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Direct atmospheric pressure chemical ionisation ion trap mass spectrometry for aroma analysis: Speed, sensitivity and resolution of isobaric compounds

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Abstract

Atmospheric pressure chemical ionisation (APCI) sources were developed for real time analysis of volatile release from foods using an ion trap (IT) mass spectrometer (MS). Key objectives were spectral simplicity (minimal fragmentation), response time and signal to noise ratio. The benefits of APCI-IT-MS were assessed by comparing the performance for in vivo and headspace analyses with that obtained using APCI coupled to a quadrupole mass analyser. Using MS–MS, direct APCI-IT-MS was able to differentiate mixtures of some C6 and terpene isobaric aroma compounds. Resolution could be achieved for some compounds by monitoring specific secondary ions. Direct resolution was also achieved with two of the three isobaric compounds released from chocolate with time as the sample was eaten.

Keywords: Ion trap MS; MS–MS; Terpene; Terpenoid; Aroma; Isobaric; In vivo

1. Introduction

Gas phase analysis of volatiles by direct mass spectrometry (DMS) has been applied to various research areas including aroma [1], air pollution [2,3], human breath [4] and medical analysis [5]. DMS is defined here as the direct introduction of analyte mixtures into the MS source with no prior separation or concentration. The interest in our laboratory is measuring aroma release in vivo. By sampling exhaled air from the nostril of a person (whilst consuming a food) directly into the MS source, time-release curves of aroma compounds can be obtained at low levels (nL aroma per L air) over the time of a single breath (typically 5 s) [6]. The data obtained from such studies are important for flavour scientists as they give a measure of aroma release from foods under real physiological conditions and can relate well to perceived aroma [7].

In vivo aroma analysis is particularly challenging for direct MS as both speed, sensitivity and resolution are required for high quality, time-release curves. Direct MS of volatile compounds has been reported by different ionisation techniques, atmospheric pressure chemical ionisation (APCI) [8], proton transfer reaction (PTR) [5] and selected ion flow tube (SIFT) [9]. In APCI, molecules are ionised using the "soft" proton transfer mechanism and protonated molecules dominate the spectra. Identification of the molecular species present is difficult, especially if isobaric compounds are present. Aromas typically contain isobaric compounds, which can have very different odours and the capability to measure these compounds would be desirable for flavour scientists.

In this paper, a gas phase interface for a commercial ion trap (IT) MS was developed by comparing spectral simplicity, response time and signal to noise ratios from three interfaces. The performance of the IT-MS fitted with the selected APCI source was then compared with a commercial APCI-quadrupole MS. The potential of MS–MS to discriminate between some positional isomers of aroma compounds was also tested on a series of aroma terpenes and terpenoids (as

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well as some oxygenated compounds containing six carbon atoms). Given the molecular size of the analyte molecules (30–250 Da) the strategy adopted with IT-MS was to form the protonated molecule by APCI, then fragment once to provide structural information. The last part of this work evaluated whether IT-MS was sufficiently sensitive and fast enough to perform breath-by-breath analysis in MS–MS mode on real food samples.

2. Experimental

2.1. Ionisation source description

APCI ionisation sources were evaluated on a Thermo Finnigan LCQ Deca Xp ion trap mass spectrometer. The standard liquid phase orthogonal APCI source provided with the IT-MS was modified for gas phase analysis following similar principles to those described elsewhere [10,11]: the vaporisation nozzle was removed and replaced with a PTFE tube delivering nitrogen flow and creating a venturi region around the sampling tube.

An in-line source, capable of operating at reduced and atmospheric pressure was built (Fig. 1a and b). In all the sources, the gas phase sample was introduced via a deactivated fused silica capillary (0.53 mm i.d., Supelco) held at 150 °C to minimize dead volume and prevent condensation of the volatile compounds. Except for the reduced pressure source, all the sampling was achieved by a venturi effect around the end of the sampling capillary [12]. Flow rate could be controlled by adjusting the position of the capillary tube in the venturi and by changing the nitrogen flow rate.

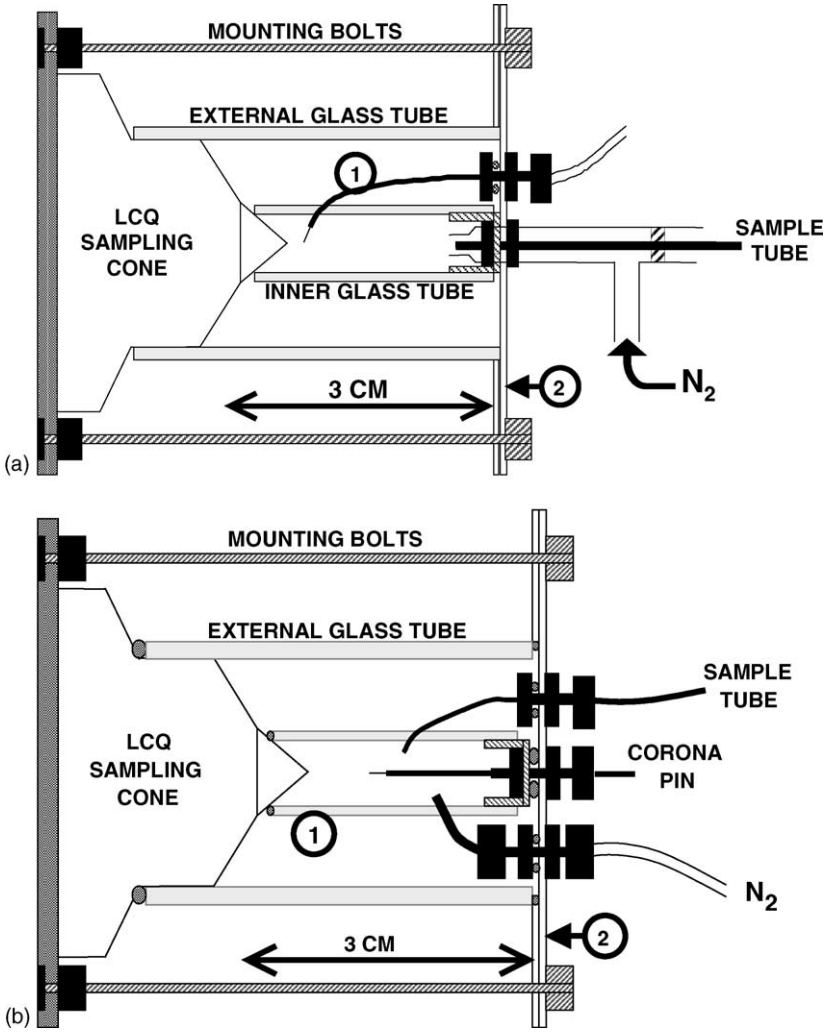

Fig. 1. (a) In-line atmospheric pressure ionisation source. The gas sample is introduced via the deactivated silica sampling tube and "pumped" into the source by the flow of nitrogen through a Venturi effect. Excess gas exits via the gap between the inner glass tube and sampling cone. Ionisation occurs within the inner glass tube (volume 1 mL) and ions are then transported to the ion trap through the standard LCQ sampling cone. (1) Corona pin, (2) Perspex plate. (b) In-line reduced pressure ionisation source. The gas sample is introduced via the deactivated silica sample tube and a flow of nitrogen is introduced separately. Pressure in the source is a balance between the pumping effect through the sampling cone (around 1.5 L/min) and the amount of gas delivered via the sample and nitrogen lines. Ionisation occurs within the internal glass tube (volume about 1 mL) which is sealed to the sampling cone by an O ring. Ions formed are then transported to the ion trap through the LCQ sampling cone. (1) Inner glass tube and (2) Perspex plate.

The quadrupole MS used for the comparison with IT-MS was a LCZ Micromass-Waters machine fitted with the APCI MS-NOSE^{TM} interface [6].

2.2. Analyser settings

The LCQ Deca Xp conditions were the same for all sources (mass range *m/z* 30–200; multipole RF amplitude 120 V; ion transfer tube 3 V and 150 °C; tube lens offset −20 V). To maximise the frequency of data collection, the IT-MS spectra were not averaged; however, this resulted in some loss of signal stability and lower signal to noise values.

2.3. Source comparison

Four compounds with different physical, chemical and fragmentation properties were chosen for study; 2-methylbutanal (Acros, Loughborough, UK), 2,5-dimethylpyrazine (Aldrich, Gillingham, UK), 1-hexanol (Sigma, Poole, UK) and ethyl butyrate (Firmenich SA, Geneva, Switzerland). Each compound was dissolved separately in water to give a headspace concentration of 100 nL/L based on their respective air/water partition coefficient [13], converted from their Henry's law constant (Table 1). The relation between the concentration in liquid (μL/L) and the headspace concentration (nL/L) is given in Eq. (1).

$$C_w = \frac{C_a MW}{22.4 \times 10^6 K_{aw} d} \quad (1)$$

where C_w is the concentration of the molecule in water in μL/L, C_a the concentration of the molecule in air in nL/L, *MW* the molecular weight in grams, K_{aw} the dimensionless air/water partition coefficient and *d* the density in g/mL. The value of 22.4×10^6 includes a coefficient to convert the headspace concentration from μL/L into nL/L and a conversion for the density value. Headspace from above the standard solutions was sampled at 5 mL/min into the ionisation sources and samples were initially analysed in triplicate with the source removed and re-mounted for each replicate, in case this caused subtle variation in ionisation source geometry and changes in performance. No significant differences were noted with source remounting. Each ionisation source was evaluated with the aim of obtaining minimal fragmentation, a dynamic response to sample introduction and maximal signal to noise values.

2.4. MS–MS of isobaric compounds

For the evaluation of isobaric compound resolution by MS–MS, headspace was sampled from aqueous solutions of the following compounds: β-Citronellal (Sigma), γ-terpineol, *trans*-2-hexenal, 1,8-cineole, terpinolene, linalool, menthone, α-pinene, β-pinene, β-myrcene, 4-hexen-3-one, *trans*-2-hexen-1-ol, *cis*-3-hexen-1-ol, 2-hexanone, 2-hexanol (Aldrich) and (+)−limonene, hexanal, 1-hexanol (Acros). Since it was a qualitative study, solutions were prepared at concentrations that gave a satisfactory signal on the MS.

3. Results and discussion

3.1. Principles for construction of a gas phase APCI source

APCI is a simple ionisation process which proceeds via several stages. The first stage can be generalised as the formation of reagent ions (H_3O^+) and the second is the transfer of protons from the reagent ions to the volatile compounds. The geometry of the source is important to perform these stages efficiently and therefore achieve good sensitivity [14–16]. Focusing the charge onto one ion species is preferred for optimum sensitivity and spectral simplicity.

The APCI source supplied with the Thermo Finnigan IT-MS was designed for liquid samples and had a relatively large volume chamber to allow for solvent vaporisation along with orthogonal delivery of ions to the sample cone to exclude potentially undesired contaminants. For analysis of volatile compounds in the gas phase, a source with a smaller volume can be constructed and this can be "in-line" rather than orthogonal as there are no contaminating compounds present. A low volume ionisation source will minimise "tailing" from sample signals, concentrate the sample around the corona pin and minimise the spreading of the analyte ions from the corona discharge [16].

A low volume in-line source was constructed to operate at atmospheric (Fig. 1a) or reduced pressure (Fig. 1b). Op-

Table 1
Air/water partition coefficients (estimated from Henry's law constant) and calculated concentration of aroma compounds required in water to give 100 nL/L in headspace

Compound	CAS	*MW* (Da)	Density (g/mL)	Converted ($K_{aw} \times 10^3$)	Compounds concentration in water to give 100 nL/L in headspace (μL/L)
Ethyl butyrate	105-54-4	116.16	0.879	15[a]	0.039
2-Methyl butanal	96-17-3	86.13	0.804	6.5[b]	0.074
1-Hexanol	111-27-3	102.17	0.814	0.68[a]	0.82
2,5-Dimethylpyrazine	123-32-0	108.14	0.990	0.14[b]	3.340

The density of the aroma compounds was included in the calculation.
[a] Converted from reference [25].
[b] Converted from reference [24].

erating conditions were optimised by monitoring the water cluster ions at different sample and nitrogen flow rates. The monomer and dimer water ions are most efficient at proton transfer [2,14,15] so conditions that produce these species were favoured. At atmospheric pressure, a nitrogen flow of 3 L/min and a sample flow rate in the range 5–20 mL/min showed no signs of the higher water cluster ions ($(H_2O)_nH^+$) at m/z 55 ($n = 3$) and 73 ($n = 4$), and the dimer (m/z 37) was the major ion. The IT-MS system did not detect the protonated water monomer (m/z 19) as its mass was below the cut-off level for the ion trap.

The effect of running the in-line source under reduced pressure was also studied. Reducing the pressure will modify many parameters, such as gas phase collision rates (including production of H_3O^+ and proton transfer reactions), ion velocities, residence times in the ionisation region and corona discharge electrical field. Hence, the gas phase ion chemistry involved in sample ionisation is altered by changing the pressure, and the net effect of operating the in-line source under reduced pressure was determined experimentally. Initially, the ionisation source was operated at the lowest source pressure (i.e., with introduction of headspace from the solutions of volatile compounds with no nitrogen make-up gas). Under these conditions, neither water reagent ion signal nor sample signal was observed. Signal started to appear when nitrogen flow was around 200 mL/min and increased with increasing nitrogen flow. A lack of water reagent ion was not responsible for the poor signal observed, given the small amounts of water needed to promote ionisation in APCI sources. This was confirmed by addition of extra water vapour into the ionisation source which only led to more water cluster formation and was detrimental to overall performance as reported by Sunner et al. [14]. The lack of signal was probably due to poor transmission of ions into the MS as the pressure in the ionisation region was very similar to the pressure in the first vacuum stage of the MS, and ions were therefore not drawn into the MS by differential pressure. Measurement of flow rate into the IT-MS through the sampling orifice showed a maximum transmission of 1.5 L nitrogen/min and, with a flow of 1 L nitrogen/min into the source, the best sensitivity was observed. This nitrogen flow represented a compromise between the amount of analyte drawn into the ionisation source by differential pressure (APCI sensitivity is determined by amount in the source, not concentration) and the transmission of ions from the source to the IT-MS. Under these conditions, the convectron pressure gauge in the ion transfer tube showed a reading of 1.2 torr, just below that measured for full atmospheric introduction of sample. The results showed that there was a narrow operating window in which good sensitivity was achieved.

3.2. Ionisation source comparison

3.2.1. Spectral simplicity

With the conditions in the reduced pressure source now set, the fragmentation of four different compounds (2-methylbutanal, 2,5-dimethylpyrazine, 1-hexanol and ethyl butyrate) was studied with the three ionisation sources (Table 2). All three sources showed similar patterns of fragmentation from the four test compounds. 2,5-

Table 2
Fragmentation of four test aroma compounds in the three ionisation sources compared on IT-MS

Compound/source	Ethyl butyrate	2-Methyl butanal	1-Hexanol	2,5-Dimethylpyrazine
Modified manufacturer's APCI	117 (100%)	87 (100%)	85 (100%)	109 (100%)
	89 (12%)	69 (32%)	101 (16%)	
		45 (38%)	121 (9%)	
[0,1-5]				
In-line APCI	117 (100%)	87 (100%)	85 (100%)	109 (100%)
	89 (5%)	69 (30%)	101 (18%)	
		45 (30%)	121 (25%)	
[0,1-5]				
In-line APCI reduced pressure	117 (100%)	87 (100%)	85 (100%)	109 (100%)
		69 (26%)	101 (11%)	
		45 (17%)	121 (35%)	
		105 (12%)		
[0,1-5]				
MS nose	117 (100%)	87 (100%)	85 (100%)	109 (100%)
		69 (10%)	57 (10%)	
		45 (15%)	43 (5%)	
[0,1-5]				
PTR	117 (100%)[a]	87 (100%)[b]	43 (100%)[a]	109 (100%)[c]
	89 (53%)	69 (34%)	85 (53%)	
	43 (14%)	45 (64%)	57 (36%)	
			41 (33%)	

Results were also compared with fragmentation reported in two commercial sources: PTR-MS (values obtained from literature) and MS nose (experimental values). Values show the relative proportions of ions expressed as a percentage of the base peak. Fragments below 5% not included.

[a] From reference [26].

[b] From reference [27].

[c] Assumed from fragmentation of other pyrazines from reference [27].

Dimethylpyrazine did not fragment in any of the sources under the conditions used. Protonated ethyl butyrate fragmented to form *m*/*z* 89 while 1-hexanol and 2-methyl butanal showed more complex fragmentation and adduction patterns. The protonated molecule was the most abundant species for 2-methyl butanal, with a fragment corresponding to the loss of water at *m*/*z* 69, adduction of water at *m*/*z* 105 and an ion at *m*/*z* 45 from the α-cleavage of the carbonyl group. The most abundant ion for 1-hexanol was at *m*/*z* 85 corresponding to the loss of water. The ion at *m*/*z* 121 was a water adduct and the fragment at *m*/*z* 101 suggests loss of a hydrogen molecule. Lowering the pressure in the ionisation region did not decrease fragmentation. On some other APCI–MS systems, the sampling cone voltage can play a major role in fragmentation but, with the IT-MS system [6], varying the sampling cone voltage (capillary tube voltage) did not have an impact on fragmentation until high values (above 25 V) were reached when it led to more fragments. As a comparison, fragmentation patterns obtained for these molecules with a commercial PTR-(Ionicon, Innsbruck, Austria) and an APCI-quadrupole machine (MS-NOSE^{TM}, Micromass) are also presented in Table 2. The MS-NOSE^{TM} shows the lowest fragmentation as cone voltage could be optimised for each compound to minimise fragmentation and therefore maximise the protonated molecule intensity.

3.2.2. Rise and decay time

Rise and decay times for the four compounds on the three ionisation sources were determined as the time taken for the signal from the headspace of the four test compounds (100 nL/L) to reach 90% of the maximum values and time taken to decay to 10% of the maximum value. For all the ionisation sources, ethyl butyrate showed the fastest rise and decay times whereas 2,5-dimethylpyrazine was the slowest; the other compounds lay in between these extremes. For the modified manufacturer's source the rise and decay times were respectively 5.2 and 5.8 s for ethyl butyrate and 7.8 and 15 s for 2,5-dimethylpyrazine. For both in-line sources, the values decreased to 0.4 s (rise and decay for ethyl butyrate) and 0.9 and 1.8 s for 2,5-dimethylpyrazine. The differences between the sources can be explained by the differences in dead volume. Raising the temperature of the source and sampling tube might further speed up the times obtained, but it could also cause thermal degradation.

3.2.3. Signal to noise

Fig. 2 compares the average signal height, noise and signal to noise values of the most intense ions of the four compounds studied in the three sources. Noise was determined by calculating the standard deviation over 50 baseline data points, and averaged for each source mounting. The signal height reported, included the subtraction of the noise height.

The modified manufacturer's source showed low signal level and hence, overall poor signal to noise values. The in-line source operating under reduced pressure gave signal heights that were twice those obtained under atmospheric

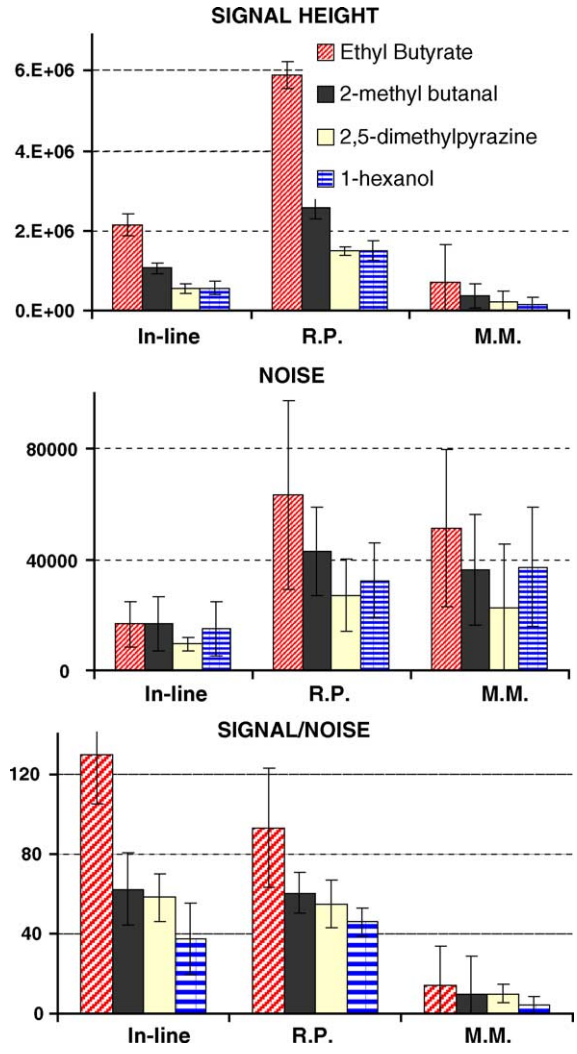

Fig. 2. Average signal height, noise and signal to noise values for all compounds on each source. In-line: in-line APCI source, R.P.: in-line reduced pressure ionisation source, M.M.: modified manufacturer APCI source.

conditions. The increase in signal height may be due to a more favourable ion chemistry condition for analyte ionisation or the fact that, for this source, the whole sample entered the MS, ionised or not. However, the reduced pressure in-line source also exhibited a higher noise level and, overall, the source working at atmospheric pressure actually gave better performance. All sources resulted in the same order of signal intensity for the four compounds with ethyl butyrate having the highest signal height, followed by 2-methylbutanal, and 2,5-dimethypyrazine and 1-hexanol having the same signal height.

Comparison of spectral simplicity, response time and signal to noise ratios showed that low dead volume sources are ideal for gas phase analysis and the in-line APCI source, operating at atmospheric pressure was the most suitable interface for gas phase aroma analysis. It offered good performance, coupled with the fact it was easy to operate and was used for subsequent experiments.

3.3. Comparison of speed and sensitivity with ion trap and quadrupole mass analysers

The in-line APCI-IT-MS system was compared to a quadrupole mass spectrometer fitted with the MS Nose™ APCI interface [6]. For aroma analyses, the ion trap has two potential advantages over a quadrupole mass analyser. First, ion trap can perform MS^n analysis and secondly, in full scan mode, it has a faster scan rate and duty cycle compared to a quadrupole analyser. On the other hand, quadrupole MS systems operate faster and are more sensitive in single ion monitoring mode.

Each MS was set to its optimum performance mode. The quadrupole mass analyser was set in single ion monitoring mode and the data point collection interval (DPCI) was controlled by the dwell time and the interscan delay, which could be both set at minimum values of 10 ms, leading to a minimal quadrupole DPCI of 20 ms. The ion trap MS was operated in full scan mode as speed is the same whether working in single ion or full scan mode. However, modifying the DPCI is not as straightforward as for the quadrupole analyser. Automatic gain control (AGC) is an option controlling the optimum ion injection time to avoid space charging effects if too many ions enter the trap [17]. DPCI was decreased by reducing the maximum ion injection time, imposing an upper limit for AGC. The fastest DPCI obtained with the ion trap mass analyser was 140 ms, even for an injection time of a few milliseconds. This relative slowness was due to the time required for the AGC prescan and the electronic calculation between data collection which limited the data point frequency of the ion trap, rather than the ion trapping time itself.

Given the different operating principles, and given that in vivo aroma analysis requires speed with sensitivity, the parameter studied was the sensitivity achieved as a function of the data point frequency. This was determined by individually analysing the four test compounds at headspace concentrations of 100, 50, 20, 10, and 5 nL/L on each MS system over a range of DPCI values. From these plots, the DPCI value at which the signal to noise ratio was 10 (limit of quantification), was calculated for each compound at each concentration and plotted in Fig. 3. The 100 nL/L point has been excluded from the plots to show the region of interest more clearly. Fig. 3 shows that the quadrupole could achieve good sensitivity at DPCI values down to 100 ms; the ion trap system gave similar sensitivity but only above a DPCI value of 200 ms. For in vivo monitoring of volatile release, speed of analysis is important as the release profile is governed both by the time of a breath (typically 5 s) and the mastication frequency (typically 0.5–1 chews per second). For monitoring a single ion, the quadrupole mass analyser will offer the advantage of speed and sensitivity. However, it is worth noting that the two systems become comparable when seven ion species are scanned simultaneously as the DPCI of the quadrupole then becomes 7 × 20 or 140 ms equivalent to the DPCI of the ion trap with AGC ON

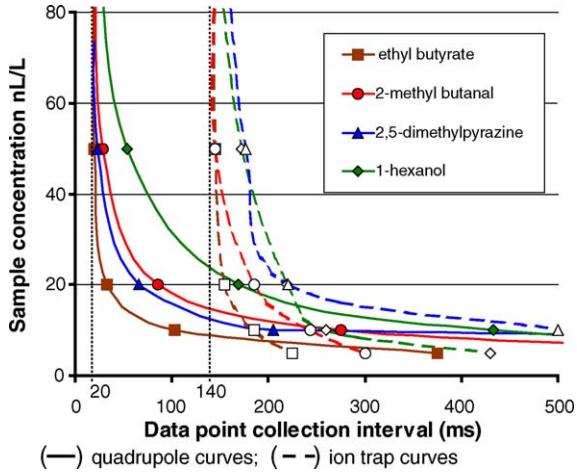

Fig. 3. Limits of quantification (set as $S/N = 10$) as a function of data point collection interval and sample headspace concentration for four aroma compounds in a quadrupole and IT mass analyser. The quadrupole mass analyser was operated in single ion mode and the ion trap mass analyser in full scan mode with AGC on. Each sample was run in triplicate and the data points are the mean values; variation was typically 30% for the Quadrupole and 16% for the Ion trap.

in full scan mode. Thus, when more than seven ion species are scanned, the IT-MS system provides the better sensitivity.

The effect of switching AGC OFF was also studied. This eliminated the prescan time required with AGC and the DPCI was only dependent on the time needed for the ions to enter the trap. Saturation of the ion trap was unlikely, given the dilute aroma concentrations. A faster DPCI of 80 ms was achieved by turning the AGC function OFF. However, noise levels also increased with slower DPCI, and there was no advantage in operating the IT-MS in the AGC OFF mode.

3.4. MS2 for the resolution of isobaric compounds

Two groups of isobaric compounds commonly encountered in the flavour of natural products were studied: the first group consisted of C6 compounds and the second group contained terpenes and terpenoids. Some of the MS2 fragments were generated using wideband activation, an option delivering a band of energy wide enough to fragment the parent ion and all masses down to 20 amu below [18]. This option is typically used when the fragment obtained in MS2 is only a loss of water. Wideband activation would further dissociate the resulting fragment (without going for MS3), and generate more fragments in MS2. The percentages of MS2 fragments are not reported below as the relative amounts depend on the conditions applied and the data are qualitative, not quantitative.

Eight C6 compounds with molecular weights (MW) of 98, 100, and 102 were subjected to APCI-IT-MS and the *m*/*z* values for the MS1 and MS2 ions recorded (Table 3). Some differentiation of compounds can be achieved by comparing the ion patterns in the MS1 and MS2 categories.

Table 3
MS1 and MS2 ions obtained on the APCI in-line source after introduction of headspace samples of some isobaric C6 aroma compounds Unstable in the MS2 column means that more than 90% of the parent ion was lost during the isolation process

Compound	Molecular weight	[0,3-4]MS1	MS2	
		m/z	%	
trans-2-Hexenal	98	57	10	29
		81	10	77, 53
		99	100	81, 57
4-Hexen-3-one	98	99	100	81,57, 43
trans-2-Hexen-1-ol	100	55	30	29, 39
		83	100	55
		99	20	83, 57
cis-3-Hexen-1-ol	100	83	90	55
		101	100	83, 55
Hexanal	100	83	30	55
		101	100	83, 55
		119	20	Unstable
2-Hexanone	100	101	100	83, 55, 45
2-Hexanol	102	85	100	57, 43
		101	20	83, 55
1-Hexanol	102	85	100	57, 43
		101	20	83, 73, 55
		121	25	Unstable

Both the unstable ions were water adducts, which were probably not stable enough to survive the isolation process.

The MS1 spectra for these compounds are composed of 2 or 3 ion species, mostly as a result of loss or adduction of water, except for the 2 ketones, which did not show any fragmentation. The MS1 spectra for the C6 compounds were distinct and provided some selectivity. However, in the in-line interface, some of the compounds produced MS1 ions which did not correspond to the protonated molecule. For instance, the hexanols did not give a protonated molecule at *m/z* 103, nor did they dehydrate as has been observed previously to form $[M–H_2O+H]^+$ but lost a hydrogen molecule to form an ion at *m/z* 101. This type of behaviour makes the monitoring of mixtures difficult as compounds not having the same molecular mass may give the same ions in MS1.

MS2 brought further selectivity for the mixture of C6 compounds. The two ketones gave specific spectra (2-hexanone MS1 ion at *m/z* 101, specific MS2 ion at *m/z* 45; 4-hexen-3-one MS1 ion at *m/z* 99, specific MS2 ion at *m/z* 43) and could be resolved from all the other molecules presented in Table 4. 1-Hexanol gave a MS1 ion at *m/z* 101 and was the only compound to yield a fragment at *m/z* 73. For these three compounds, MS2 brought selectivity. However, for other isobaric compounds, the MS1 ion gave identical fragments in MS2, as can be seen in Table 3 where the common ions observed in MS1 (*m/z* 83, 85, 99 and 101) give the same MS2 ions (e.g., *m/z* 83 fragmented to *m/z* 55, *m/z* 85 broke down to *m/z* 57 and 43, *m/z* 99 broke down to *m/z* 81 and 53 and *m/z* 101 broke down to *m/z* 83 and 55). This shows the experimental limitations of identifying small isobaric compounds using MS–MS.

The second case presented here included five terpenes (MW 136) and five terpenoids (MW 154) which have more diverse structures than the C6 compounds, i.e., cyclic structure (e.g., limonene, terpinene,), bicyclic structure (e.g., pinenes) or acyclic structure (e.g., myrcene, linalool). Table 4 lists the MS1 ions obtained for both terpenes and terpenoids. These MS1 spectra were dominated by ions at *m/z* 137, 155 and 169, and MS1 gave some resolution which was enhanced when MS2 was taken into account. Table 5 presents the data in a format which allows easy comparison of the MS ions and shows which compounds can be differentiated. Parent ions at *m/z* 137 and 81 were not included in this table as they

Table 4
MS1 ions from some isobaric terpenes (MW 136) and terpenoids (MW 154) obtained using APCI- IT-MS of the compounds in the gas phase

Terpenes	m/z	%	Terpenoids	m/z	%
α-Pinene	137	60	β-Citronellal	137	40
	153	100		155	100
	155	20		171	30
	171	20		173	70
β-Pinene	137	100	1,8-Cineole	137	30
	153	30		155	100
	155	60	Menthone	155	100
β-Myrcene	81	10	γ-Terpineol	137	50
	137	100		153	20
	153	60		155	100
	155	50		169	6
	169	60		171	20
Limonene	137	60	Linalool	137	36
	153	80		81	100
	155	80			
	169	100			
Terpinolene	137	100			
	153	65			
	155	20			
	169	60			

Table 5
MS2 fragments obtained from targeted masses of terpenes and terpenoids

MS1 ion	MS2 fragments	81	95	107	109	111	123	133	135	137	151	153
m/z 155	ß-Citronellal											
	1,8-Cineole	w	w									
	Menthone	w	w									
	γ-terpineol	w	w									
	a-Pinene											
	ß-Pinene	w	w									
m/z 153	γ-terpineol											
	a-Pinene											
	ß-Pinene											
	ß-Myrcene											
	Limonene											
	Terpinolene											
m/z 169	γ-terpineol											
	ß-Myrcene											
	Limonene											
	Terpinolene											
m/z 171	ß-Citronellal											
	γ-terpineol								w			
	a-Pinene				w				w			

w in a cell indicates that wideband activation is required to generate these fragments by MS–MS.

gave identical MS2 fragments and made no contribution to selectivity. For the other MS1 ions, some compounds formed unique fragments in MS2, which could allow their individual monitoring from the mixture of the compounds reported here. Limonene produced a unique fragment at *m*/*z* 111 from parent ion *m*/*z* 169, β-myrcene alone formed *m*/*z* 81 from *m*/*z* 153, and γ-terpineol was the only compound to produce *m*/*z* 107 from *m*/*z* 155. MS2 also allowed the differentiation of α- and β-pinene. The feasibility of using MS3 was studied but, due to the common structural elements in these molecules gave no further improvement.

The MS–MS studies show that some differentiation of C6 and terpene compounds is possible in the simple mixtures used. However, in flavour analysis, the resolution limit of MS2 would be affected by the number of isobaric compounds present as well as the relative proportions of each compound.

3.5. MS2 breath-by-breath analysis

The preceding sections have demonstrated the strengths and limitations of IT-MS. The IT-MS system was then applied to differentiate isobaric compounds during the consumption of a real food sample (chocolate). A full scan mass spectrum of the headspace above a commercial chocolate sample showed the major ion occurred at *m*/*z* 87. In chocolate, three compounds could contribute to this ion; 2-methyl butanal, 3-methyl butanal and diacetyl [19,20]. Experiments on the authentic compounds showed that in MS2, these three compounds fragmented as follows; 2-methyl butanal *m*/*z* 69, 45 and 41, 3-methyl butanal *m*/*z* 69 and 41, and diacetyl *m*/*z* 59 alone. Chocolate samples were spiked with 100 mg/kg of each compound alone and the breath-by-breath release of parent ion *m*/*z* 87 was monitored in MS2 for each spiked chocolate sample. Fig. 4 shows the relevant MS2 ion trace

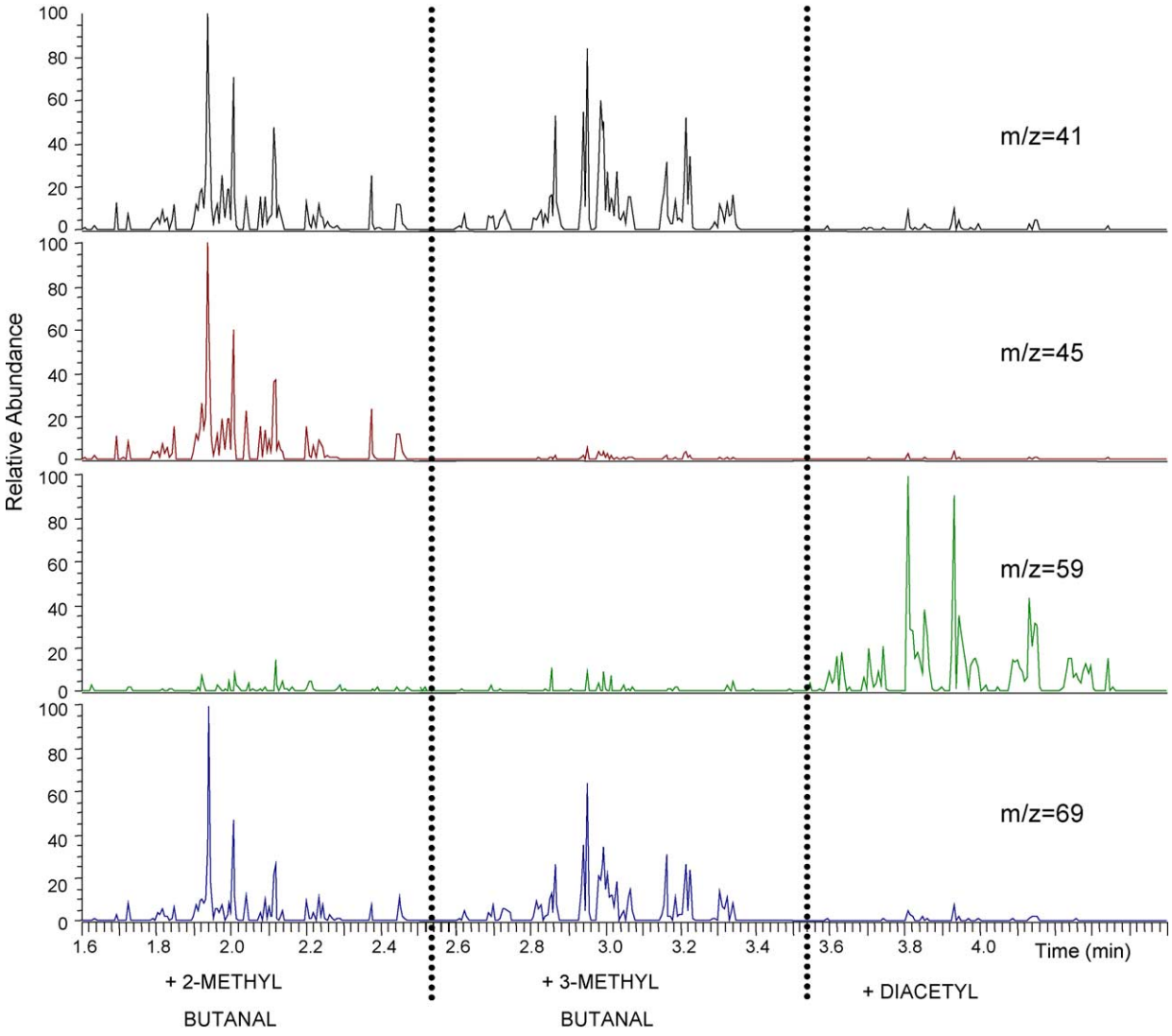

Fig. 4. Monitoring release of isobaric aroma compounds from chocolate during eating. Chocolate samples spiked with either 2-methyl butanal, 3-methyl butanal or diacetyl (all with molecular mass 86) were placed in mouth at 1.6, 2.6 and 3.6 min, respectively and eaten. The traces show breath by breath release of all three compounds by following the MS2 fragments of parent ion 87 during consumption of the chocolate samples.

for each spiked chocolate sample. Sampling flow rate was increased to 20 mL/min to improve the signal intensity. AGC was maintained ON and the maximum injection time set for this MS2 event was 200 ms, which resulted in a data point every 400 ms. This scan frequency was fast enough to follow breath inhalation and exhalation, but was too slow to show the fine detail of the chewing and swallowing actions which have been previously noted with APCI–MS [21]. A faster MS2 DPCI was attempted, but resulted in a severe loss of sensitivity.

At these concentrations, it was possible to differentiate the three compounds satisfactorily by monitoring ions at *m*/*z* 41, 45, 59 and 69 (Fig. 4). Diacetyl and 2-methylbutanal were directly resolved as they produced unique MS2 fragments at *m*/*z* 59 and *m*/*z* 45 respectively. The trace of 3-methylbutanal could be retrieved if required from ions at *m*/*z* 41 and 69 by removing the part of the signal due to 2-methylbutanal.

When this technique was applied to normal chocolate samples (i.e., without spiked compounds), the MS2 fragment from 2- and 3-methylbutanal were successfully monitored, while the signal at *m*/*z* 59 from diacetyl was rather weak (APCI is not very sensitive to diacetyl [22]) and the presence of diacetyl could not be confirmed with certainty (data not shown).

This study on chocolate illustrates the limits of MS2 breath-by-breath analysis. It has the potential to improve resolution for in vivo analysis, but was still short both in sensitivity and speed to be fully operational on a real food system.

4. Conclusions

An APCI source was optimised for use on the IT-MS machine through design and careful choice of operating conditions. Performance was monitored by ion fragmentation, sensitivity and by duty cycle. For in vivo analysis, the APCI-IT-MS was slower and showed more fragmentation than the commercial APCI quadrupole machine. However, when speed is not imperative or if several ions need monitoring, the IT-MS showed a better sensitivity. The IT-MS system could resolve some isobaric compounds by MS–MS in the simple mixtures used and could resolve isobaric compounds released from a real food (chocolate) during eating where the predominant ion monitored (*m*/*z* 87) was a mixture of ions from three compounds.

Therefore the principle of using MS–MS to resolve some isobaric odour compounds was established but the technique currently lacks sensitivity and speed for further use on real food systems. IT-MS instrument developments will provide faster duty cycles with even better sensitivity and hybrid mass analysers [23] also have great potential for flavour analysis. These improvements in instrumentation will no doubt allow further progress to be made.

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