JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of Linear Response in the Detection of Aroma Compounds by Atmospheric Pressure Ionization−Mass Spectrometry (API-MS)

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Linearity and detection thresholds of atmospheric pressure ionization−mass spectrometry (API-MS) were determined for 11 aroma compounds in air at concentrations ranging from 50 ppb to ~450 ppm (moles of volatile per mole of air). In most cases, the protonated molecular ion (i.e., $m/z = M + 1$) was the base peak throughout the range; however, some compounds showed an increase in fragmentation at lower concentrations. Detection limits varied greatly (from 50 ppb to 14 ppm) depending upon the aroma compound being measured. The linear range was also strongly dependent upon the aroma compound, with values ranging from <10-fold change in concentration to >4000-fold change in concentration depending upon the volatile being studied. The two volatiles with poor detection thresholds also exhibited the smallest linear range. Most compounds had linear ranges of >200. There was no apparent relationship between gas-phase basicity and either detection limit or linear range.

KEYWORDS: APCI-MS; aroma compounds; linear response; breath analysis

INTRODUCTION

It is generally recognized that it is not only the quantity and concentration of aroma compounds present in a food but also the release of these compounds in the mouth during eating that determine sensory quality. This recognition has led to substantial research on aroma−food matrix interactions because these interactions influence the driving force for release (i.e., vapor pressure) and on the factors determining release (e.g., breakdown of the food structure in the mouth to produce surface area for evaporation). Basic aroma−food matrix interactions can be readily studied using static methods such as equilibrium headspace analysis or dialysis. The determination of aroma release during eating (or simulations thereof) has typically necessitated dynamic measurements. These methods have involved simulated mouth systems (*1*−*4*), food residue analysis (*5*), exhaled odor measurement (*5*, *6*), or real time breath analysis of human subjects (*7*, *8*).

Early techniques to measure volatiles in the nose or mouth during eating involved the use of Tenax trapping (gas chromatography, GC) or membranes (mass spectrometry, MS) to separate aroma compounds from air and moisture (*9*−*13*). Gathering time-release data by Tenax trapping methods is slow, and one typically does not get individual breath-by-breath data but pooled data over several breaths (the method from ref *14*, however, collects individual breaths). Results have shown that the time-release profile is very dynamic, and one may wish to collect breath-by-breath data to get a more detailed picture of this phenomenon. Thus, researchers have sought more rapid measurement techniques.

Passing breath (or food headspace) through traditional MS membrane separators allows the introduction of volatiles into electron impact sources while excluding air and water (*9*, *10*). These methods enable the analysis of time-release profiles in almost real time, but suffer from selective permeability of the membrane for different compounds. This can complicate both quantification and time profiling. Most recent methods rely on atmospheric pressure ionization−mass spectrometry (API-MS), which does not require membranes to separate the gas sample from the ionization region of the MS (*15*). Unfortunately, initial efforts to directly introduce breath into MS ion sources caused difficulties due to interferences from breath components, for example, ammonia, moisture, and acetone. Later, improved API systems for direct sampling of exhaled human breath have been described (*16*).

Linforth and Taylor (*7*, *8*, *17*, *18*) have pioneered the development of direct API-MS breath analysis. They have used a single-quadrupole MS with a proprietary venturi inlet system (*19*). Since their original work, several other researchers have developed variations on this approach. Taylor and Linforth's venturi inlet has been compared to a simple direct inlet where a vacuum is applied to the source to draw the sample into the ionization chamber through a glass tube (*20*, *21*). This direct inlet is considered to be more sensitive (up to 100×) than the venturi inlet, but less linear and more specific in compound

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10.1021/jf0486545 CCC: $30.25
Published on Web 01/14/2005

response. It suffers from quenching in the source and has a relatively noisy baseline. One should note that this system was interfaced with a Finnigan LCQ-MS. This is an ion trap system as opposed to a quadrupole system, so some of the differences may be related to the MS as well as the inlet system.

Other researchers have also adapted the standard API inlet of a Finnigan LCQ-MS for breath sampling, but incorporated a venturi inlet similar to that patented by Linforth and Taylor. They focused on adding moisture to the source to improve sensitivity by decreasing fragmentation (*22*). In addition, there have been reports on the use of a proton-transfer-reaction MS (PTR-MS) for both breath analysis and monitoring volatile formation during coffee roasting (*23*−*26*).

The API process involves the formation of an initial reactant ion that can then transfer its charge to any molecule with a higher proton affinity. Water is an excellent choice for the reagent molecule as its proton affinity lies between those of the main components of air (nitrogen, oxygen and carbon dioxide) and those of most volatile organic compounds. There are two main advantages of this: first, water is a requirement for the analysis and not an obstacle; second, none of the air components are ionized, but a wide range of organic compounds that possess odor are ionized. The basic reaction is therefore the generation of a protonated ion to be detected by the mass spectrometer:

$$H_3O^+ + M \rightarrow MH^+ + H_2O$$

According to this equation, the ionization of compounds depends on their gas-phase basicity, that is, their ability to accept a proton (*27*).

It has also been demonstrated that there is a linear response to M as long as depletion of H_3O^+ is negligible. This is characterized by the equation

$$\sum I(MH^+(H_2O)_b) = \frac{[M]}{[H_2O]} R_{eq} \sum I(H_3O^+(H_2O)_h)$$

where R_{eq} is a constant at a given temperature, $[H_2O]$ is the ion source water concentration, I is the ion intensity noted [e.g., on the right-hand side of the equation above, I is the ion intensity of $(H_3O^+(H_2O)_h)$], and $(H_2O)_b$ and $(H_2O)_h$ indicate the formation of water clusters besides H_3O^+ (*28*). Because depletion of H_3O^+ poses an inherent limitation to the analytical abilities of API-MS, determination of linearity becomes essential to obtain reliable data (*28*).

The objective of this research was to better understand the strengths and weaknesses of an ion trap API-MS system for breath analysis. To this end, this paper presents our studies on detection thresholds and the linearity in the response of a Finnigan LCQ API-MS when coupled with a venturi inlet system.

MATERIALS AND METHODS

Volatiles. Eleven compounds of different chemical functionalities and organoleptic characteristics were analyzed by API-MS for their ionization patterns and linear responses: ethyl butyrate, *cis*-3-hexenol, ethyl isovalerate, isoamyl isovalerate, γ-decalactone, methyl dihydrojasmonate, ethyl 3-methyl-3-phenylglycidate, benzaldehyde, 2-octanone, 2-methylbutyric acid, and 2-methylpyrazine. These compounds were kindly provided by Robertet Flavors Inc. (Piscataway, NJ).

Sample Preparation. Approximately 0.4 g of each compound was dissolved in 10 mL of pentane, an inert and volatile saturated hydrocarbon that has been proven not to interfere with the analysis of odorants (*22*). The complete volatilization of 50 μL of this solution in 1 L of air yields 2000 μg/L of air of odorant in a closed environment under equilibrium conditions. Thus, we prepared a progressive dilution (factor of 2) of this stock solution in pentane to ultimately obtain a range of gaseous concentrations suitable for analysis: 0.5−2000 μg/L of air.

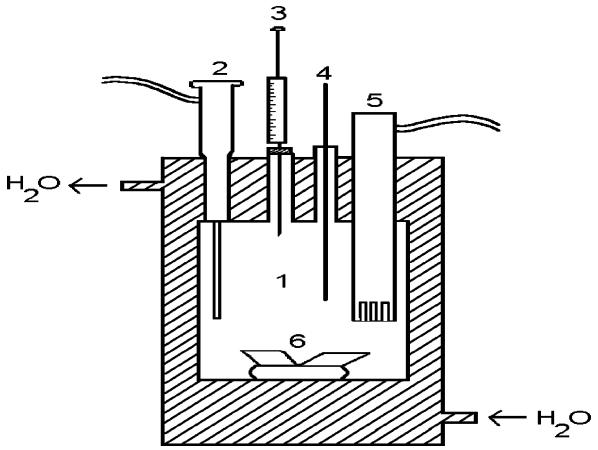

Figure 1. Diagram of the glass vessel used for preparing known concentrations of test volatiles for API-MS: 1, water-jacketed glass vessel; 2, temperature sensor; 3, port for syringe injection of test volatiles; 4, capillary outlet; 5, hygrometer; 6, magnetic stirrer bar with two blades attached.

A 50 μL aliquot of each dilution was injected through a septum into a 1 L glass vessel, water-jacketed at 38.5 °C (**Figure 1**). To accelerate equilibration of the odorants, the air inside the vessel was continuously circulated by a magnetic stirrer. After 3 min of stirring (sufficient to achieve 100% volatilization of test compounds), the capillary outlet (4 in **Figure 1**) (20 cm long, 0.53 mm i.d., 0.73 mm o.d.; Chrompack, Middelburg, The Netherlands) was connected to the MS source inlet (30 cm long, similar characteristics) via a plastic connector. The source capillary was heated to 75 °C by a heated transfer line to avoid odorant condensation.

Gas-Phase Analysis. To introduce gaseous samples in sufficient quantities into the API interface of the instrument, the original API probe assembly was modified as described elsewhere (*22*). Briefly, the original capillary (0.15 mm i.d., 0.39 mm o.d.) was replaced with the aforementioned methyl-deactivated capillary and installed in the instrument through a hole (0.78 mm in diameter) made by drilling the sample tube inlet fitting, manifold, vaporizing flange, and nozzle.

Once a test volatile had been added to the glass evaporation vessel and equilibrated for 3 min, the vessel was sampled for ∼45 s. The vessel was then rinsed with methanol and dried by flushing hot air (120 °C) through it. Each sample was run in duplicate.

API-MS operating conditions were as follows: vaporizer temperature, 200 °C; capillary temperature, 150 °C; capillary voltage, 15 V; corona discharge needle voltage, 5 kV; plasma current, 5 μA; sheath gas, nitrogen; pressure, 80 arbitrary units (5.7 L/min); auxiliary gas, nitrogen; pressure, 60 arbitrary units (7.5 L/min); flow rate of sample into the source, 85 mL/min; and full MS scanning in positive ion mode (1 scan/s). To increase the relative humidity of both sheath and auxiliary gases, which enhances sensitivity and decreases fragmentation, two stainless steel washing bottles were filled with 100 mL of HPLC grade water and kept at a constant temperature of 38.5 °C. Humidification was achieved by passing the gas through a frit (5 μm pore size), with a resulting relative humidity of ∼65% for both gases after leaving the bottles (*19*).

Linearity and Detection Limits. The lower detection limit for each volatile was determined as the concentration of the dilution that yielded an MS response at least 3 times the background noise. The linear range was determined as the quotient of the highest concentration dilution giving a linear MS response on the log:log plot of MS response versus concentration and the volatile concentration in the lowest dilution that was linear on this plot. In neither case were the data interpolated to give finer detail. In some cases, more than one ion was used in this calculation. Depending upon the MS fragmentation pattern, the M + 1

Table 1. Detection Limits and Linearity of Volatiles

	current study			literature	
volatile	detection limit[a,b]	linear range[b] (ppm)	dynamic range[c]	detection limit	dynamic range
ethyl butyrate	97 ppb	0.097–100.5	1038	10 ppbv[d]	240[d]
cis-3-hexenol	110 ppb	0.11–474	4230	6 ppbv[d]	200[d]
ethyl isovalerate	90 ppb	0.09–22	220		
isoamyl isovalerate	66 ppb	0.066–67	1019		
γ-decalactone	65 ppb	0.065–17	261		
methyl dihydrojasmonate	50 ppb	0.05–12.6	255		
ethyl 3-methyl-3-phenylglycidate	3 ppm	3–28	<10		
2-octanone	350 ppb	0.35–96	270		
2-methylpyrazine	127 ppb	0.127–65	511		
2-methylbutyric acid	14 ppm	14–455	32		
benzaldehyde	210 ppb	0.21–438	2085		
toluene				200 ppt[e]	10000[e]
benzene				200 ppt[e]	10000[e]

[a] Taken as lowest concentration where signal-to-noise ratio is >3. [b] Concentration expressed as moles of volatile per mole of air. [c] Calculated as a ratio of upper limit of linearity divided by detection limit. [d] Parts per billion on a volume basis, estimated from data presented in Figure 5 of ref *27*. [e] Parts per trillion (*23*).

ion alone may have been used in calculations or up to two other ions may have been summed to obtain MS response. This depended upon the fragmentation of the volatile and is noted in the discussion that follows.

Gas-Phase Basicity Determination. To determine the proton affinity of flavor compounds in the gaseous phase, experiments were carried out in a dual-cell Fourier transform mass spectrometer (FTMS) (model 2001 Finnigan MAT/ThermoQuest, San Jose, CA) equipped with a 3.0 T superconducting magnet. For the proton affinity measurements of the flavor compounds, the following scheme was used:

$$MH^+ + B \rightarrow M + BH^+ \qquad (k_f)$$

$$BH^+ + M \rightarrow B + MH^+ \qquad (k_r)$$

where M is the flavoring species of unknown gas-phase basicity, B is the reference compound of known basicity, MH^+ and BH^+ are the corresponding protonated forms, k_f is the reaction constant for the forward reaction, and k_r is the reaction constant for the reversed reaction. The MH^+ ions were prepared in the FTMS by the protonation of compound M with H_3O^+ generated by the electron ionization of H_2O at 6 eV. The MH^+ ions were then transferred to the second cell, where the requisite base (B) was present at constant pressure. In the reversed direction, the base B was protonated with H_3O^+ and the resulting BH^+ was transferred to the second cell, where compound M was present at constant pressure. The ions of interest were isolated by applying SWIFT (stored-waveform inverse Fournier transform) waveform and/or chirp broad-band excitations (*29*). (Argon was pulsed into the cell at pressures of 10^{-5} Torr in an attempt to thermalize the ions before any proton-transfer reactions were examined.) All neutral reagents were introduced via slow leak valves, and the subsequent reactions were monitored over time. The following series of standard reference compounds in increasing order of gas-phase basicity was used to determine the gas-phase basicity (proton affinity) of the flavoring compounds: nitrobenzene, acetone, tetrahydrofuran, benzaldehyde, ammonia, acetophenone, 2,3-dihydrofuran, and aniline.

RESULTS AND DISCUSSION

Table 1 presents our data on detection limits, linear ranges, and gas-phase basicity for some volatiles using our LCQ API-MS. These volatiles were selected for study because we used them in previous studies (*22*, *30*). Our results are compared with data from the literature (*23*, *27*) whenever possible.

Ethyl Butyrate. Two major ions were observed for ethyl butyrate: the protonated molecule (*m*/*z* 117) and the protonated butyric acid fragment (*m*/*z* 89). The former was the base peak throughout the concentration range analyzed, with a relatively constant 117:89 fragmentation ratio (100:12.5) over the range of concentrations studied. The lower detection limit and linear range of the instrument for this volatile were 97 ppb and 0.097–100.5 ppm, respectively (**Figure 2**).

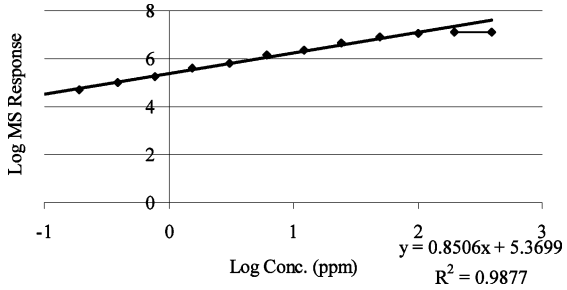

Figure 2. Log:log relationship between ethyl butyrate concentration and API-MS response (R = sum of *m*/*z* 117 parent ion and *m*/*z* 89 fragment). Concentration is expressed as parts per million (μmol of volatile/mol of air).

The linearity of ethyl butyrate for the venturi inlet–quadrupole API-MS system has been reported to be linear over ~3 orders of magnitude (*27*). Applying our method of calculating the linear range to these data on ethyl butyrate, the above system would have a linear dynamic range slightly greater, calculated to be 1038× for this volatile. Although our system may have a slightly higher linear range for this volatile, the venturi inlet–quadrupole API-MS system has a 10× lower detection limit (10 ppbv) for it.

***cis*-3-Hexenol.** Two primary ions were found for *cis*-3-hexenol: the parent protonated molecule *m*/*z* 101 and the fragment *m*/*z* 83, corresponding to the loss of one water molecule from the parent ion. Neither ion could be characterized as the base peak because the fragmentation ratio varied widely throughout the concentration range: from 100:73 at the lowest concentration studied to 100:116 at the highest concentration. Therefore, these ions were summed in the determination of instrument detection limit (110 ppb) and linearity (4230×). In general, ionization of this compound was weak, as reflected by the MS response in comparison with other chemicals in our study. Interestingly, the linearity of this volatile was exceptional, covering nearly 4 orders of magnitude (**Figure 3**).

Data from the literature for *cis*-3-hexenol indicate a detection limit of ~6 ppbv for the venturi inlet–quadrupole API-MS system, certainly vastly more sensitive than ours (*27*). However, these data also suggest that *cis*-3-hexenol has a linear range of perhaps 2 orders of magnitude, which is as much as a 20× smaller linear range than our ion trap MS system.

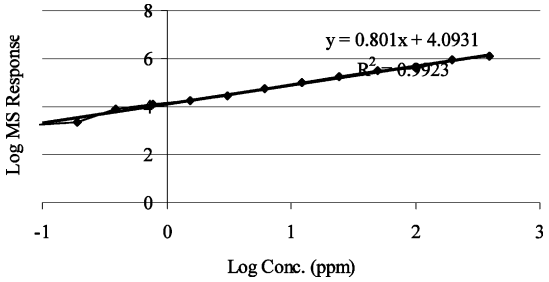

Figure 3. Log:log relationship between *cis*-3-hexenol concentration and API-MS response (R = sum of m/z 101 parent ion and m/z 83 fragment). Concentration is expressed as parts per million (μmol of volatile/mol of air).

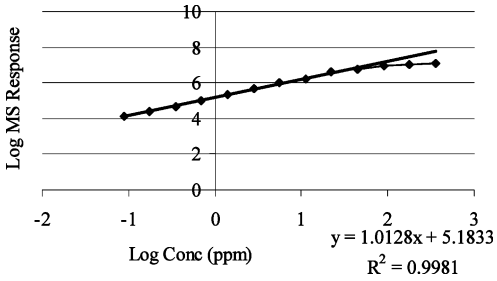

Figure 4. Log:log relationship between ethyl isovalerate concentration and API-MS response (R = sum of m/z 131 parent ion and m/z 103 fragment). Concentration is expressed as parts per million (μmol of volatile/mol of air).

Ethyl Isovalerate. The protonated molecular ion m/z 131 was the base peak with one major fragment (m/z 103) that corresponded to the protonated isovaleric acid. However, the fragmentation ratio increased at lower concentrations (ranging from 100:46 at the lowest concentration to 100:16 at the highest concentration). We found a lower detection limit of 90 ppb for this compound. Although response did not reach a plateau, the slope of the plot decreased at concentrations above ~22 ppm (**Figure 4**). The data were linear for only slightly more than 2 orders of magnitude of change in concentration. There are no data in the literature for comparison.

Isoamyl Isovalerate. Similar to ethyl isovalerate, the protonated molecular ion (m/z 173) was the most abundant ion over the concentration range, whereas m/z 103 was the second ion observed from the isovaleric acid fragment. Similar to the previously discussed compounds, the 173:103 fragmentation pattern changed from the lowest (100:74) to the highest (100:21) concentration. This ester had a slightly lower detection limit (66 ppb) than the other two esters and a linear range of 0.066−67 ppm.

γ-Decalactone. This intramolecular ester provided the protonated molecular ion m/z 171 and two ions corresponding to the loss of one and two water molecules: m/z 153 and m/z 135. It had the steepest slope of response versus concentration among all volatiles studied ($y = 1.097x + 5.841$). The detection limit was 65 ppb and the linear range 0.065−17 ppm. The parent ion m/z 171 was the base peak over the concentration range studied.

Methyl Dihydrojasmonate. Two ions were identified in the MS profile of this complex ester: the protonated molecular ion m/z 227 and the m/z 209 fragment resulting from the loss of one water molecule. The fragmentation ratio 227:209 increased slightly at lower concentrations (100:7 to 100:22). It had a detection limit (50 ppb) similar to those of other volatiles and a small linear range (0.050−12.6 ppm).

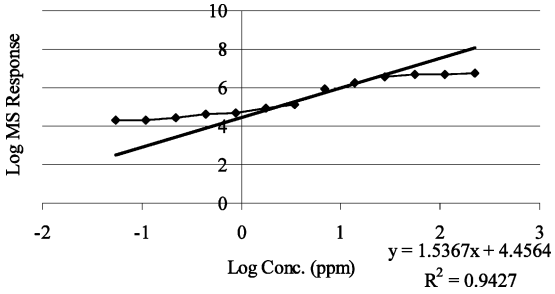

Figure 5. Log:log relationship between ethyl 3-methyl-3-phenylglycidate concentration and API-MS response (R = m/z 207 parent ion). Concentration is expressed as parts per million (μmol of volatile/mol of air).

Ethyl 3-Methyl-3-phenylglycidate. This volatile goes by several other names in the industry including aldehyde C-16 or strawberry aldehyde. The parent protonated molecular ion m/z 207 was the base peak over two fragments of much lesser importance: m/z 133 and m/z 105, which were consequently discarded. This compound yielded very poor data (**Figure 5**). The detection limit was 3 ppm and the linear range 3−28 ppm.

Benzaldehyde. Only the protonated molecular ion was detected for benzaldehyde: m/z 107. The detection limit was slightly lower than that of some of the other volatiles studied (210 ppb), but it exhibited a wide linear range (0.21−438 ppm). This range may have been even greater because the MS response did not plateau within the range of concentrations studied.

2-Octanone. Two ions were identified in the MS of this volatile: the protonated molecular ion m/z 129 and the fragment m/z 111, derived from the loss of one water molecule from the parent ion. The ratio of 129:111 was reasonably constant (100:5) down to a concentration of 730 ppb, but then increased rapidly to 100:62 at the lowest concentration studied. This volatile exhibited a behavior similar to that of benzaldehyde, with an S-shaped log:log plot and lower detection limit and linear range of 350 ppb and 0.35−96 ppm, respectively.

2-Methylbutyric Acid. The parent protonated molecule m/z 103 and the fragment m/z 85 resulting from the loss of one water molecule were the two ions present in the spectrum of this organic acid. Although the former was the base peak for most of the concentration range studied, there was an increase in the fragmentation ratio (103:85) as the concentration decreased. The MS is not particularly sensitive to this volatile, having a detection limit of ~14 ppm. The MS provided a linear response up to the highest concentration used in this study (455 ppm); thus, the linear range may have been greater than reported in **Table 1**.

2-Methylpyrazine. The protonated molecular ion, m/z 95, was the only ion detected in the analysis of this heterocyclic flavorant. The system showed average sensitivity with a detection threshold of 127 ppb. The system was linear from this threshold to the 65 ppm dilution.

Gas-Phase Basicity. Gas-phase basicity values for each of the volatiles studied are presented in **Table 2**. Earlier work noted that compounds with gas-phase basicities of ≥200 kcal/mol are readily ionizable by API-MS and give similar responses (*28*). This is evidently related to the fundamental protonation reaction, as compounds must have a higher gas-phase basicity than that of water (158 kcal/mol) for the reaction to take place.

The volatiles chosen for our study have gas-phase basicities very close to the 200 kcal/mol cutoff aforementioned and, thus, we would expect similar MS responses and behaviors. Although our volatiles with basicities >200 had similar detection limits, they did not have a wide basicity range that would allow any

Table 2. Gas-Phase Basicity Values[a]

ethyl butyrate	204
cis-3-hexenol	202
ethyl isovalerate	205
isoamyl isovalerate	207
γ-decalactone	205
methyl dihydrojasmonate	207
ethyl 3-methyl-3-phenylglycidate	209
2-octanone	198
2-methylpyrazine	211
2-methylbutyric acid	n/a
benzaldehyde	199.3[b]
toluene	187.4[b]
benzene	179.3;[b] 181.9[c]

[a] All values are measured in kilocalories per mole. All values are from in-house measurements unless otherwise referenced. [b] U.S. Secretary of Commerce on behalf of the United States, http://webbook.nist.gov/chemistry/. [c] From ref *23*.

firm conclusions in this respect to be drawn. The variation we see in thresholds (and to some extent linearity) may be partially due to the background level of the selected ions in our environment/instrument. Because the detection threshold was defined as the volatile concentration where MS response equaled or exceeded the background noise by a factor of 3, the background presence of given ions would influence our observed instrument thresholds.

Instrument background also influences the linear range observed for our volatiles because this was calculated as the ratio of the highest linear concentration divided by the lowest linear concentration (influenced by instrument background). It is therefore evident that one must work in a clean environment (*27*). We would not expect instrument background to have a significant effect on the upper limit of the linear range. Here we found considerable variability among compounds but no relationship to gas-phase basicity.

Conclusions. Our results bring several points into consideration. One point is that there is substantial variability in API-MS sensitivity to different aroma compounds. Although gas-phase basicity may be a predictor of sensitivity, the small variability in gas-phase basicity values of the volatiles used in our study preclude the drawing of any conclusions in this respect. However, we expect the cleanliness of the instrument and laboratory environment to be key factors influencing the detection limits. A high instrument background would decrease instrument sensitivity.

A second observation from a comparison of our data to the literature is that different types of API-MS instruments (or source inlets) give different sensitivities and linearities for the same aroma compounds. The venturi inlet−quadrupole API-MS system (*19*) offers much better sensitivity than our ion trap instrument across the two volatiles analyzed in common (ethyl butyrate and *cis*-3-hexenol). Linearity may have been better for our ion trap system, but absolute comparisons between the data are difficult. Other researchers reported on the sensitivity and linearity of a system similar to ours (Finnigan LCQ API-MS) for breath-by-breath analysis, comparing it to the venturi inlet−quadrupole API-MS system (*20*). Although they reported that their direct inlet was ~100× more sensitive, they did not present quantitative data to support these observations. They remarked that their direct inlet was also very nonlinear, but no data were presented in support of this statement either.

Literature claims of superior sensitivity have been made for the PTR-MS (*23*). Unfortunately, no data have been presented that permit a direct comparison of this instrument to our ion trap or the venturi inlet−quadrupole under similar sampling conditions and similar volatiles. Thus, it is difficult to speculate as to the relative performance of this instrument.

ACKNOWLEDGMENT

We thank Robertet Flavors, Inc., for supporting this work, as well as Ashutosh Singh and Dr. Stephen Cass (Department of Chemistry, University of Minnesota, Minneapolis, MN) for performing the gas basicity measurements for us.

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Received for review August 10, 2004. Revised manuscript received November 14, 2004. Accepted November 28, 2004.

JF0486545