimental

Samples of 2-hydroxybutyric acid (>97% pure, solid at room temp), 3-hydroxybutyric acid (95% pure, viscous liquid at room temperature) and 2-hydroxyisobutyric acid (97% pure, solid at room temperature) were purchased from Sigma–Aldrich, UK. Acetoacetic acid, only available as its lithium salt, readily hydrolyses when exposed to humid air producing acetone and so the reactions of acetoacetic acid could not be studied in the gas phase.

The SIFT-MS analytical technique has been described in many previous papers [8,11–18] and only a very brief description is required here. These experiments were carried out using a *Profile 3* SIFT-MS instrument. The precursor ions are formed in a microwave discharge source and are selected according to their mass-to-charge ratio, *m*/*z*, by a mass filter and injected into flowing helium carrier gas where they are convected as a thermalised swarm along a flow tube. Air/breath samples are introduced at known flow rates into the carrier gas and the precursor ions and the product ions of the reactions of the trace gases in the sample are detected and counted by a downstream analytical mass spectrometer system. From the data obtained, together with the rate coefficients of the reactions, the partial pressures of the trace compounds in the sample are obtained. To determine the rate coefficients for the reactions of the precursor ions with particular compounds their flow rates into the helium must be sufficiently high to reduce the precursor ion count rates at the downstream mass spectrometer by about an order-of-magnitude or more [17,19]. In this way, the rate coefficients for the reactions of H_3O^+, NO^+ and O_2^+ have been determined with many types of compounds see for example [20,21] and thus a large kinetics library has been built up in support of SIFT-MS analyses (see Refs. [12,22] and references therein).

Unfortunately, the liquid/solid hydroxybutyric acids involved in this study have low vapour pressures and so the flow rates of their vapours into the helium carrier gas cannot be made sufficiently high to significantly reduce the count rates of the precursor ions to allow measurements of the rate coefficients for the reactions. So we have had to resort to calculating the collisional rate coefficients for the reactions, as outlined in Section 2.1 below. However, the determination of the product ions of the reactions of these hydroxyacids is straightforward, because only low flow rates of the compounds into the helium are required. So, in this study, the vapours above the compounds were introduced directly into the SIFT-MS instrument via an axial sample inlet positioned 4 cm upstream of the analytical mass spectrometer sampling orifice, during which time a full scan (FS) of the analytical mass spectrometer was taken over an appropriate range of ion mass-to-charge ratio, *m*/*z*, in order to detect and identify all product ions. Sample spectra obtained for H_3O^+ and NO^+ precursor ions are given in Fig. 1a and b respectively when 3-HBA is sampled into the instrument. In this way the product ion distributions for the reactions were identified as shown in the spectra and as discussed in Section 3.1. Then, by targeting these product ions using the multi-ion monitoring (MIM) mode of the SIFT-MS instrument, accurate product ion distributions were determined [11,12,23], which requires that the product ion intensities are corrected for mass discrimination and differential diffusion [22]. It was immediately apparent that O_2^+ precursor ions resulted in excessive fragmentation of the nascent parent cations of the hydroxyacids (formed via charge transfer) and thus O_2^+ is not useful as a precursor ion for trace gas analysis of these compounds; such is quite common for polyatomic organic compounds [12]. So O_2^+ was not used as a precursor ion in this study.

Obviously, the headspace of aqueous liquids and urine, and indeed exhaled breath, are humid, so it is imperative to identify the product ions used for the analysis of trace compounds in the presence of water molecules with which they might react. Thus, the vapour samples above the liquid/solid samples were humidified prior to their introduction into the helium carrier gas of the SIFT-MS and the product ion distributions determined as a function of the sample humidity using the MIM mode of data acquisition. These experiments are quite straightforward, because a unique feature of SIFT-MS is that the humidity of any sample is routinely analysed, as explained in several papers [11,12,24,25], by measuring the relative count rates of the precursor ions and their hydrates e.g., $H_3O^+(H_2O)_{0,1,2,3}$ [24,25]. Sample data on the variation of the signals levels of the latter ions against the percentage humidity as derived from them are shown in Fig. 2.

Using SIFT-MS with the appropriate kinetic data for the reactions, the concentration of the ketone body 3-HBA was measured in the headspace of a dilute aqueous solution of this compound and then after the solution was acidified using hydrochloric acid. Further, the headspace of the urine from two volunteers was examined for this compound before and after the addition of hydrochloric acid. In both cases, acetone was also measured in parallel, in the case of the aqueous solutions to check for the extent of hydrolysis of the hydroxy acid, and for the urine to determine the acetone/hydroxy acid relative concentrations. Additionally, the exhaled breath of both volunteers was analysed for acetone and 3-hydroxybutyric acid immediately following the donation of the urine samples.

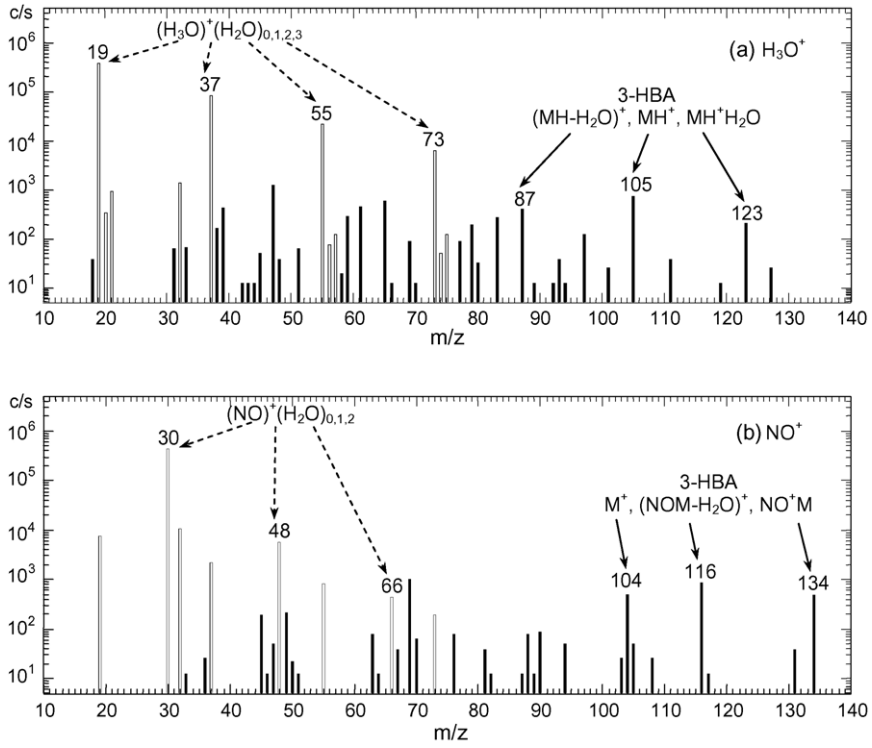

Fig. 1. Full scan (FS) SIFT-MS spectra of ion count rate per second, c/s, against mass-to-charge ratio, *m/z*, obtained when the vapour above 3-hydroxybutyric acid, 3-HBA, is introduced into the SIFT-MS helium carrier gas using (a) H_3O^+ and (b) NO^+ precursor ions (shown as open bars). The product ions for the 3-HBA reactions are as indicated in the form of M^+, MH^+, MH^+H_2O, NO^+M, etc., where M is 3-HBA with a molecular weight of 104 u. See the text, especially Eq. 1 and 2 for interpretation of these spectra.

2.1. Calculation of rate coefficients

Exothermic proton transfer reactions invariably proceed at the gas kinetic or collisional rate. Since it is highly likely that the proton affinities of the hydroxy acids exceed that of water molecules (in common with all carboxylic acids for which the proton affinities are known [26]) then it can be confidently assumed that the H_3O^+ reactions with the hydroxyl acids included in this study will proceed at the collisional rate [17,18]. The collisional rate coefficients, k_c, can be calculated using the theoretical approach developed by Su and Chesnavich [27], but this requires that the polarizabilities and dipole moments of the reactant neutral molecules be known. Unfortunately, these are not available in the literature for these hydroxyacids; however, they can be estimated from the values for similar compounds [28]. Thus, the polarizabilities were interpolated between the $C_4H_8O_2$ stochiometry (9.7, 10.0, 8.6 and 9.44 in units of 10^{-24} cm^3) and the $C_5H_{11}O_3$ stochiometry (11.3×10^{-24} cm^3) as $(10 \pm 2)10^{-24}$ cm^3 and the dipole moments were taken as 2.5 ± 0.4 D from a recent measurement [29] The estimates for these parameters are given in Table 1 together with the respective k_c values calculated according to the equation given in [27]. It cannot be assumed that charge transfer and other reaction processes that both NO^+ and O_2^+ undergo at thermal energies will proceed at the collisional rate, but we have no option at this stage other than to assume this. So the k_c for these reactions are also included in Table 1, noting that these values are somewhat lower than those for the H_3O^+ reactions in accordance with the larger reduced mass of the ion/molecule systems, as required in the theoretical treatment [27]. Further discussion of this approach is given in [17]. We have some evidence that the rate coefficient for the NO^+ reaction with 3-HBA is lower than the equivalent k_c value, as will be indicated in Section 3.2. The rate coefficient for the association reaction of NO^+ with

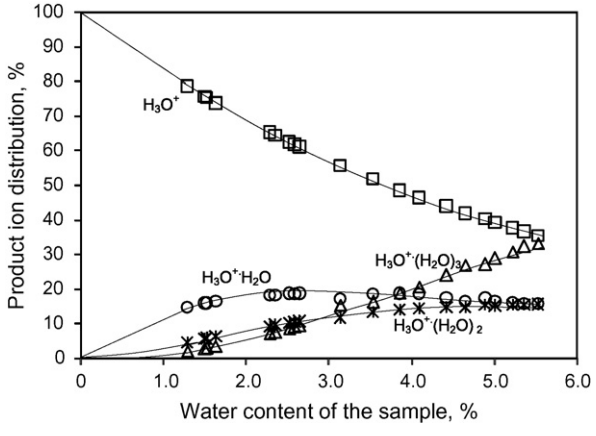

Fig. 2. The loss of the injected precursor H_3O^+ ions and the formation of the hydrated ions $H_3O^+(H_2O)_{1,2,3}$ with the addition of water vapour/air mixture into the helium carrier gas of the SIFT-MS instrument. The count rate of each ion species is expressed as a percentage of the total ion count rate on the ordinate. From such data the number density of H_2O molecules in the carrier gas and hence the humidity of the air sample is derived [24], which is given on the abscissa as a percentage of the water content of the sample.

Table 1
Rate coefficients and the parameters used for their calculation for the reactions of the precursor ions given with the hydroxy acids indicated

Molecule	M u	α[a] 10^{-24} cm^3	μ[b] D	k_c H_3O^+ 10^{-9} cm^3 s^{-1}	k_c NO^+ 10^{-9} cm^3 s^{-1}	k_c $O_2^{\bullet+}$ 10^{-9} cm^3 s^{-1}
2-Hydroxybutyric acid $CH_3CH_2CH(OH)COOH$	104	10 ± 2	2.5 ± 0.4	[3.6]	[3.0]	[2.9]
3-Hydroxybutyric acid $CH_3CH(OH)CH_2COOH$	104	10 ± 2	2.5 ± 0.4	[3.6]	[3.0]	[2.9]
Isohydroxybutyric acid $(CH_3)_2C(OH)COOH$	104	10 ± 2	2.5 ± 0.4	[3.6]	[3.0]	[2.9]
Acetoacetic acid $CH_3C(O)CH_2COOH$	102	10 ± 2	2.5 ± 0.4	[3.6]	[3.0]	[2.9]

The α and μ are respectively the polarizabilities (10^{-24} cm^3) and dipole moments (Debye, D) and M are the molecular weights of the reactant neutral molecule (atomic units, u). k_c are the values of the collisional rate coefficients in 10^{-9} cm^3 s^{-1} calculated according [27].
[a] Estimated from the values for similar compounds given in [28].
[b] From a measurement for hydroxybutyric acid [29].

acetone is well known to be smaller than the collisional rate coefficient [20].

3. Results

3.1. Determination of product ion distributions

3.1.1. H_3O^+ reactions

As mentioned above, it is advisable to determine the product ion distributions at various humidities in order to ensure that the correct product ions are included for SIFT-MS analyses of trace compounds in ambient air (typically 1–2% humidity) and in breath and above aqueous liquids/urine at body temperature (both media typically 5.5–6.5% humidity). Sample data, again choosing 3-HBA, considered as the most important biologically, are shown in Fig. 3a for H_3O^+ precursor ions. It can be seen that there appears two significant primary product ions:

$$H_3O^+ + CH_3CH(OH)CH_2COOH \rightarrow C_4H_8O_3{\cdot}H^+ + H_2O \quad (1a)$$

$$H_3O^+ + CH_3CH(OH)CH_2COOH \rightarrow C_4H_7O_2{}^+ + 2H_2O \quad (1b)$$

Reaction (1a) involves proton transfer from H_3O^+ to the neutral molecule, M, producing MH^+. Reaction (1b) involves the loss of a water molecule from the nascent protonated molecule $(MH^+)^*$ forming an $(MH\text{-}H_2O)^+$ ion, a process that commonly occurs for alcohols [17] and carboxylic acids [18], It is important to note that the monohydrate ions MH^+H_2O are observed as secondary products, becoming the major product ion at the higher humidities, as can be seen in Fig. 3a. A trace of the dihydrate ion, $MH^+(H_2O)_2$ is seen, but this is a very small fraction and can be ignored in the analysis of this hydroxy acid. Further, it should be noted that the actual product distributions are not required for SIFT-MS analyses, but it is essential for accurate analyses that all significant product ions be included.

Similar primary product ions are formed in the reactions of the 2-hydroxybutyric acid and the 2-hydroxyisobutyric acid, viz. MH^+ and $(MH\text{-}H_2O)^+$ ions, but the degree of secondary ion production varies with humidity, as can be seen in Table 2, the dihydate ions $MH^+(H_2O)_2$ becoming important products at the higher humidities.

3.1.2. NO^+ reactions

The major reaction process occurring in these reactions, and the single process that occurs for the 2-hydroxybutyric acid and isohydroxybutyric acid isomers, is adduct ion formation, NO^+M. But, as can be seen in Fig. 3b, in the 3-hydroxybutyric acid, 3-HBA, reaction there are two major product ions and a minor

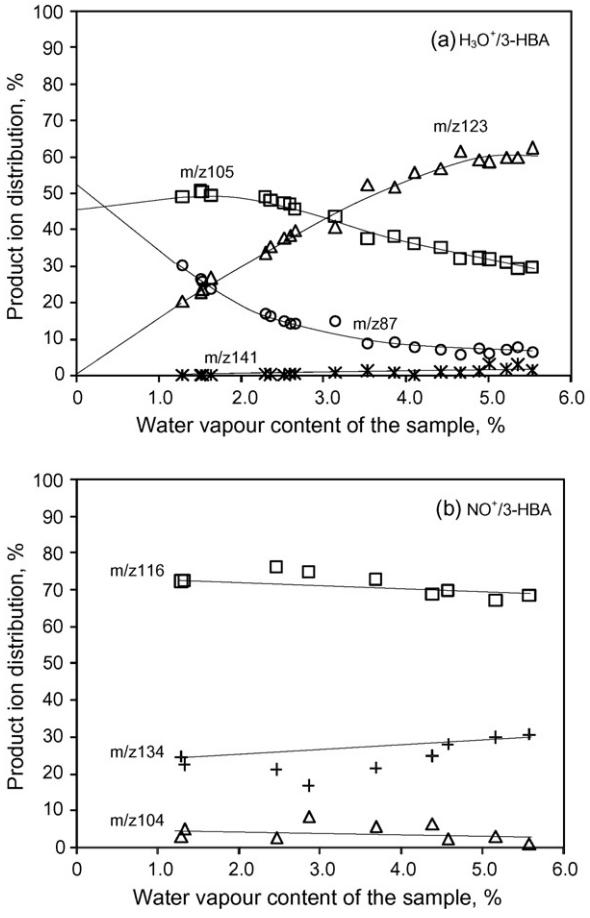

Fig. 3. The relative count rates of the product ions at the mass-to-charge ratio, m/z, values indicated expressed as percentages, as 3-hydroxybutyric acid, 3-HBA, is introduced into the carrier gas of the SIFT-MS instrument and as the humidity of the sample is varied and measured according to the description given in Fig. 2, in (a) using H_3O^+ precursor ions and in (b) using NO^+ precursor ions. The designations for the ions at each m/z value are indicated in Table 2.

Table 2
The product ion distributions in percent for the reactions of H_3O^+ and NO^+ with the hydroxy acids indicated in the presence of water vapour at the absolute humidities typical of laboratory air (1.5%) and moist air (5.5%), close to that of exhaled breath and aqueous liquid headspace at 37 °C

Molecule[a]	H_3O^+ Product	Lab air 1.5% H_2O	Moist air 5.5% H_2O	NO^+ Product	Lab air 1.5% H_2O	Moist air 5.5% H_2O
2-Hydroxybutyric acid	$C_4H_8O_3H^+H_2O$(*m/z* 123)	65%	60%	$NO^+C_4H_8O_3$ (*m/z* 134)	100%	100%
	$C_4H_8O_3H^+$ (*m/z* 105)	20%	5%			
$CH_3CH_2CH(OH)COOH$	$C_4H_7O_2^+$ (*m/z* 87)	5%	5%			
	$C_4H_8O_3H^+(H_2O)_2$(*m/z* 141)	10%	30%			
3-Hydroxybutyric acid	$C_4H_8O_3H^+H_2O$(*m/z* 123)	25%	60%	$NO^+C_4H_8O_3$ (*m/z* 134)	15%	30%
	$C_4H_8O_3H^+$ (*m/z* 105)	50%	30%	$C_4H_6O_2NO^+$ (*m/z* 116)	75%	65%
$CH_3CH(OH)CH_2COOH$	$C_4H_7O_2^+$ (*m/z* 87)	25%	5%	$C_4H_8O_3^+$ (*m/z* 104)	5%	5%
	$C_4H_8O_3.H^+(H_2O)_2$(*m/z* 141)	0%	5%			
2-Hydroxyisobutyric acid	$C_4H_8O_3H^+H_2O$(*m/z* 123)	55%	75%	$NO^+C_4H_8O_3$ (*m/z* 134)	100%	100%
	$C_4H_8O_3H^+$ (*m/z* 105)	35%	10%			
$(CH_3)_2C(OH)COOH$ IE = 10.9 eV	$C_4H_7O_2^+$ (*m/z* 87)	5%	0%			
	$C_4H_8O_3H^+(H_2O)_2$(*m/z* 141)	5%	15%			

[a]Both the mass-to-charge ratios, *m/z*, of the product ions and their molecular formulae are given. IE is the ionisation energy of the molecule [26].

product ion:

$$NO^+ + CH_3CH(OH)CH_2COOH(+He) \rightarrow NO^+C_4H_8O_3 + (He) \quad (2a)$$

$$NO^+ + CH_3CH(OH)CH_2COOH(+He) \rightarrow NOC_4H_6O_2^+ + H_2O \quad (2b)$$

$$NO^+ + CH_3CH(OH)CH_2COOH(+He) \rightarrow C_4H_8O_3^+ + NO \quad (2c)$$

Reaction (2a) is the helium-mediated three-body association reaction producing NO^+M and reaction (2b) is the result of the loss of a water molecule from the nascent $(NO^+M)^*$ ion. Note that the major product ion over the range of humidities is that formed in reaction (2b) appearing at a mass–charge ratio, *m/z*, of 116. Reaction (2c) is the result of charge transfer between the NO^+ and M producing M^+. That the last reaction occurs for thermalised reactants at 300 K (the conditions in the SIFT-MS flow tube) indicates that the ionisation energy of 3-HBA is close to that of the NO molecule, which is 9.24 eV [26]. Of the three hydroxy acids the ionisation energy of only the 2-hydroxyisobutyric isomer is reported and is 10.9 eV [26], which is consistent with the energetics i.e., charge transfer cannot occur between this hydroxyacid molecule and NO^+ ions. That a single product ion NO^+M results from the NO^+ reactions with the other two isomers and this adduct ion is a minor product of the 3-HBA reaction over the range of humidities explored offers a way to distinguish the 3-HBA from the 2-hydroxy isomers.

The relative signal levels of the various product ions for the three reactions of H_3O^+ and NO^+ precursor ions under the particular conditions of the SIFT-MS instrument are given in Table 2 at just two absolute humidities that are typical of room air (1.5%) and exhaled breath and aqueous liquid headspace at a temperature of 37 C (6%), since these are the most commonly used conditions for trace gas analysis. As mentioned above, for accurate SIFT-MS analyses it is only necessary to include all the product ions for each precursor ion. But sometimes for the analysis of complex mixtures it is necessary to use just one of the product ions to avoid overlapping peaks (see Refs. [11,12]). Then scaling factors must be used, as derived from laboratory data such as those given in Table 2, to account for the omission of some product ions. This is exactly the situation in analysing urine headspace for 3-HBA, because there are potential overlaps between the product ions formed when these are analysed in the presence of pentanone, as we discuss below.

3.2. Analysing headspace of aqueous solution of 3-hydroxybutyric acid

A solution of approximately 0.1 mL of 3-HBA in 20 mL of water was made up equating to a liquid concentration of about 50 mmol/L. By dilution of this, solutions of 5, 10, 20 and 40 mmol/L were made up and 10 mL of these were placed into glass bottles sealed with septa. The bottles were placed into a water bath held at a temperature of 40 C and the headspace of each was analysed for both 3-HBA and acetone by SIFT-MS using both H_3O^+ and NO^+ precursor ions. Thus, the rate coefficients for the hydroxyacid given in Table 1 and the observed product ions indicated in Table 2 were included in the SIFT-MS kinetics library. The kinetic data entry for acetone is ever present in the SIFT-MS kinetics library, being utilized for the on-going studies we are carrying out on breath acetone [12,22,30].

3-HBA is indeed seen to be present in the headspace of its aqueous solution and these SIFT-MS measurements indicated that a liquid phase concentration of 20 mmol/L equates to a headspace partial pressure of 10 parts-per-billion, ppb, when using NO^+ precursor ions and 15 ppb when using H_3O^+ precursor ions. This discrepancy most probably results from the assumption that the rate coefficient used for the NO^+ reaction with 3-HBA is the collision rate coefficient as given in Table 1, which is seemingly too high. It is not unusual for effective two-body ion-molecule association rate coefficients to be less than

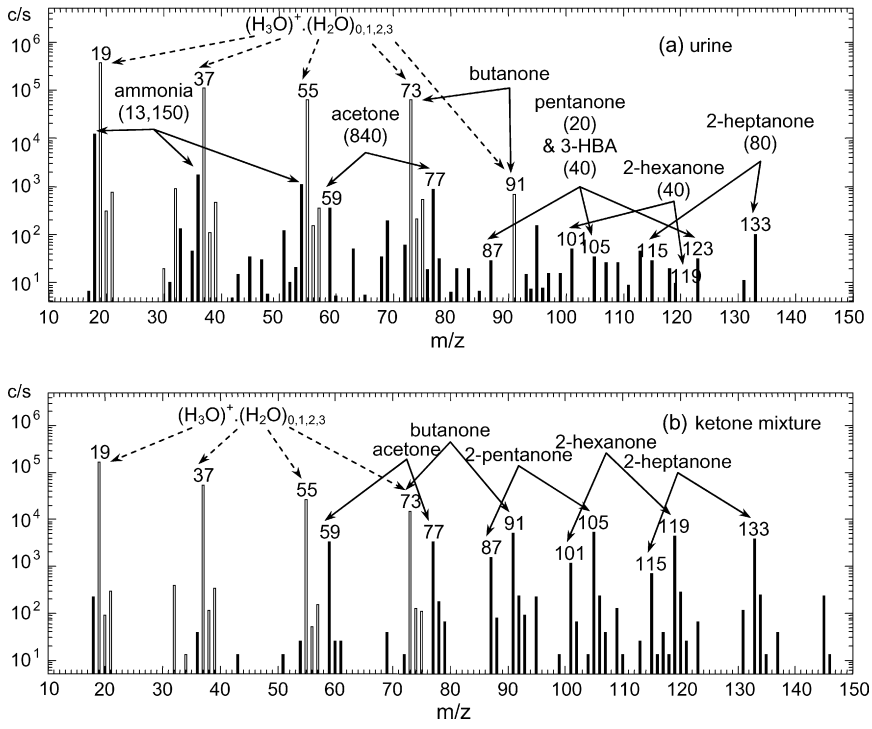

Fig. 4. Following Fig. 1, full scan (FS) SIFT-MS spectra of ion count rate per second, c/s, against mass-to-charge ratio, *m/z*, obtained using H_3O^+ precursor ions when (a) the headspace of urine and (b) humid air containing the ketones indicated are sampled into the SIFT-MS instrument. The number in parentheses below each compound in (a) is the estimated concentration of the compound in parts-per-billion, ppb. Note the overlap of some peaks for 3-HBA and 2-pentanone.

the collision rate coefficients [18,31,19,20]; indeed, to repeat, this is the case for the association reaction of NO^+ with acetone [31,19,20] that is used to measure the headspace concentrations of this compound. So we expect the higher concentration of the 3-HBA given by the H_3O^+ analysis to be closer to the proper value. The acetone level in the headspace of this solution is somewhat lower than that of the 3-HBA at 10 ppb for a liquid phase 3-HBA concentration of 20 mmol/L, but when the liquid is acidified using 1 mL of 0.1 molar hydrochloric acid, moving the pH of the liquid down to 2, then the acetone and 3-HBA levels reverse, that of 3-HBA being about 5 ppb and that of acetone being about 10 ppb. We tentatively interpret this as being due to enhanced hydrolysis of the 3-HBA, which results in the further production of acetone:

$$CH_3CH(OH)CH_2COOH \rightarrow CH_3COCH_3 + H_2O + CO_2 \qquad (3)$$

3.3. Analysing urine samples

Two volunteers provided mid-stream samples of urine, which were immediately analysed in an identical manner to those of the aqueous solutions, as described above. Firstly, FS spectra were taken using both H_3O^+ and NO^+ precursor ions to identify the major product ions of the reactions of the trace gases in the headspace of the urine. Most obvious species present are ammonia, acetone and higher order ketones. Of great interest is the presence of the characteristic ions of 3-HBA, as indicated in the sample spectra shown in Figs. 4a and 5a. The sequence of ions seen in the homologous series beginning with acetone at *m/z* 59 (continuing with 73, 87, 101 and 115) using H_3O^+ and starting at *m/z* 88 (continuing with 102, 116, 130 and 144) using NO^+ are surely those ketones indicated in the figures. We have not previously observed ketones other then acetone in urine headspace using SIFT-MS, although they have been detected in urine using gas chromatography mass spectrometry [32]. The observation of this ketone series is probably due to the increased sensitivity of current SIFT-MS instruments.

Whilst the ketones were not a major concern of this paper, it became clear that there are product ion at common *m/z* values for 3-HBA and pentanone in their reactions with both H_3O^+ and NO^+ precursor ions. Therefore, it became imperative to look at the ketone reactions and product ions produced under the conditions existing in this SIFT-MS instrument. So an aqueous cocktail of approximately equal amounts of five ketones, M, was made up and a few drops of this cocktail were injected into a Nalophan bag (made up from of wall thickness 25 microns supplied by Kalle UK Ltd, to have an inflated volume of about 1 L) which was then inflated with clean cylinder air. FS spectra were taken as this humid gas mixture was introduced into the helium carrier gas of the SIFT-MS instrument. The spectra obtained using both H_3O^+ and NO^+ are shown as Figs. 4b and 5b below the corresponding urine FS spectra. As can be seen, using H_3O^+ ions both MH^+ product ions (ketone·H^+) and the corresponding MH^+H_2O monohydrate ions are seen, as expected, and traces of the $MH^+(H_2O)_2$ dihydrate ions are also present (importantly, <1% of the total product ions for pentanone; see below). Using NO^+ the spectra are simpler in that only the adduct ions, NO^+M, are formed, which do not readily form hydrates; indeed, there is barely a trace of NO^+MH_2O ions in the spectra (see Fig. 5b). So this well illustrates why NO^+

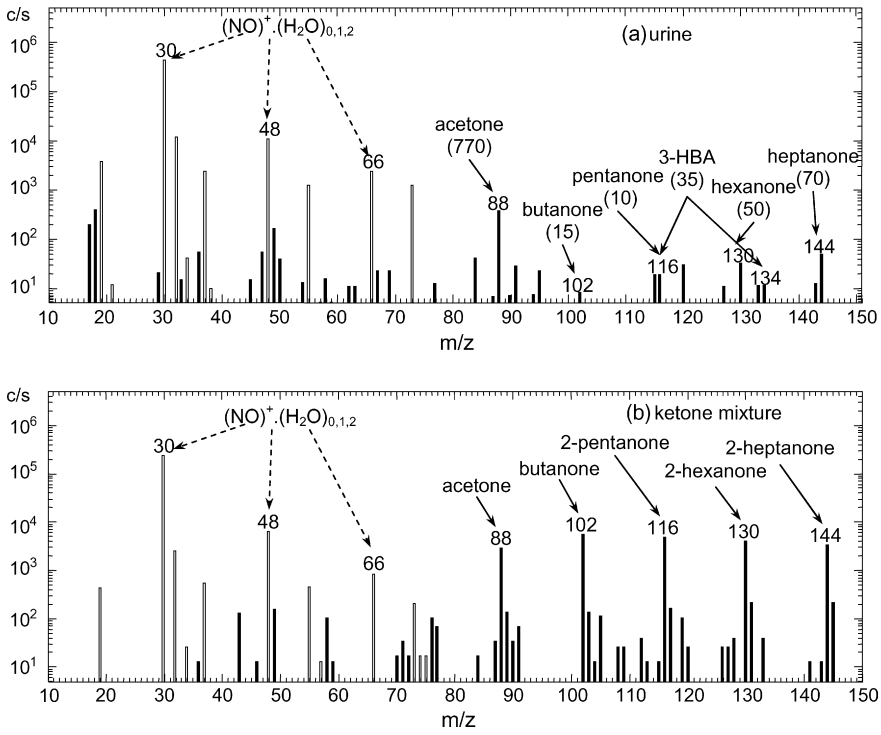

Fig. 5. Following Fig. 1, full scan (FS) SIFT-MS spectra of ion count rate per second, c/s, against mass-to-charge ratio, *m/z*, obtained using NO^+ precursor ions when (a) the headspace of urine and (b) humid air containing the ketones indicated are sampled into the SIFT-MS instrument. The number in parentheses below each compound in (a) is the estimated concentration of the compound in parts-per-billion, ppb. Note the overlap of some peaks for 3-HBA and 2-pentanone.

is the preferred precursor ion for the analysis of ketones using SIFT-MS [12,19,20,31]. Indeed, H_3O^+ cannot be used to analyse butanone, because the MH^+ product ion at *m/z* 73 overlaps with the trihydrate ion $H_3O^+(H_2O)_3$ which is always relatively large in the H_3O^+ spectra.

Further inspection of the urine spectra and the corresponding spectra of the ketone cocktail, together with the data given in Table 2, shows that although there are indeed overlaps between the 3-HBA and pentanone product ions, importantly there are ions that are characteristic of the 3-HBA, viz. *m/z* 123 for H_3O^+ and *m/z* 134 for NO^+ precursor ions. So using appropriate branching ratios to account for the overlapping ions (see Table 2) the level of 3-HBA in the urine headspace can be estimated using these ions only. Note, again, that it is a more accurate procedure to use the MIM mode of analysis rather than analysing FS spectra such as shown in Figs. 4a and 5a. If there is a need to distinguish the isomers of 2-hydroxybutyric acids and 3-HBA then *m/z* 116 can be used as a characteristic ion of 3-HBA.

The acetone levels in the urine headspace for one volunteer were measured as 600 ppb and for the other as 800 ppb. Essentially parallel measurements of breath acetone resulted in acetone levels within 10% of the urine levels. This remarkable result indicates that the acetone is clearly systemic and equilibrated amongst the body fluids. Recent detailed studies of several breath metabolites by sampling exhaled breath via the mouth and via the nose indicate that acetone is indeed systemic and is not produced in the oral cavity, as are several other metabolites, including ammonia and ethanol [33]. The other ketones in the homologous series (pentanone, hexanone and heptanone), which are present in the urine headspace are at much lower levels, typically at 20–80 ppb.

Both H_3O^+ and NO^+ precursor ions were used to analyse the urine headspace of the fresh urine and low levels of 3-HBA were detected in the samples from both volunteers. However, on acidifying the urine, again to a pH close to 2, then both precursor ions revealed the presence of 3-HBA in the samples from both volunteers at levels of typically 40 ppb, some 20 times lower concentration than that of acetone. Unlike the aqueous solutions of the 3-HBA discussed above, increases in the acetone level on acidification could not be clearly discerned, because of the relatively high acetone levels in the headspace (given above). However, small increases (maximum about 40%; see also Ref. [32]) in the levels of the ketones in the headspace of the aqueous ketones cocktail were observed on acidification and similar small decreases were observed on alkalation with NaOH. These results require further investigation. Neither 3-HBA nor any ketones other than acetone were detected in the exhaled breath of either volunteer, but further studies of this are planned concomitant with the expected increase in the sensitivity of SIFT-MS instruments.

4. Concluding remarks

This study of the ion chemistry of hydroxbutyric acids, and in particular the biologically important 3-hydroxybutyric acid, 3-HBA, has provided the required kinetic data to allow a search for and the successful detection of 3-HBA in the headspace of urine samples from two healthy volunteers. The significance of

this work is that 3-HBA is one of the ketone bodies (the other two being acetoacetatic acid and acetone) important in the diagnosis of ketoacidosis [9]. Thus, we are now able to study the levels of 3-HBA, in parallel with acetone, in the headspace of urine of patients with known clinical diseases, including diabetes. The current detection limit of 3-HBA in urine headspace using SIFT-MS is a few ppb, which equates to a liquid phase concentration of 1 or 2 mmol/L and is thus comparable to the urine concentrations typical of healthy individuals [34]. It is reported that for diabetic patients the urine levels of 3-HBA can be as high as 300 mmol/L. This equates to headspace levels of several parts-per-million, ppm, which is easily quantified by SIFT-MS. The cut-off concentration in urine when considering the onset of ketoacidosis is 5 mmol/L [9]. This also equates to a headspace level easily accessible to SIFT-MS analysis, thus providing a very valuable and rapid analytical method for the detection of 3-HBA along with acetone in urine. Unfortunately, we have been unable to acquire kinetic data for acetoacetic acid, the other recognised ketone body, because only its lithium salt is readily available and this hydrolyses and decomposes to acetone in the presence of water vapour, indicating that acetoacetic acid is unstable in the gas phase.

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